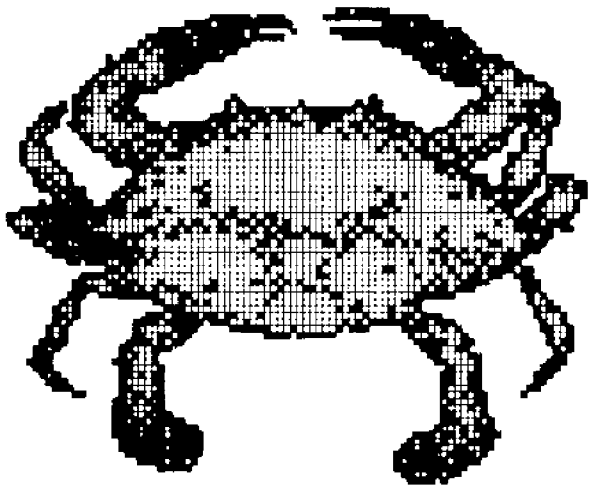
The Evaluation of Water Quality Variations in BLUE CRAB SHEDDING SYSTEMS



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Water Quality Fluctuations in Response to Variable Loading in a Commercial Closed Blue Crab Shedding Facility

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ABSTRACT

A commercial, closed recirculating seawater facility using biological filters for the control of nitrogenous metabolites is described. The volume of each system was 7,560 liters. Loading values of over 1000 crabs (Callinectes sapidus Rathbun) were maintained in each system. Water quality parameters (NH_3-N, NO_2-N, NO_3-N, pH, dissolved oxygen, salinity, temperature, alkalinity) affecting molting survival were monitored for a two-month period and safe operational ranges established. Alkalinity and pH values declined in the systems, demonstrating a limited buffering capacity of the system. NO_3-N values exceeding 350 mg/l were observed with no apparent effects to the crabs. Increased molting mortality was observed when concentrations of nitrite approached 1.6 mg/l NO_2-N. Nitrite accumulations were associated with depressed oxygen levels induced by peak system loadings or equipment failure. Successful molting rates of over 95 percent were associated with nitrite and ammonia concentrations below 1 mg/l.

KEYWORDS: Aquaculture, biological filter, blue crab, closed system, molting, water quality.

INTRODUCTION

Reported landings for soft crab species have declined drastically in most states harvesting the resource (Jaworski, 1971; Otwell, et al., 1980; Perry, et al., 1982a). According to Jaworski (1971), this lowered production is attributed to a deterioration of coastal zones and accompanying decline in water quality. Despite the decline in landings, the value of soft crabs has continued to rise in the Gulf of Mexico area averaging $4.50/Kg as compared with $1.94/Kg in 1970 (Perry et al. 1982b).

Traditionally, premolt blue crabs were collected and held in natural waters in floating boxes or pens until they molted (Haefner and Garten, 1974). The continuing decline in coastal water quality and subsequent increase in molting mortality have forced fishermen to turn to shore-based facilities to reduce molting (shedding) losses (Jaworski 1982). The potential value of using closed, recirculating seawater systems for maintaining molting crabs has been demonstrated by a few successful commercial operators (Perry et al. 1982a). Recirculation systems reduce labor requirements and eliminate the exposure of crabs to deleterious environmental effects during the vulnerable molting period; however, their success has been marginal because of the lack of established design criteria and management guidelines (Van Gorder and Fritch 1980; Ogle et al. 1982).

In the Gulf of Mexico, where crab fishermen are often limited by the availability of molting blue crabs, shedding operators strive for a molting mortality of less than 5 percent to maintain production and commercial viability. On the eastern coast of the United States, commercial shedding systems generally receive an abundance of crabs and therefore can absorb a higher crab mortality loss (5 to 40 percent).

The operation of a successful, closed, recirculating aquaculture system depends on the maintenance of acceptable water quality. The ability of biological filters in the closed systems to convert ammonia (NH_3), the principal nitrogenous excretory metabolite of Crustacea (Hartenstein 1970), to the relatively nontoxic nitrate (NO_3) by bacterial nitrification is summarized by Wheaton (1977) and Spotte (1979).

In 1982, a project was initiated to establish production levels and operating parameters for closed, recirculating seawater systems currently used to hold shedding crabs. This approach provided a unique

opportunity to complement experimental research on molting crabs (Malone et al. 1984) with direct observations and data from the commercial sector. In this report we describe the influence of commercial operating procedures on water quality in a large scale shedding facility.

MATERIALS AND METHODS

Description of Commercial Facility

The commercial shedding operation was located in an uninsulated building in Lacombe, Louisiana. The facility consisted of two separate systems (Figure 1), each with eight holding tanks, two biological filters, one algal tank, and a reservoir. The reservoir was located outside the building and was partially buried in the ground. This facility is a modification of that described by Perry et al. (1982a). Descriptions and dimensions of the fiberglass tanks are presented in Table 1.

TABLE 1

Dimensions of Commercial System

Description	Length (m)	Width (m)	Depth (m)	Water Depth (m)	Area (m^2)	Volume (m^3)
Crab Tank	2.44	1.07	.30	.13	2.61	.34
Biological Filter	2.44	.91	.30	.24	2.22	.53
Shell Filter Bed	2.29	.91	.08	---	2.08	.17
Dolomite Filter Bed	2.29	.91	.04	---	2.08	.08
Carbon Filter Bed	2.29	.91	.03	---	2.08	.06
Algae Filter	2.44	.91	.30	.24	2.22	.53
Holding Reservoir	2.74	1.37	1.52	1.07	3.75	4.02

Water levels in the crab tanks were controlled by a 12.7 cm standpipe constructed from 3.2 cm polyvinylchloride (PVC) pipe. Water input to the discharge nozzle in the crab tanks consisted of capped 1.3 cm PVC pipe with two 0.3 cm holes to promote active aeration in the tanks.

The biological filters were constructed with a fiberglass partition that was 15.2 cm from one end of the tank with holes drilled in the lower 2.5 cm of the partition. This head chamber received the overflow from the crab tanks through the standpipe that discharged beneath the water level in the head chamber. The function of the head chamber was to direct flow under the submerged updraft biological filter. The biological filter consisted of 7.6 cm of washed clam shells (Rangia cuneata) (2 to 3 cm in diameter) on the bottom, overlaid by 3.8 cm of dolomite (3 mm grain size) and 2.5 cm of activated carbon (1 to 3 mm grain size) (Figure 2). Each layer of media was separated by nylon window screen. The filter bed rested on 1.3 cm egg-crate louvering supported by lengths of 2.5 cm PVC pipe. The water level in the biological filter was approximately 5 cm above the top of the activated carbon bed; overflow to the algal tank was provided through a 5.1 cm PVC pipe. Theoretically, the biological filters performed two main functions: mineralization and nitrification. These functions take toxic nitrogen waste products produced by the crabs and convert them to relatively nontoxic forms. By design rational, buffering was accomplished using carbonate filtrants (shell and dolomite), and the physical adsorption of dissolved organic carbon occurred on activated marine carbon.

The algal tank contained 11 baffles that alternately extended to within 7.6 cm of the tank's sides. Historically, attached algae

grew on the sides of the baffles and water flowed in a serpentine fashion from one end of the tank to the other. Another 5.1 cm PVC pipe, from the other four crab tanks and biological filter in each system, drained into the algal tank half way through each filter. Each algal filter was illuminated by two 1.2 m fluorescent fixtures with four 40-W Grow Lux® lights. Plants in the systems included water milfoil (Myriophyllum sp.), floating on top of the algal filter, and attached filamentous green algae on the filter walls. No algal biomass was harvested from either of the systems and the algal filters were provided with a constant light regime. Water flowed by gravity from the algal filters and was carried by 7.6 cm PVC pipe through the wall of the building to the partially buried reservoir outside. Incorporation of algal filters into the system design was for the removal of nitrate, which is the end product of nitrification.

The large reservoir helped to buffer rapid water quality changes in the systems. Rapid water quality changes (typically transitional increases in ammonia and nitrite) can be associated with the introduction of a large number of crabs to a system acclimated to a lesser number of crabs, a common practice in commercial operations. Water was constantly circulated from the reservoir via a 5.1-cm PVC pipe to a 0.25-kW pump (model Dayton® 6K695). The pipe was screened to prevent intake of large debris. The water was then distributed through a 3.2-cm overhead PVC pipe to the crab tanks by a series of 1.9-cm PVC valves and tees. Water was sprayed under pressure into the crab tanks at a constant total system flow rate of 83.3 l/min.

Methods

Artificial seawater (Rila Mix®) was used in the commercial facility. The two systems were constructed and operated one year prior to the study and were shut down in the winter of 1982-83. Start-up in March of 1983 consisted of turning on the pumps and diluting the systems to volume with fresh well water. Fresh water was added to the systems to offset evaporation, but no water changes were made during the period of observation. Intermolt blue crabs and miscellaneous estuarine fish were used to acclimate the biological filters until April. During the study, premolt blue crabs ranging from 10 to 15 cm in carapace width were taken from Lake Pontchartrain, Louisiana, and held in the systems. Vinyl-coated, wire-mesh enclosures 0.3 cm in diameter isolated crabs that had molted. System management included visual inspections every three to four hours to collect soft-shelled crabs and to remove dead animals, debris, and exuviae. Crabs in the shedding systems were not fed at any time. Mean residence time for a typical crab in the system was estimated to be seven days.

Systems were monitored at 9:00 a.m. each day for temperature, salinity, dissolved oxygen, ammonia, nitrite, and pH. A one-liter sample of water was taken from each system and analyzed immediately for total ammonia and nitrite. Determinations of alkalinity levels and nitrate concentrations were made on a weekly basis. The techniques and instrumentation used to measure these parameters are listed in Table 2. Crab densities in each system were recorded daily after crab additions were made at 2:00 p.m. Data were collected through the spring shedding season of 1983, and systems were numbered (1 and 2) for reporting and identification during the interpretation of results.

RESULTS AND DISCUSSION

Alkalinity, pH, Salinity, and Nitrate

Water quality observations for pH, alkalinity, and nitrate are illustrated in Figure 3. Both systems behaved similarly with regard to the monitored parameters. Each system initially exhibited a pH of 7.7, and declined to values between 7.0 and 7.2. This reduction of pH was associated with a decline in alkalinity. The systems displayed initial alkalinities of 70 mg/l $CaCO_3$, declining to values as low as 30 mg/l $CaCO_3$, suggesting a limited capability of the dolomite and shell layers to buffer pH changes. These findings are consistent with the observations of Bower et al. (1981), who noted the limited ability of calcareous filtrants to maintain pH above 8.0. In this study, pH values fell within the 7.0 - 8.5 range of optimum nitrification rates for biological filters (Wheaton, 1977), although filters can be acclimated to lower pH values than 7.0 (Haug and McCarty, 1972). We conclude that the dolomite/shell bed was sufficient for control of pH above 7.0, even after two years of operation with no filter maintenance, and that this pH apparently does not adversely affect the crabs. In fact, the lower pH may be beneficial in that it reduces ammonia toxicity caused by the equilibrium reaction between NH_4^+ and NH_3 (Spotte, 1979; Wheaton, 1977).

Nitrate in both systems accumulated throughout the course of the study. Nitrate increases paralleled the crab loadings of both systems. Nitrate values for systems 1 and 2

TABLE 2

Measurements Taken and Techniques

Parameter	Instrument or Test	Reference
Total Ammonia as NH_3-N	Orion 95-10 Ammonia Electrode/ Orion 701 A Digital Ionalyzer	APHA (1980)
Nitrite as NO_2-N	Bausch and Lomb Spectronic 20, Spectrophotometer	Sulfanilamide-based colorimetric reaction, APHA (1980)
Oxygen as O_2	Yellow Springs Instrument Co. Dissolved Oxygen Meter, Model 51	
Salinity	American Optical Refractometer	
pH	Mini (Model 47) pH Meter	
Nitrate as NO_3-N	Modified Hydrazine Reduction	Spotte (1979)
Alkalinity as $CaCO_3$	Titration	APHA (1980)

were 171 and 214 mg/l NO_3-N, respectively, at the beginning of the observation period. Differences in these accumulated nitrate concentrations apparently resulted from unequal crab densities and the length of time the individual systems were operated in the year prior to the study (system 2 was operated for a longer period in 1982). Visual observations of the algal filters revealed little or no algal growth despite efforts to reintroduce algae from local sources and another commercial system. Values exceeding 350 mg/l NO_3-N were observed at the end of this study with no apparent deleterious effects. Nitrate is generally not toxic to marine organisms even at elevated levels (Hirayama, 1974; Siddall, 1974). Salinity in the systems remained constant at 4 and 5 ppt in systems 1 and 2, respectively.

Ammonia, Nitrite, Temperature, Dissolved Oxygen

Ammonia and nitrite increases were closely correlated with increases in crab densities (Figure 4), and both systems showed comparable results in terms of water quality and crab loadings. In system 1, total ammonia concentrations remained under 0.4 mg/l NH_3-N regardless of crab density. Nitrite concentrations, however, increased to 1.6 mg/l NO_2-N during a period of heavy loading. During the heaviest crab loading in system 2, ammonia levels approached 1.0 mg/l NH_3-N, with a similar increase in nitrite. On May 5, increased mortality of molting crabs was observed in system 1 when nitrite concentrations approached 1.6 mg/l NO_2-N. A pump malfunction occurred in system 2 on May 28, and nitrite concentrations subsequently increased. Concentrations above 1.2 mg/l NO_2-N were observed on May 29, but returned to low levels the following day. Mevel and Chamroux (1981) found that during similar pump malfunctions, the concentration of nitrate decreased and nitrite levels increased. They concluded that nitrate is reduced to nitrite when oxygenation of the environment is deficient, and that bacteria are responsible for the dissimilarity in nitrate reduction. This might explain the increase of nitrite during the pump malfunction observed in our study.

Figure 5 illustrates the temperature and dissolved oxygen levels displayed by the systems under study. Water temperature in the systems equilibrated rapidly to ambient air temperature, ranging from 11° to 27°C. Observations indicated that higher temperatures decreased the overall carrying capacity

of the systems. This is supported by the inverse effect of temperature on the saturation levels of dissolved oxygen (Figure 5). Higher temperatures also increase the metabolic rates of both the heterotrophic and nitrifying bacteria (Wild et al. 1971), and the crabs (Laird and Haefner 1976). Oxygen levels in both systems were influenced by this increased biological activity. Oxygen measurements in the commercial systems were taken after the biological filter and before the algae tank. Because of the large open-water surface area on the top of the upflow biological filters, it was thought that some surface reaeration occurred before D.O. measurement; however, in both commercial systems the lowest dissolved oxygen values were concurrent with peak values of nitrite and crab loadings, suggesting intense activity in the biological filter. These observations are consistent with those of Manthe et al. (1984) who identified dissolved oxygen as the factor limiting the efficiency of nitrification beds in experimental crab shedding systems of the same design. That study also demonstrated that as D.O. concentration decreased, toxic nitrite concentrations increased, resulting in significant crab mortality.

In the latter part of the study, the biological filters began to overflow in the head chambers. Accumulations of detritus were observed in the nylon screens separating the different layers of media in the filter bed. These accumulations may have led to the short circuiting of the filter bed, thus reducing its nitrification ability. Annual biological filter breakdown and cleaning should be considered using this design.

CONCLUSIONS

Throughout the study, acceptable water quality was maintained with this filter design and successful molting rates of more than 95 percent were observed in the facility. Loading values of over 1,000 crabs were maintained by each system over the observed period. Table 3 summarizes observed operational ranges for selected water quality parameters in the systems studied.

Both systems exhibited a decline in pH and alkalinity demonstrating a limited capacity of the dolomite and shell layers to buffer the systems over the two-year period. Although pH values were below recommended standards for seawater culture systems (Spotte, 1979), the observed pH range did not adversely affect molting success. Nitrate levels in both systems accumulated throughout the course of the study. Values exceeding 350 mg/l NO_3-N were observed with no apparent deleterious effects.

TABLE 3

Observed Operational Ranges for Selected Water Quality Parameters

Parameter	Range
Total Ammonia	0 - 1 mg/l
Nitrite	0 - 1 mg/l
Nitrate	0 - 350 mg/l
pH	7 - 8
Temperature	11 - 27°C

Decreased molting success was observed when concentrations of nitrite approached 1.6 mg/l NO_2-N. Total ammonia levels did not rise above 1.0 mg/l NH_3-N and these levels had no apparent harmful impact on the molting crabs.

In both systems the lowest dissolved oxygen values were concurrent with peak values of crab density and nitrite, indicating an intense oxygen demand in the biological filters to process the increased production of nitrogenous waste. The monitoring of nitrite and dissolved oxygen concentrations appears to be of critical importance to commercial softshelled crab production in closed systems.

ACKNOWLEDGEMENTS

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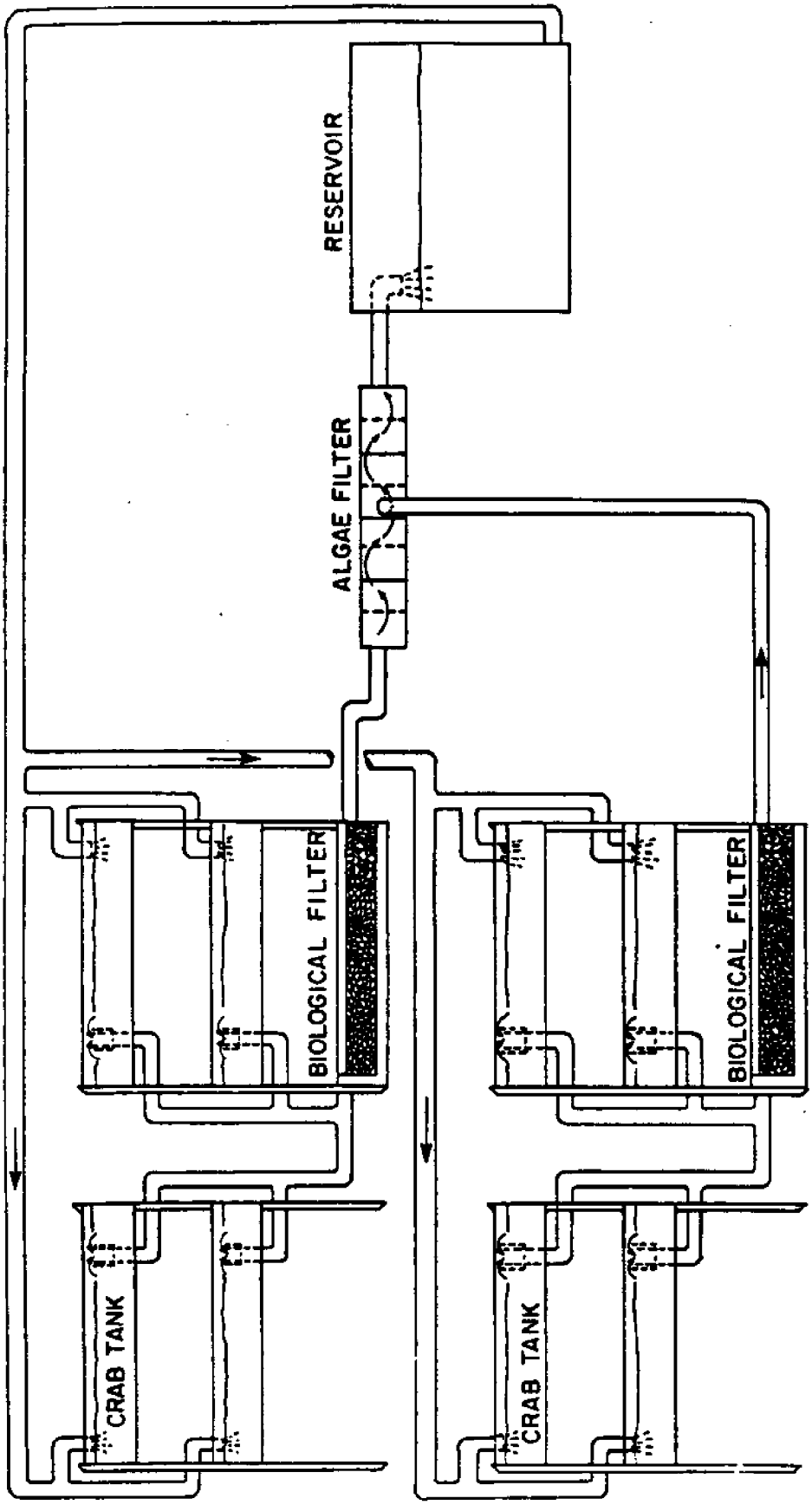

Figure 1. Schematic diagram of one of the commercial systems.

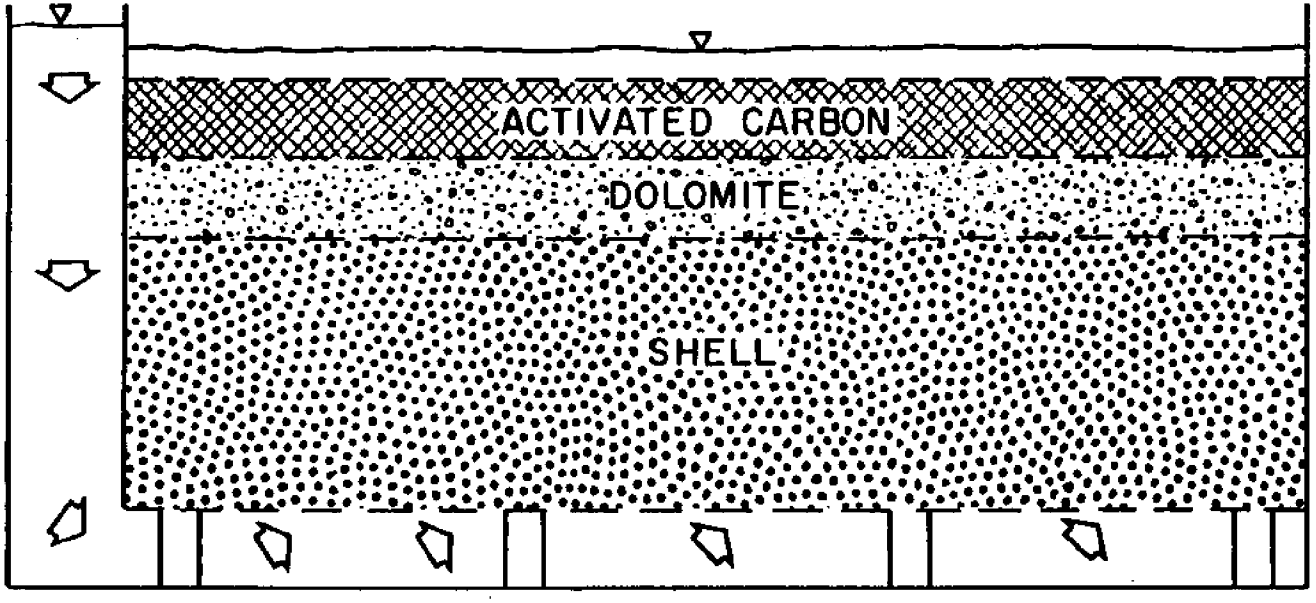

Figure 2. Biological filter cross section showing the different media layers.

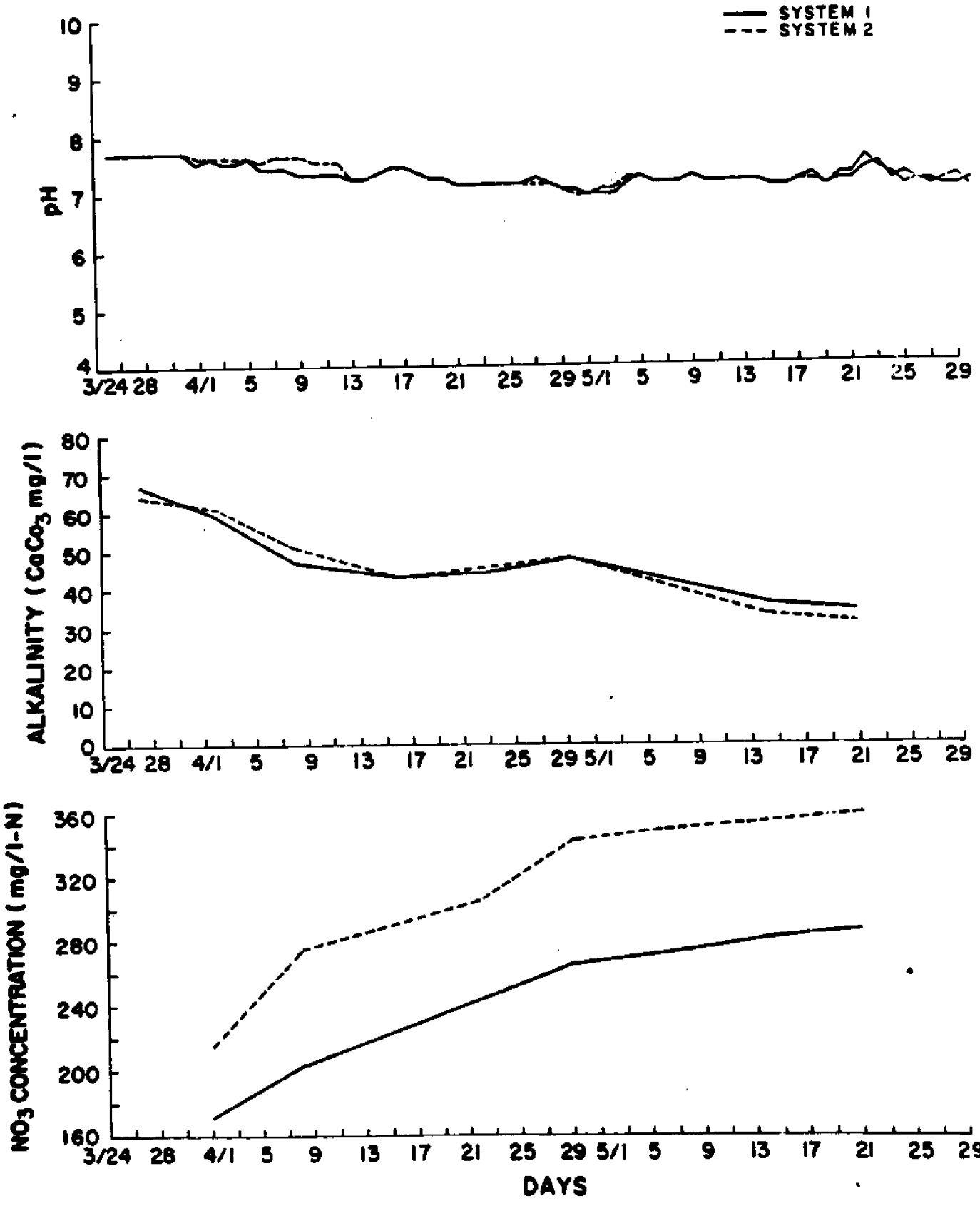

Figure 3. Supporting water quality parameters for the commercial systems (pH, alkalinity, and nitrate).

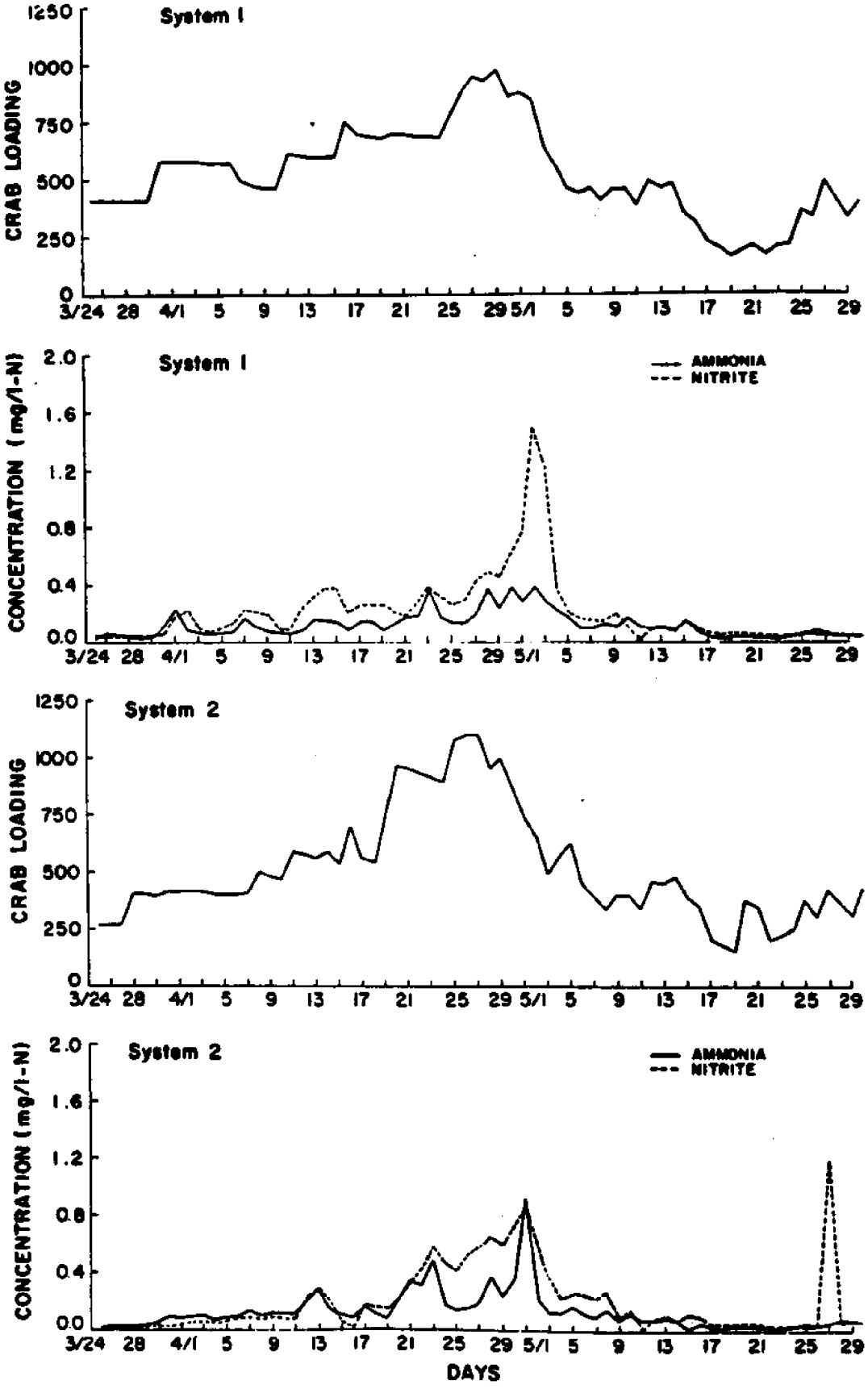

Figure 4. Ammonia and nitrite concentrations in relation to crab densities (systems 1 and 2).

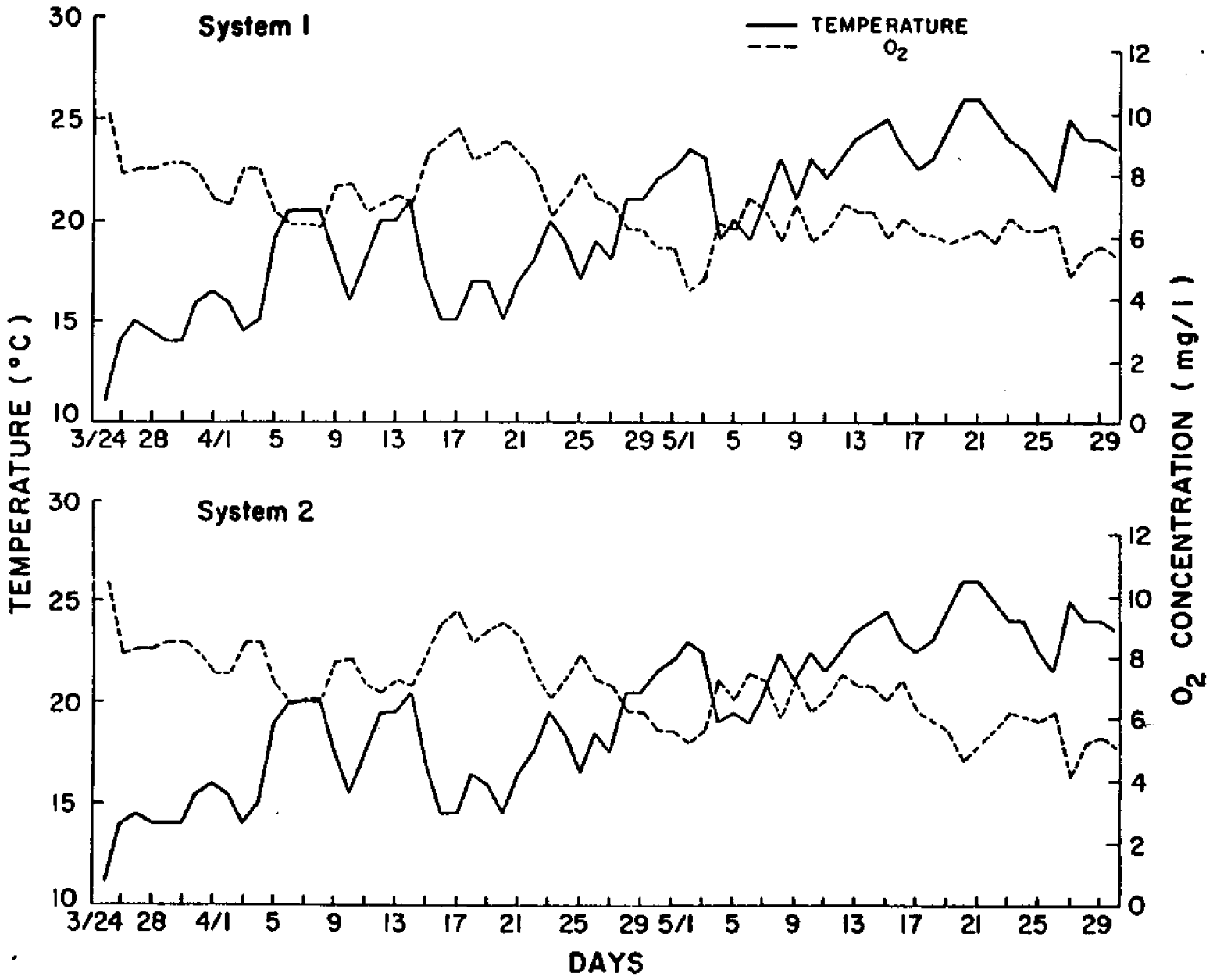

Figure 5. Temperature and dissolved oxygen values (systems 1 and 2).

Scaled-Down Experimental Units: Method and Rationale

Scaled experimental units were developed from critical volumetric and surface area relationships derived from a successful commercial system operated in Lacombe, Louisiana. Two 3 ft x 6 ft tanks were determined to be a convenient and manageable experimental size. To reduce the original commercial system to this size, a reduction factor of 0.167 was calculated. The use of the 8 percent scale experimental systems was warranted by the need to limit the cost associated with anticipated crab mortalities and for replication of results.

Because of biological considerations and the need to duplicate the original system as closely as possible, this reduction method provided to be a satisfactory one as volume and surface area ratios of various components were kept close to the original commercial system (shown in Table A). This is especially desirable in the crab, shell, and algae sections. In the reservoir, the volume, but not the surface area, was kept constant. The rationale given is that the reservoir has no biological criteria, but serves only as a volumetric buffer for the system. The original system incorporated baffles in the algae tank, allowing the water to weave in a serpentine fashion through the tank. The surface ratio in the scaled-down experimental system does not allow for baffles in the algae section. However, since the flow is reduced by the same reduction factor, the retention time of the water is the same, and therefore, the lack of baffles should not be a deleterious factor.

TABLE A

Comparison of Critical Design Parameters With Calculated Ratio (.167) in Reduced System

Description	Calculated Ratio	Volume	Bottom Area	Media Area
Crab Tank	.167	.167	.167	--
Biological Filter	.167	.156	.156	.167
Algae Filter	.0835	.0833	.0833	.0876
Reservoir Compartment	.0835	.0828	.30	--

Limiting Factors Associated with Nitrification in Closed Blue Crab Shedding Systems

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ABSTRACT

A study of factors limiting crab densities in closed blue crab (Callinectes sapidus) shedding systems was conducted using scaled-down experimental units. Nitrification beds, activated carbon, dolomite, and plants were used to maintain water quality. Nitrite (NO_2-N) was found to be the most critical toxic element accumulating in the system as a result of the nitrification process. Concentrations of approximately 20 mg/l NO_2-N and above caused increased mortality in intermolt crabs. Mortality in molting crabs was observed at concentrations as low as 2 mg/l NO_2-N. Dissolved oxygen (DO) was identified as the factor limiting the efficiency of the nitrification beds. As DO concentrations decreased, the rate of nitrification slowed, apparently causing nitrification to be inhibited at the nitrite-to-nitrate conversion step. As nitrite concentrations increased, high mortalities resulted, further increasing the loading in the systems and depressing DO concentrations, because of the high BOD exerted by the dead crabs. Elevated crab populations were maintained in the systems when aeration and flow increases were supplied to the nitrification beds.

INTRODUCTION

Production of softshelled crabs in the state of Louisiana has decreased dramatically over the last 20 years. The decline in the blue crab (Callinectes sapidus) has been principally attributed to the deterioration of the coastal zones and accompanying decline in water quality (Jaworski, 1971). Because of the declining natural water quality, the percentage of successful molts (ecdysis) has decreased in open-water shedding boxes and softshelled crab fishermen in Louisiana have been forced to turn to other methods to "shed" blue crabs (Jaworski, 1979). The valuable softshelled form of the blue crab currently brings prices upwards of $25 per dozen. The potential value of using closed recirculating systems for the shedding operations has been demonstrated by a few successful commercial operators (Perry et al. 1982). These closed systems reduce labor requirements and eliminate the exposure of crabs to many deleterious environmental conditions during the vulnerable molting period. However, the widespread adoption of this technology has been inhibited by the lack of established design criteria and management guidelines (Van Gorder and Fritch 1980).

The operation of a successful closed-circulation aquaculture system depends on the maintenance of acceptable water quality. The ability of biological filters in the closed systems to convert ammonia (NH_3), which is the principal excretory metabolite of crustacea (Hartenstein, 1970), to the relatively nontoxic nitrate (NO_3) by bacterial nitrification is well summarized by Spotte (1979) and Wheaton (1977).

The objective of this study was to examine the carrying capacity of a successful filter configuration and to identify factors limiting filter efficiency.

MATERIALS AND METHODS

Scaled experimental units were developed from critical volumetric and surface area relationships derived from a successful commercial system operated by Mr. Cultus Pearson of Lacombe, Louisiana (Malone et al. 1984). The use of the 8 percent scale experimental systems was warranted by the need to limit the cost associated with anticipated crab mortalities and for replication of results. The four experimental systems each consisted of two fiberglass tanks with length, width, and depth of 6 feet, 3 feet, and 1 foot (183 x 91 x 30 cm), respectively. Of the two tanks used in each scaled-down system, the upper tank was devoted to holding crabs and the lower tank to the various

filtration units (Figure 1). The interior of the upper tanks was coated with a smooth white gel. Dimensions of the experimental systems are shown in Table 1.

TABLE 1

Dimensions of Experimental Systems

Description	Length (ft)	Width (ft)	Depth (ft)	Water Depth (ft)	Area (ft^2)	Volume (ft^3)
Crab Tank	6	3	1	0.438	18	7.875
Biological Filter	1.25	3	1	0.958	3.75	3.59
Shell Filter Bed	1.25	3	0.25	---	3.75	0.938
Dolomite Filter Bed	1.25	3	0.125	---	3.75	0.469
Carbon Filter Bed	1.25	3	0.083	---	3.75	0.311
Algae Filter	0.667	3	1	0.792	2	1.583
Reservoir Compartment[1]	4.083	3	1	0.917	12.25	11.229

[1]Includes head chamber.

Water level in the upper tank was controlled by a 5-inch (12.7 cm) standpipe constructed from 1.25-inch (3.2 cm) polyvinylchloride (PVC) pipe with gravity feed to the lower tank. The discharge nozzle into the upper tank consisted of a capped 0.5-inch (1.3 cm) PVC pipe with two 0.125 inch (0.3 cm) holes to assure active agitation (aeration) in the tank. Crabs were placed on the clean bottom of the tank. At times, a vinyl coated wire mesh enclosure (0.3 m in diameter) was added to the tank to isolate crabs in the process of molting.

The lower tank was subdivided by fiberglass partitions into sections containing a head chamber, biological filter, algae filter, and reservoir compartment. The head chamber received the overflow from the crab tank through the standpipe, which discharged beneath the water level in the head chamber. The primary function of the head chamber was to direct flow under the upflow biological filter. In some experiments, air stones were placed in this compartment to provide additional aeration ahead of the biological filter. The submerged biological filter consisted of 3 inches (7.6 cm) of washed clam shell (Rangia cuneata, 2-3 cm in diameter) on the bottom, overlaid by 1.5 inches (3.8 cm) of coarse dolomite, and 1 inch (2.54 cm) of coarse activated marine carbon (Caribsea brand, 1-3 mm particle size). Each layer was separated by nylon window screen. The biological filter was supported by 0.5 inch (1.3 cm) egg-crate louvering placed on 2-inch (5.1 cm) sections of PVC pipe. The supporting pipe columns were perforated to minimize dead water volumes and filter channelization. The water surface in the biological filter was approximately 5 cm over the top of the activated carbon bed with overflow to the algal filter provided by a 1-inch (2.54 cm) hole near the top corner of the fiberglas partition. The bottom of the algal compartment was covered with 2 inches (5.1 cm) of pea gravel which was used to maintain the calculated surface area ratio observed in the commercial systems. A 48-inch (1.2 m) fluorescent fixture was mounted so that two 40-watt grow lights were 15 cm above the water surface. Aluminum foil shields were used to minimize illumination of the biological filter and holding compartment. Diagonal flow was provided in

the algae compartment by one 1-inch hole (2.54 cm) placed above the gravel bottom in the corner opposite to the inflow point. The reservoir compartment (which evolved in the commercial systems to buffer rapid water quality changes) held the filtered water until it was pumped to the crab tank. A 0.05 horsepower chemical solution pump (Teel, model #1P677) circulated the water at a constant rate of 6.6 liters per minute. The intake line of the pump was fitted with a screened check valve to prevent the loss of pump prime during power outages and the uptake of large debris. The water was pumped through a 5/8-inch (1.6 cm) flexible plastic tubing to the spray nozzle in the upper crab tank.

The experimental systems were set up during the spring of 1983 in an open-air building at the Huey P. Long Fish Hatchery, located in Lacombe, Louisiana. Artificial seawater (Rila Sea Salts) was mixed with local well water in the experimental systems at a 5 ppt salinity, duplicating the commercial system. Freshwater additions were made only to replace losses caused by evaporation, sample collection, and spillage. To duplicate the commercial system as closely as possible and to establish accelerated nitrification in the new systems (Bower and Turner, 1981), the biological and algae filters of each experimental system were inoculated with media from the commercial system. Dominant forms of algae in the experimental systems included water milfoil (Myriophyllum sp.) floating on top of the algae filter, and attached filamentous green algae on the filter walls. No algal biomass was harvested from the systems and the algae filters were provided with a constant light regime throughout the study.

Crabs were obtained daily from a local commercial softshelled crab fishermen. These crabs were taken from Lake Pontchartrain by crab traps according to local practice. One experimental system was dedicated to the function of holding incoming crabs. This holding system was used to maintain constant crab populations in the systems being used for active experiments. The majority of the crabs used for experimental purposes were intermolt crabs (crabs between molting cycles). In some cases, smaller populations of crabs experiencing molting were placed in the systems to permit examination of the effects of water quality fluctuations on the molting crabs. This approach was taken since it was anticipated that mortalities associated with high risk experiments would exceed our limited source of shedding crabs.

When necessary, these shedding crabs were isolated from the general population by the vinyl coated wire enclosures. All crabs used in the study ranged in size from 10 to 15 cm across the carapace (top shell). Following local commercial practice, crabs in the closed shedding systems were not fed. Mortalities resulting from the experiments were removed continually and populations restored to constant levels once a day. Periodically, the entire population of crabs was replaced to minimize the effects of adaptation and selective processes. Debris from the systems was periodically removed as accumulation became apparent.

All systems were monitored daily for temperature, salinity, dissolved oxygen, ammonia, nitrite, and pH. Determinations of alkalinity levels and nitrate concentrations were undertaken weekly. Records of mortalities and visual observations were continually maintained. Routinely, a one-liter sample of water was taken from the reservoir compartment of each of the four experimental systems and analyzed immediately for the laboratory determinations. Total ammonia as NH_3-N was measured with an Orion 95-10 ammonia electrode in conjunction with an Orion model 701A digital ionalyzer. Nitrite as NO_2-N was determined using a sulfanilamide-based colorimetric reaction and a Bausch and Lomb Spectronic 20. Both tests were conducted in accordance with Standard Methods (1980). Oxygen and temperature data from the systems were obtained with a Yellow Springs Instruments (model 51) dissolved oxygen meter. Salinity was measured with a refractometer (American Optical Corporation) and pH with a Mini (model 47) pH meter. Nitrate (NO_3-N) determinations were undertaken by a modified hydrazine reduction (Spotte, 1979), and alkalinity by titration (Standard Methods, 1980).

RESULTS

Overview

Four experiments were undertaken during the spring shedding season of 1983. The four experimental units were numbered to permit identification of system histories during interpretation of results. Figure 2 presents loading curve and critical nitrogen forms for System 4, which was used as a holding system during the first three experiments. This unit most closely approximates commercial operation of the closed shedding systems, in contrast to the other units which were purposely overloaded to test the limits of the system. The limited number of mortalities observed in this system was attributed to harvesting and handling operations and not to the accumulation of toxic metabolites. These results illustrate the capability of this design to accommodate highly variable crab densities normally associated with commercial systems.

Figure 3 illustrates supporting water quality parameters for the first three experiments. As can be seen from this illustration, all four experimental systems behaved very similarly when differences in crab densities are taken into account. All systems initially showed a pH of 8.3 and slowly declined to pH values in the range of 7.0 to 7.2. The decline in pH was associated with a decline in alkalinity. These results illustrate the limited capability of the dolomite blankets to buffer pH changes under the conditions of this experiment. These observations are consistent with those of Bower et al. (1981), who noted the limited ability of calcareous filtrants to maintain pH above 8.0. Similar observations were also made in commercial operations using the same design and water sources (Malone et al. 1984). These systems displayed alkalinities as low as 30 mg/l $CaCO_3$ and pH values in the range of 7.0 after two years of operation with no water changes. There is some disagreement on pH values for optimum nitrification rates, but it appears that Nitrosomonas has a high constant oxidation rate between pH 7.0 and 9.0, while Nitrobacter conversion rates are satisfactory between 6.5 and 8.5 (Wheaton, 1977). Submerged filters can be acclimated to lower pH values if given time (Haug and McCarty, 1972). It is concluded that the dolomite bed is sufficient for the maintenance of pH above 7.0 and that this pH apparently does not adversely affect the crabs. In fact, the lower pH may be beneficial in that it reduces the ammonia toxicity caused by the equilibrium reaction between NH_4^+ and NH_3 (Spotte, 1979; Wheaton, 1977).

Temperatures in the four systems were dramatically influenced by the open-air nature of the systems and rapidly came to equilibrium with the ambient air temperature. All systems therefore displayed identical temperatures. During the course of the study, the temperature ranged from a low of 11°C to a high of 27°C. The rate of nitrification increases with temperature. Wild et al. (1971) in their studies found that nitrification rates increased in the range from 5 to 30°C. For this reason, comparable experiments were run simultaneously when possible. However, this temperature variation must be recognized when experimental results are interpreted.

Nitrate levels in the experimental systems increased throughout the course of the study. The rates of increase parallel the loading histories of each system. Values over 140 mg/l NO_3-N were observed by the end of the study period. Nitrate toxicity was not thought to be a factor even at the high concentrations observed. Nitrate is generally not toxic to marine organisms, even at elevated levels (Hirayama, 1974; Siddall, 1974). Commercial systems monitored at this time experienced upwards of 360 mg/l NO_3-N with no deleterious effects (Malone et al. 1984). Clearly, the plant filter (despite heavy plant growth) failed to significantly influence the accumulation of nitrate in these systems.

Dissolved oxygen (DO) concentrations for the first three experiments are shown in Figure 4. These values were obtained from measurements in the holding compartments of each system. Dissolved oxygen will be discussed in greater detail for each experiment.

Experiment No. 1

Experiment No. 1 was undertaken to determine reasonable crab densities for acclimation of the nitrification beds in the biological filter units. After inoculation of the filters, each system was populated with intermolt crabs at varying densities. Systems 1, 2, and 3 were loaded with 15, 30, and 50 crabs, respectively. During the start-up period of each system, the submerged biological filters demonstrated the classical nitrogen development curve expected by immature biological filters (Wheaton, 1977). In newly established culture systems, the filter bed does not contain enough bacteria to carry out its purifying activity, thus toxic substances accumulate in the system (Hirayama, 1974). A reduction of ammonia in the system during start-up signifies the establishment of a Nitrosomonas population, which converts ammonia to nitrite and causes an increase in

the nitrite concentration. As the Nitrobacter population becomes established, nitrite concentrations decrease, as the Nitrobacter convert nitrite to nitrate.

Figure 5 shows the development of the biological filters under the different crab densities. Systems 1 and 2 (loaded light to medium) experienced no difficulty with the filters or the crab populations and the systems reached equilibrium without incidence. Mortality did not exceed 7 percent in either system. None of the mortalities in these two systems was attributed to toxic elements. System 3, which was loaded heavily, experienced upward of 5 mg/l of ammonia with no apparent effects. However, as ammonia decreased with the subsequent rise in nitrite, system failure and functional mortality in crabs was observed. Functional mortality was determined by crabs showing signs of distress, lack of movement, loss of equilibrium, paralysis of appendages, and finally, death. Functional mortalities were attributed to the toxic effects of nitrite. At concentrations greater than 20 mg/l NO_2-N, system 3 experienced mortalities as high as 20 percent per day and the behavior of the surviving crabs was acutely affected. Larger crabs were observed to be more sensitive to the nitrite toxicity than crabs of a smaller size. As the nitrite concentration decreased, crab mortality, if any, declined and became nonfunctional in character as before. In all systems, the biological filters took approximately 30 days to stabilize to the point that both nitrite and ammonia were rapidly oxidized to insignificant levels. The length of this conditioning period is consistent with the observations of Bower and Turner (1981), and significantly shorter than the period noted by Hirayama (1974). However, differences in salinity, temperature, and loading regimes make direct comparisons difficult.

Experiment No. 2

Experiment No. 2 was intended to test the response of systems acclimated at various crab densities to heavy shock loadings. Following completion of Experiment No. 1, the three experimental systems were increased in population to 100 intermolt crabs plus a small experimental population of three crabs entering ecdysis (molting). Figure 6 shows the response of the systems to the increased crab densities.

All systems displayed a small accumulation of ammonia in response to the increased crab densities. The timing of the appearance of an ammonia peak was inversely related to the number of crabs used to condition the filters. The filters quickly adjusted to the increased ammonia loading and dropped to baseline conditions.

Nitrite increased and remained at high levels throughout the experiment. Although a slight nitrite recovery was noted in each system in the latter part of the experiment, this proved to be temporary and the systems remained in the failure state. All systems failed and suffered heavy functional mortality when nitrite concentrations rose above 20 mg/l of NO_2-N. The relationship between nitrite levels and mortalities is most clearly illustrated by the results of system 1. Crab populations in systems 1, 2 and 3, suffered total daily mortalities as high as 14, 30, and 30 percent, respectively. Decreased molting success also was observed in crabs entering ecdysis at concentrations greater than 2 mg/l NO_2-N. These mortality levels are clearly above those that can be tolerated by a commercial operation in areas where crab harvesting limits production. All systems were therefore considered in a state of complete failure. After 12 days the systems showed no signs of recovery and the experiment was terminated.

This experiment provided further evidence linking the functional mortalities with the accumulation of nitrite within the system. It also illustrated that the number of crabs used to acclimate the system was not critical. The ability of the systems to carry the increased crab populations was ultimately limited by a factor common to all the systems. The limiting factor also most adversely affected the oxidation of nitrite to nitrate with little impact on ammonia oxidation. It was observed in this experiment that dissolved oxygen concentrations in the effluents of the biological filters during periods of system failure were significantly depressed. Although dissolved oxygen levels averaged 5.6 mg/l in the crab tanks, levels in the effluent of the biological filters averaged only 2.0 mg/l. This suggested that lack of dissolved oxygen may be limiting the nitrifying activity of the filters.

Experiment No. 3

In this experiment, crab densities in system 2 and 3 were reduced to populations of 75 and 85 crabs, respectively, to determine if these populations could be supported. Aeration was added to system 1 in an attempt to increase the efficiency of the nitrification bed, while maintaining 100 crabs in the system. Aeration was accomplished by placing four air stones in the head chamber and two in the reservoir compartment. As can be

seen in Figure 7, all systems continued in a state of failure. Heavy mortality continued to characterize the systems. It is believed that mortalities of this magnitude significantly increased loading of both carbonaceous and nitrogenous BOD to the biological filters. This increased BOD loading neutralized the benefits of aeration in system 1. Although the DO concentrations in the head chamber of system 1 averaged 6.1 mg/l, the exit DO concentrations of the biological filter remained depressed, averaging 1.8 mg/l. The sensitivity of the systems to oxygen limitations is demonstrated in system 1, as on May 27, the aeration was inadvertently discontinued for 24 hours. After this occurrence, a large increase in ammonia concentration dominated the system. Systems 2 and 3 rapidly recovered in three days when crab densities were reduced and removed.

Experiment No. 4

This final experiment was undertaken to examine the effect of increased oxygen supplies on the nitrification abilities of the biological filters. One of the systems (system 2) was supplied aeration by means of four air stones placed in the head chamber and the flow rate of the system was increased to 10 liters per minute. No modifications were made in the other system (system 4). Both of the experimental systems were then loaded with populations of 75 crabs each. Figure 8 shows the levels of ammonia and nitrite in both systems during this period. The unmodified system realized system failure with mortality losses as high as 55 percent. The modified system with aeration and increased flow rate showed initial increases of ammonia and nitrite, but recovered and decreased after several days. In the modified system, biological filter effluents averaged 4.2 mg/l of DO during this experiment. The performance of the biological filter was significantly enhanced by the increased oxygen supply in the modified system.

DISCUSSION

Oxygen seemed to be the most critical limiting factor affecting the biological filters. A population of more than 50 crabs could not be maintained in an unmodified system or by added aeration. Only when flow was increased from 6.6 to 10 liters per minute in combination with aeration prior to the biological filter, were increased crab densities maintained. Oxygen traces through the various components of the systems revealed dramatic decreases in dissolved oxygen concentrations through the biological filter. Figure 9 demonstrates various oxygen traces through the experimental systems in states of equilibrium and system failure. In the systems, normal aeration is supplied by the spraying of water into the crab tank. All traces reveal that the highest oxygen demand in the systems is incurred by the biological filter. Systems in a failure state are demonstrated by DO traces incurred by the heavy loading of both an aerated and a normal system without modification. Stable systems presented include a lightly loaded system without modification and a heavily loaded system with modifications consisting of aeration and increased flow. In the state of system failure, DO concentrations decreased as much as 1 mg/l after the biological filter. Dissolved oxygen measurements of the effluent of the biological filters were taken in the standing water lying over the activated carbon layer. These waters undoubtedly experienced some reaeration prior to measurement. Thus, oxygen levels within the filters were probably much lower.

Nitrification processes require 4.0 to 4.6 mg/l of oxygen to completely oxidize 1 mg of NH_3-N to NO_3-N, depending on the age of the culture (Wheaton 1977). Nitrosomonas consumes, by stoichiometric calculations, 3.02 mg of oxygen per mg of NH_3-N oxidized to NO_2-N, as compared with 1.02 mg of oxygen consumed by Nitrobacter, converting 1 mg of NO_2-N to NO_3-N (Wheaton, 1977). If oxygen is not present in the required stoichiometric amount, the nitrification rate decreases. In fact, nitrite concentrations can be increased through dissimilatory reduction of nitrate (Spotte, 1979; Mevel and Chamroux, 1981). Haug and McCarty (1972) reported that in upflow submerged filters, NH_3-N oxidation rates were the greatest at the bottom of the filter where ammonium concentrations were the highest. This could explain the increase of high levels of nitrite found in our study. If oxygen was at a critical stoichiometric level, oxygen concentrations could be depleted at the bottom of the filter in the process of oxidizing ammonia to nitrite. As near anoxic conditions occurred in the filter, the rate of NO_2-N oxidation to NO_3-N would be severely decreased. This would create a kinetic "bottleneck" and result in elevated toxic nitrite concentrations.

During the study, nitrite proved to be the most toxic form of nitrogen to the blue crab. Ammonia concentrations did not correlate well with observations of functional mortalities. Nitrite concentrations in the range of 20 mg/l NO_2-N produced functional

mortality and adverse effects on the experimental intermolt crabs. Armstrong et al. (1976) and Wickins (1976) reported similar nitrite toxicity levels in prawns. The molting crabs experienced decreased molting success at lower concentrations of nitrite. The authors observed crabs dying during molting at concentrations as low as 2 mg/l NO_2-N. These lower concentrations were also supported by observations from commercial systems monitored at the time of this study (Malone et al. 1984). The commercial production of softshelled crabs similarly decreased as the nitrite concentrations in these systems rose to nearly 2 mg/l NO_2-N. These field observations clearly indicate that nitrite toxicity was the factor limiting crab densities in the experimental systems.

The mean nitrite and DO concentrations for varying percent of mortality ranges are presented in Table 2. As nitrite concentrations increase and DO concentrations decrease in the closed systems, mortalities tend to increase. It is believed that both temperature and the highly erratic BOD loading resulting from mortalities induced significant variability in system responses. It was generally observed that increasing the temperature decreased the carrying capacity of these systems. This was expected, because of the effects of temperature on the saturation levels of DO and on metabolic rates of both the nitrifying bacteria and the crabs. Mortalities adversely affected the systems by increasing the total oxygen demand on the biological filters (decay of released organics) and by increasing ammonia loadings through mineralization. Although BOD does not inhibit nitrification rate directly (Wild et al. 1971), it does consume oxygen for breakdown processes. This tends to drive the DO in the system lower and nitrification rates are impaired. As toxic levels of nitrogen increased because of lowered nitrification rates, increased crab mortalities result. This adds to the BOD load and further contributes to the decline of water quality in the systems. Systems that were severely overloaded recovered only after crabs were removed for a short time (three to five days).

TABLE 2

Mean Dissolved Oxygen and Nitrite Concentrations Associated With Varying Percent Total Mortality Levels

Total Mortality Rate (%)	n	Dissolved Oxygen (mg/l)	Nitrite (mg/l NO_2 N
		$\bar{X}$	$\bar{X}$
0 - 5	126	6.41	5.4
5 - 20	66	3.66	17.34
> 20	12	2.88	26.92

n = number of observations

In the Gulf of Mexico where softshelled crab production is often limited by the availability of molting blue crabs, a total daily mortality rate of less than five percent is required to maintain the viability of a commercial operation. The demand for good water quality in the closed systems is therefore high. Clearly, the results of this study indicate the need for the development of specific design criteria to meet the needs of this type of system. Further research should be directed toward the development of filter designs that alleviate the nitrite accumulations in these systems.

CONCLUSIONS

The following conclusions have been drawn from the results of this study:

1. The filter configuration tested here maintained satisfactory water quality when operated with densities of 50 crabs or fewer. The systems responded quickly to variable crab populations within this range.

2. Annual water changes would be warranted with this system to alleviate long-term buildup of nitrate and long-term decreases in alkalinity.

3. Toxic accumulations of nitrite were identified as the factor limiting crab densities in these systems.

4. Intermolt crabs were adversely affected by nitrite levels of about 20 mg/l N, and molting crabs seemed to be adversely affected by levels of nitrite as low as 2 mg/l N.

5. Failure of the biological filter to provide rapid oxidation of ammonia and nitrite was attributed to a dissolved oxygen limitation in the biological filter.

6. Increased temperatures and high mortalities aggravated the oxygen deficiency in biological filters and adversely affected system performance.

ACKNOWLEDGMENTS

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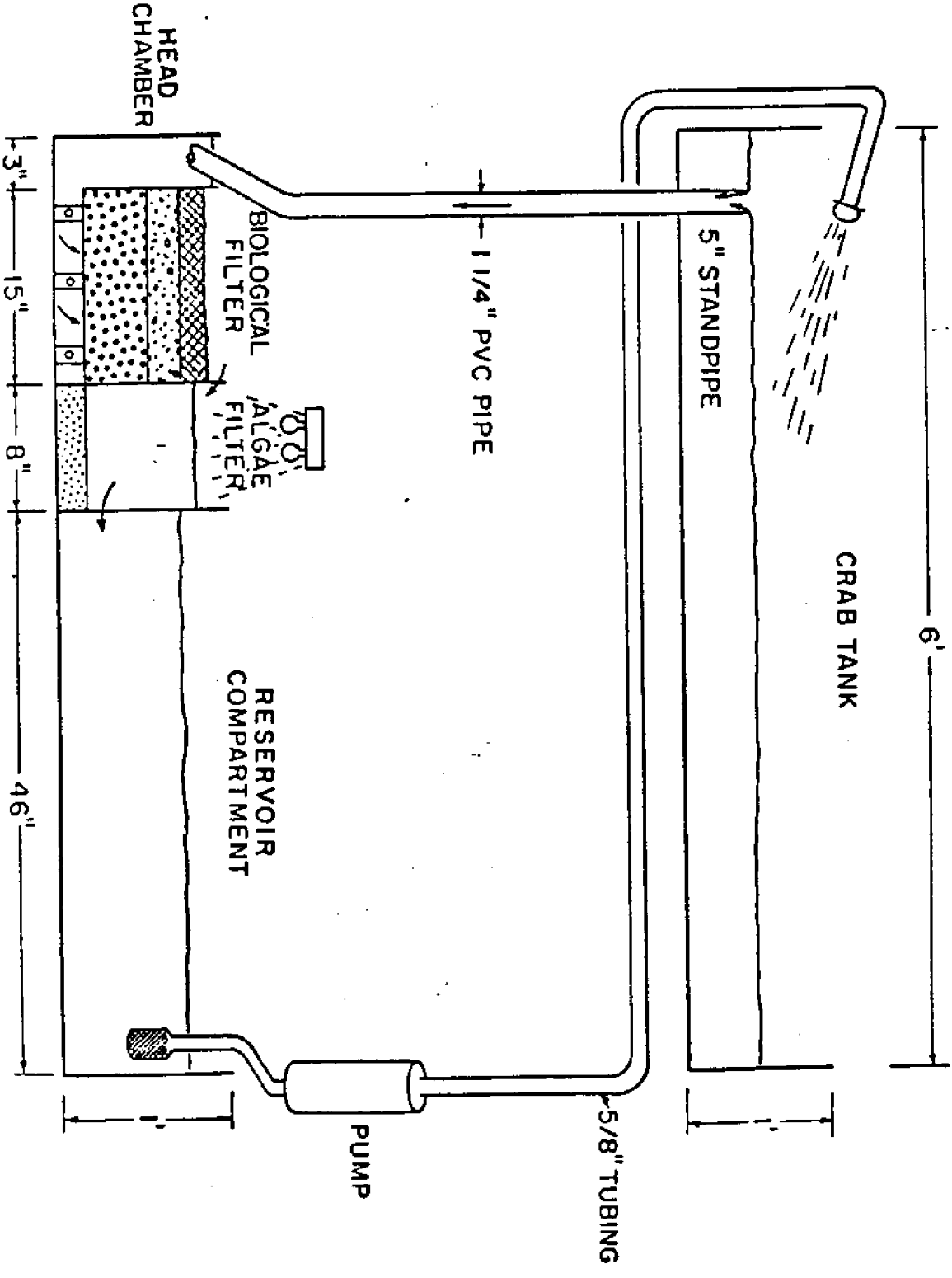

Fig. 1 - Configuration of experimental units.

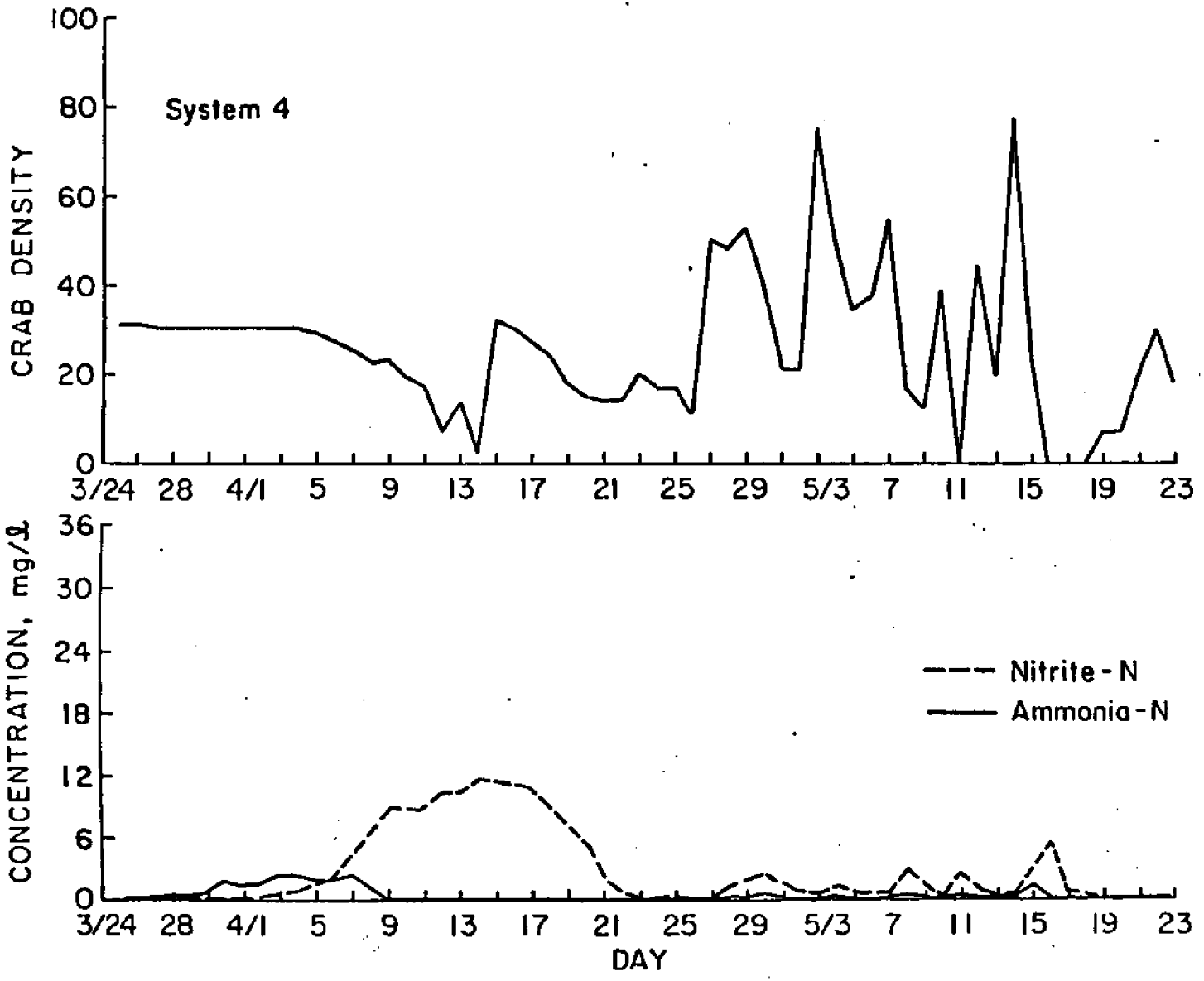

Fig. 2 - Ammonia and nitrite levels associated with crab densities in holding system (system 4).

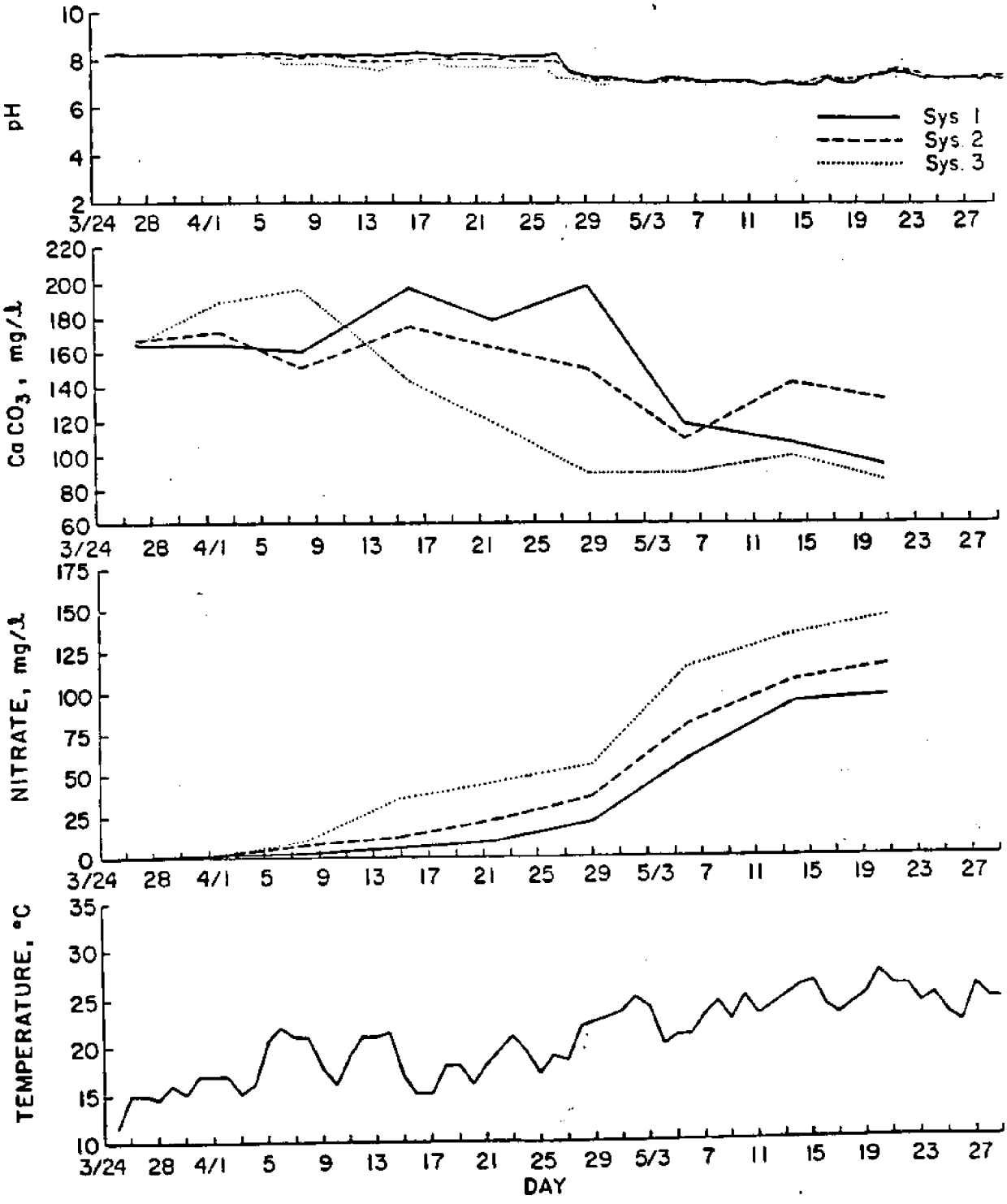

Fig. 3 - Supporting water quality parameters for experiments 1-3 (pH, alkalinity, nitrate, temperature).

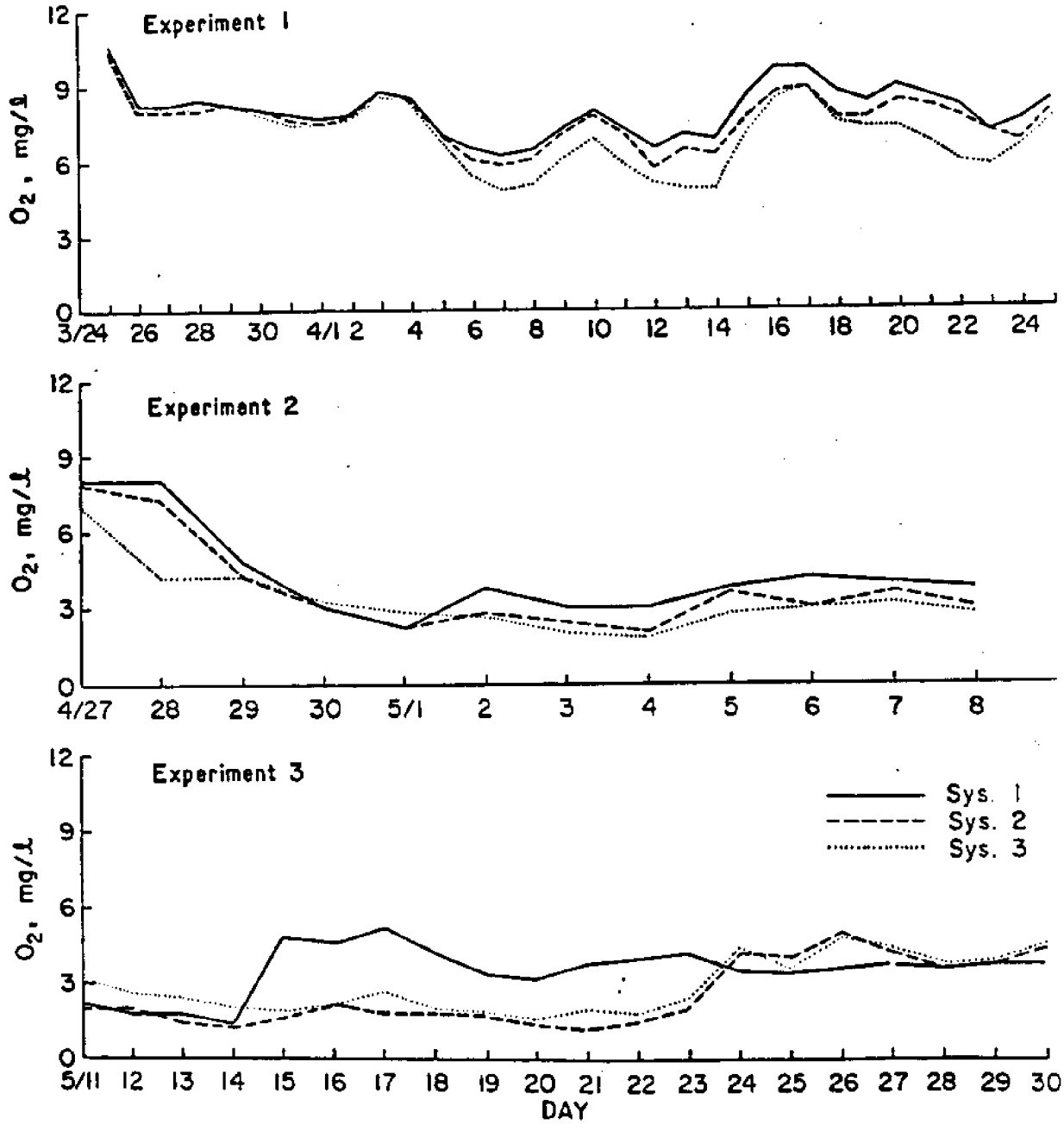

Fig. 4 - Dissolved oxygen concentrations for experiments 1-3.

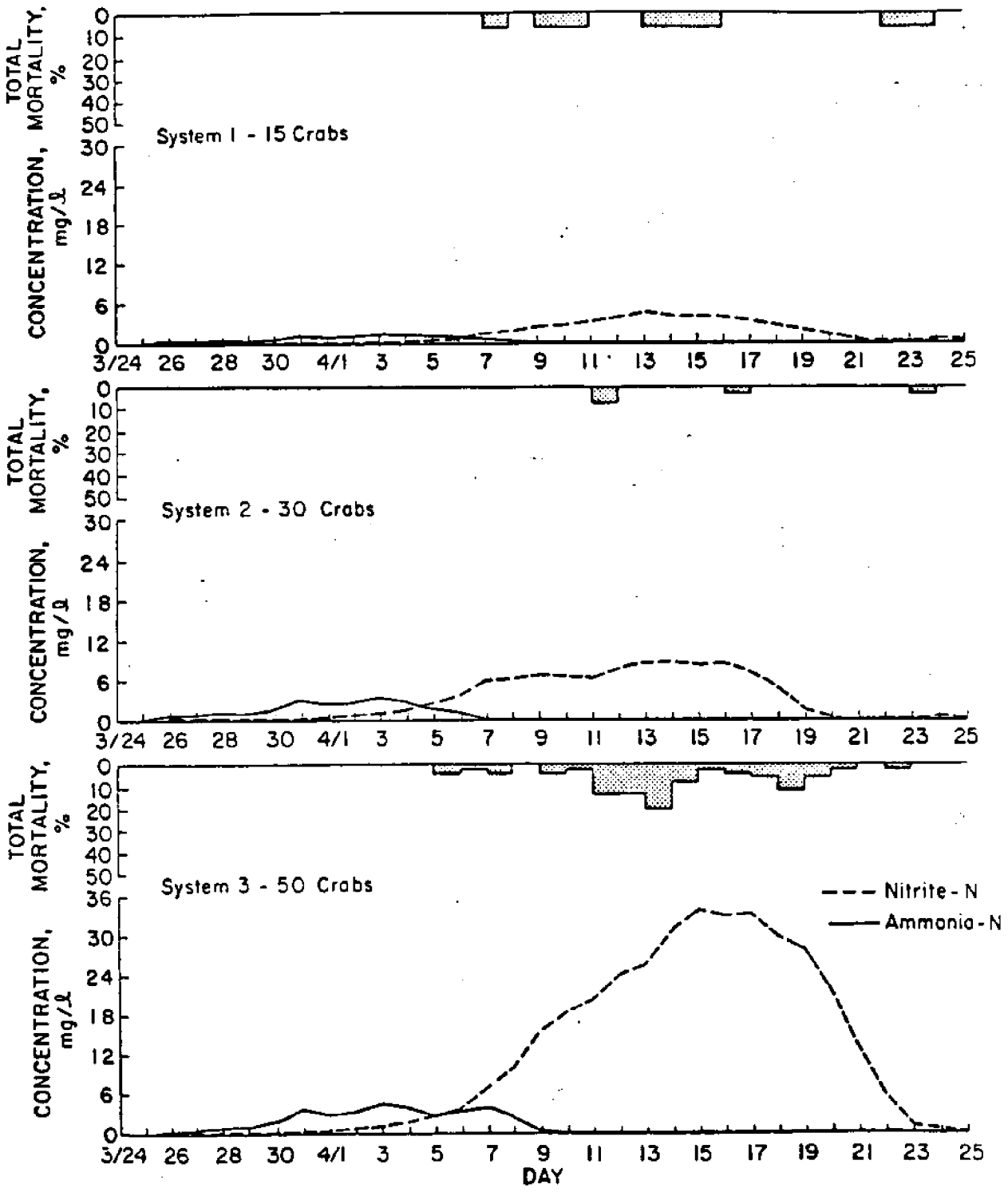

Fig. 5 - Experiemnt 1 - Ammonia and nitrite concentrations of systems 1-3 during start up period.

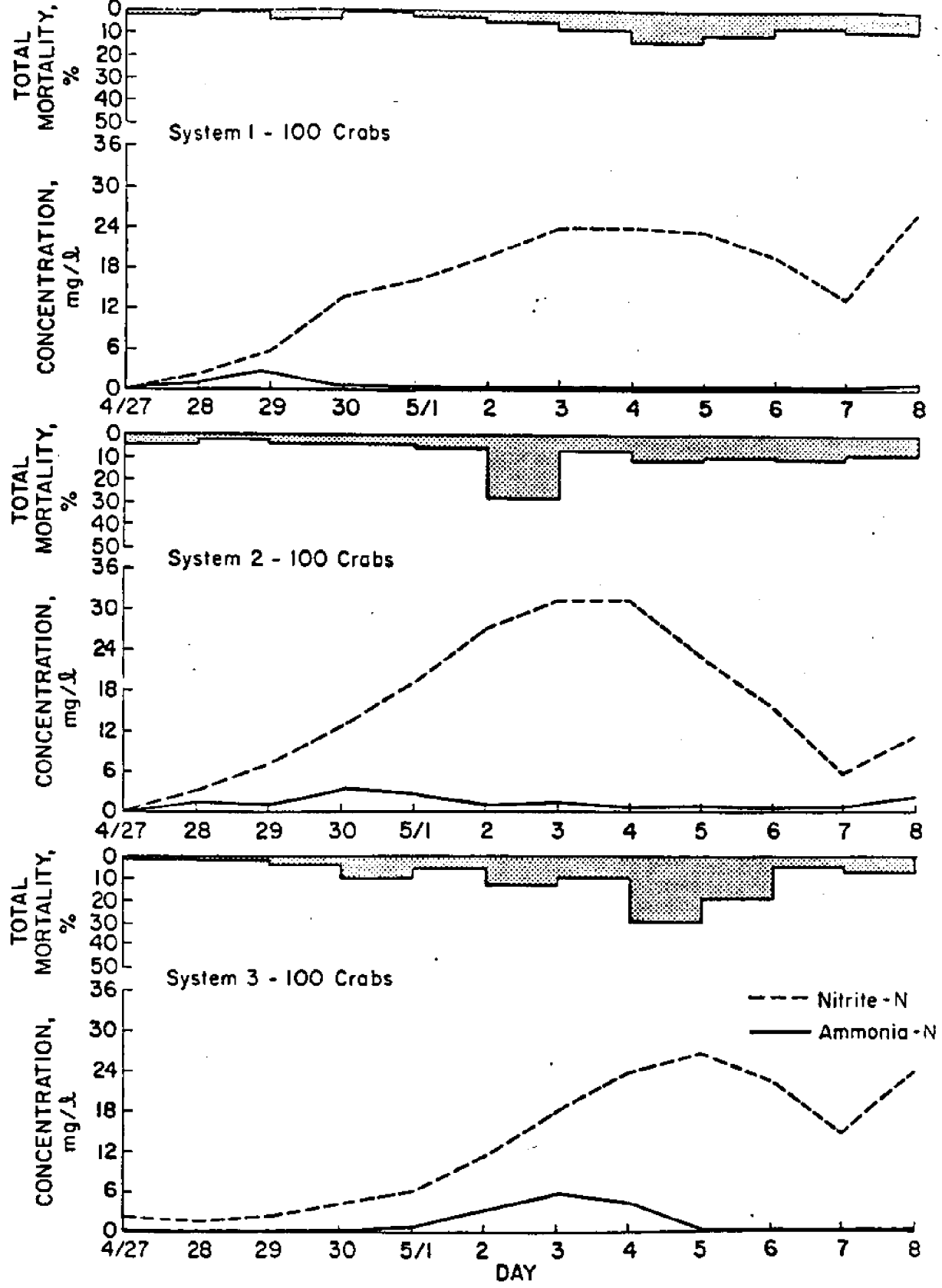

Fig. 6 - Experiment 2 - Ammonia and nitrite concentrations of systems 1-3 following shock loading.

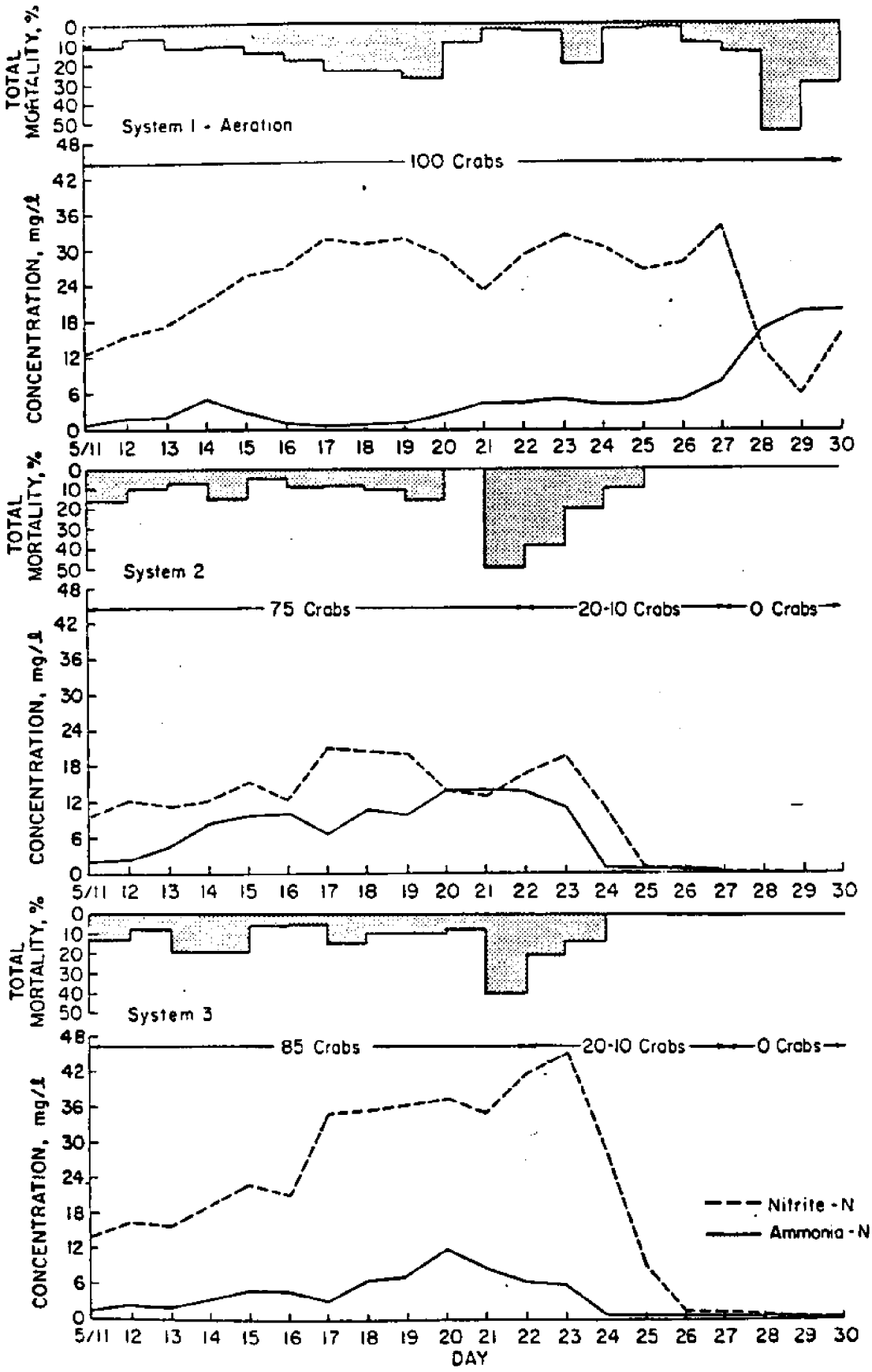

Fig. 7 - Experiment 3 - Ammonia and nitrite concentrations under conditions of aeration (system 1) and heavy loading (systems 2 and 3).

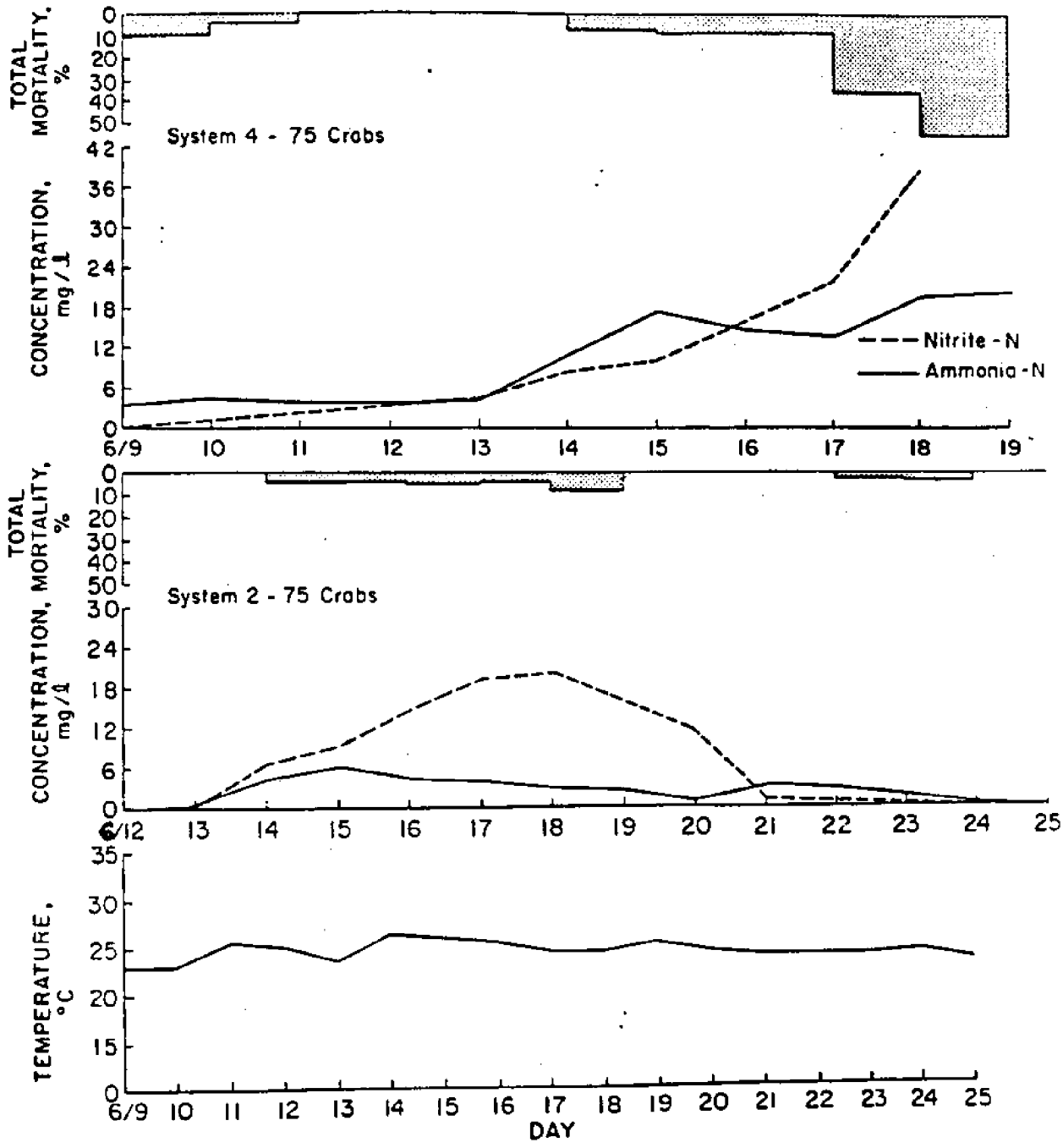

Fig. 8 - Experiment 4 - Ammonia and nitrite concentrations of an unmodified system (system 4) and a system with aeration/flow increase (system 2) under identical crab densities.

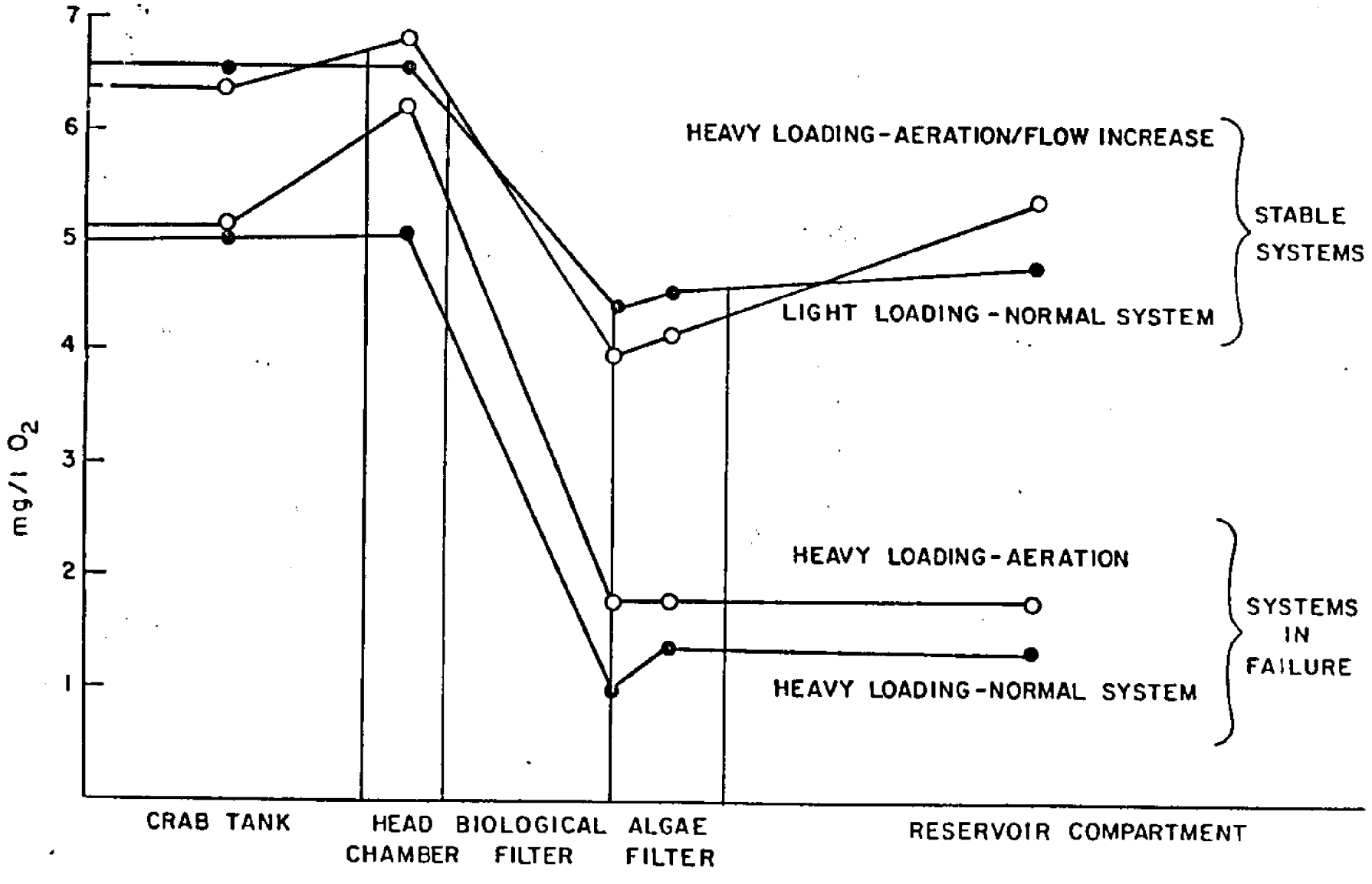

Fig. 9 - Oxygen traces through experimental systems with differing crab densities and system modifications.