THE CHEMISTRY OF A NEW WATER-RECIRCULATION AQUACULTURE SYSTEM WITH EMPHASIS ON THE INFLUENCE OF OZONE ON WATER QUALITY.

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By

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ABSTRACT

Water quality changes that occur during establishment and maintenance of nitrification in two identical recirculating aquaculture systems containing rainbow trout are described. The time taken for the nitrification process to become established was 40-45 days. Mortality of fish attributed to elevated nitrite concentrations were recorded during the initial conditioning phase of the systems. Un-ionised ammonia concentrations did not attain lethal levels during this period. Nitrate concentrations accumulated slowly throughout the study, while the pH and alkalinity of the water decreased with progressive nitrification. Levels of carbon dioxide, calcium, dissolved and suspended solids remained relatively stable until the carrying capacity of the systems was increased, upon which they increased rapidly and general water quality deteriorated. Permanently elevated concentrations of ammonia and nitrite served as an indication that the carrying capacity of the systems had been exceeded.

The use of ozone as a water enhancement treatment in aquaculture systems during one- and six-hour applications was also considered in this study. Ozonation significantly reduced nitrite levels at low concentrations (0,1-0,15 mg/l), although they returned to pre-treatment levels within a few hours of cessation of the treatment. The formation of an unstable, intermediate product that reforms as nitrite in the absence of ozone, rather than the complete oxidation of nitrite to nitrate, is proposed. Ozonation also resulted in decreased dissolved solids, and improved the clarity and odour of the water. Ozone had no effect on ammonia concentrations (at pH > 7,0), or on nitrate or calcium levels, and did not conclusively increase the redox potential of the

water. Residual ozone concentrations up to 0,04 mg/l in a six-hour treatment had no harmful effects on rainbow trout, and there were no signs of gas-bubble disease arising from supersaturation of the water with oxygen. Activated carbon filters were effective at removing residual ozone from the water after treatment. Treatment of the water with oxygen alone had no effect on nitrite concentrations.

INTRODUCTION

The potential of aquaculture in South Africa has been realized in recent years (Hecht and Britz, 1990). Rapid growth of the industry has resulted in a concomitant need for research in improving management and production practices. The establishment and subsequent maintenance of suitable environmental conditions is the key to the successful culture of aquatic animals (Hirayama, 1974; van Vuuren, 1986; Walmsley, 1986). Concern about the control of water quality is, at present, one of the main constraints on aquacultural production (Jensen, 1991). This need was also identified in 1985 which culminated in a workshop organised by the Ecosystems Programme of the Foundation for Research Development (FRD), entitled Water Quality Maintenance in Intensive Aquaculture (Walmsley, Hecht and Bruton, 1986).

Diminishing natural sources (Liao and Mayo, 1974; Colt and Tchobanoglous, 1976; Toerien, 1986; Hecht and Britz, 1990), and regulations regarding fish farm effluent discharge (Boersen and Westers, 1986; Jensen, 1991) have seen the emergence of water recirculation systems and associated technology as a major factor in modern aquaculture (Speece, 1973; Bohl, 1977; Kaiser and Wheaton, 1983; Morrison and Piper, 1986; Hortle, 1988; Lucchetti and Gray, 1988; Huner, 1991). Recirculating the water has several advantages over open, flow-through systems (Burrows and Combs, 1968). Greater control of environmental factors (especially water temperature, Burrows, 1971; Gaigher, 1986), and disease (Wheaton, 1977) result in faster growth and better condition of culture animals (Kaiser and Wheaton, 1983) and allow increased stocking densities (Hirayama, 1974; Stickney, 1979). Liao and Mayo (1972) maintained that if properly designed, a water recirculation system can increase the carrying capacity of

a hatchery tenfold over a single-pass system. Other advantages include water conservation and increased flexibility in aquaculture site location (Lucchetti and Gray, 1988). On a per kilogram of fish produced per year basis, water recirculation units require less tank space, lower flow rates and pumping costs (Kaiser and Wheaton, 1983). This reduces operation and production costs. On the basis of the aforementioned evidence, recirculating systems will have an important role in countering the two main factors limiting fresh water aquaculture in southern Africa, as specified by Hecht and Britz (1990), namely a lack of suitable water supplies, and a wide seasonal range of water temperatures.

Despite the widespread incorporation of water recirculation in aquaculture today, there have been relatively few studies documenting changes in general water quality during the operation of a recirculating system. Several reports have monitored variations in nitrogenous metabolite levels during the initial conditioning stages of a biological filter (Forster, 1974; Collins, Gratzek, Shotts, Dawe, Campbell and Senn, 1975; Carmignani and Bennett, 1977; Mevel and Chamroux, 1981). There is relatively little literature, however, that documents changes in water quality that occur once nitrification has been established. Such studies are important because many water quality parameters are closely associated with one another, and small changes in one relatively harmless character may initiate variations in another that can induce stress and cause mortality among the captive aquatic animals.

The primary aim of any aquaculture facility is to produce the maximum amount of usable biomass as cost efficiently as possible, within the available amenities. The maximum bioload that a

recirculating system can support (carrying capacity) is limited by several factors, and when that bioload has been exceeded, rapid deterioration of culture conditions are known to occur (Spotte, 1970). This may result in mass mortality of fish. It is thus important to be able to recognise any "early warning" to prevent such a situation arising. A knowledge of the changes that occur in different parameters in a recirculating system, obtained by monitoring the water chemistry routinely, will help provide this information. In addition, by being able to predict when optimal culture conditions are likely to be exceeded, it will be possible to sustain densities of fish at the maximum levels that a system can handle.

A knowledge of the changes in water quality and their possible effects on system performance is therefore important for the efficient planning and operation of recirculating systems. Furthermore, an understanding of the processes that occur in the water will ultimately result in improved management practices and reduced operating costs, the two main constraints for closed systems, as stated by Wheaton (1977).

A major drawback of closed systems is the accumulation of nitrogenous metabolic wastes (ammonia and nitrite), which are toxic to culture animals (Spotte, 1970; 1979). This makes it necessary to remove or convert these wastes to less harmful substances (Mevel and Chamroux, 1980). Menasveta (1980) stated that better larval prawn production could be obtained in a closed recirculating system through the improvement of water quality. Traditional methods such as biological and mechanical filtration are used extensively for controlling levels of toxic substances and other effluent components of recirculated aquaculture waters. Research into complementing these processes and improving culture

conditions with additional water treatment is receiving increasing attention, in an attempt to improve the quality of the water, and ultimately production efficiency and output.

One such treatment is ozonation. Ozone, an allotrope of oxygen and one of the most powerful oxidising agents known (Manning and Bacher, 1975), has been used extensively in municipal and industrial water purification processes since 1898 (Rosenthal, 1981). Its application in aquaculture was tested and discouraged by Hubbs in 1930, due to its apparent toxicity to fish. Ozone is highly toxic to fish above certain concentrations (Benoit and Matlin, 1966), but this problem is easily overcome by correct system design which ensures that most of the ozone dissolved in the water is removed between treatment and returning to the culture tanks.

Interest in the use of ozone in aquaculture was rekindled by Benoit and Matlin (1966) who used it successfully to control fungal infections on rainbow trout eggs. Since then ozonation has been increasingly and more effectively applied in aquaculture (Engdal, 1991), primarily for disease control and shellfish depuration in quarantine stations (Spotte, 1970; Ciambrone, 1975; Moffett and Shleser, 1975). Ozone destroys viruses (Sproul, 1975; Snyder and Chang, 1975), bacteria (Rosenlund, 1975; Colberg and Lingg, 1978; Wedemeyer, Nelson and Smith, 1978; Graikoski, Blogoslawski and Choromanski, 1985), fungi (Combs and Blogoslawski, 1975), algae and protozoa (Thurberg, 1975). Very small doses are required, and it reacts almost instantly, leaving no persistant, toxic residual in the water (Murphy, 1975). Furthermore, ozone dissociates rapidly to oxygen (Sander and Rosenthal, 1975; Sengupta, Levine, Wackenhuth and Guerra, 1975), which is itself beneficial to culture water

below saturation levels (Rosenlund, 1975). These attributes have made ozone a preferred disinfectant to methods such as chlorination and ultra-violet radiation (Engdal, 1991), although the cost of ozone production is a major constraint (Lohr and Gratzek, 1986). The use of ozone as a biocide is a very large and diverse subject that has been well researched and documented. This aspect of its application is outside the range of the present study, and therefore will not be considered further.

Ozone has been shown to be effective at oxidising certain chemical components occurring in water. These include ammonia (Rosenthal, 1981) and nitrite (Paller and Lewis, 1988), both of which are toxic to aquatic organisms (Spotte, 1970; Liao and Mayo, 1972; Wickins, 1976; Thurston, Russo and Smith, 1978; Heales, 1985). Other recorded effects are a reduction in the biological oxygen demand (BOD), turbidity, dissolved solids, odour and colour of the water (Murphy, 1975; Colberg and Lingg, 1978; Rosenthal, 1981; Sutterlin, Couturier and Devereaux, 1984). Used in conjunction with biological filtration, there have been indications that ozonation may significantly improve the general quality of culture water, and hence the total load that a recirculating system can support (Williams, Hughes and Rumsey, 1982). Improved growth and survival of culture animals have also been shown to occur in ozonated water (Stopka, 1975; Menasveta, 1980; Sutterlin et al., 1984; Tipping, 1987; 1988; Poston and Williams, 1988).

Many of the pathways by which ozone reacts with various substances are not known, because the speed at which the reactions take place are too rapid to follow analytically (Sander and Rosenthal, 1975). This has given rise to disagreement as to whether certain reactions occur or not. For example, Colberg

and Lingg (1978), Rosenthal and Otte (1979), Rosenthal (1981) and Hsieh, Tsai, Yeh and Su (1990) report that ammonia is significantly oxidised by ozone, while other reports claim it is not (Murphy, 1975; Wheaton, 1977; Lohr and Gratzek, 1986; Paller and Lewis, 1988). Furthermore, in reactions which are known to take place, end products remain unidentified. It is well documented that nitrite (NO_2-N) is efficiently and rapidly oxidised to nitrate by ozone, (NO_3-N) (Colberg and Lingg, 1978; Rosenthal and Otte, 1979; Sutterlin et al., 1984; Paller and Lewis, 1988). There has been a suggestion, however, that nitrite is merely converted to an intermediate product and reverts back to nitrite after ozonation ceases (Honn, 1979, in Rosenthal, 1981). There is not much evidence of this however, and there have been no suggestions made as to the nature of the intermediate product, if any.

It is clear that a greater understanding of the general effects of ozone in aquaculture is still required if its full potential is to be realized. Many authors have stated their belief in the use of ozone in increasing system productivity (Moffett and Shleser, 1975; Murphy, 1975; Menasveta, 1980; Sutterlin et al., 1984; Engdal, 1991). It was considered that, due to the relative confusion surrounding the efficacy of ozone as a water-chemistry enhancement treatment in aquaculture, further studies in this respect were necessary. This would in turn provide information relating to what extent, and in what direction, further ozone research should be applied if its high production costs were to be justified.

The overall objective of this study was therefore to monitor changes in water quality in a new recirculation aquaculture system, from initial stocking until the carrying capacity of the

system had been exceeded, and to investigate the role of ozone in water quality enhancement, with respect to increasing system efficiency.

MATERIALS AND METHODS

EXPERIMENTAL SYSTEMS

This study was conducted in the hatchery of the Rhodes University experimental fish farm. Two separate and identical recirculating systems were constructed. One of these acted as a control whilst the other was used for the ozonation trials. These systems will be referred to as systems A and B respectively. Equipment manufactured and installed for the latter included a 20 cylinder (10,5 kPa) oxygen supply, a 3-way manifold with approved piping, an ozone generator and an ozone contacting chamber. The system is schematically represented in Figure 1 and was designed in collaboration with Mr. M.T.T.Davies (Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown).

With the exception of a Reavell 1,1 kW rotary blower which supplied supplementary aeration, the experimental systems were entirely independent of the other sections of the hatchery complex. The biological filter was a vital component in this study, as it is in any recirculating system. It was important that it operate efficiently as a nitrification unit, as designated by Spotte (1970, 1979) and Wheaton (1977), in order to remove toxic nitrogenous metabolites produced by the culture animals. Two "serial" biological filters were constructed, utilizing guidelines in Spotte (1970), (gravel media, shallow with large surface area, supplementary oxygenation) and Wheaton (1977), (pre-filtration sedimentation, upflow and downflow) that were to provide the necessary characteristics of a working biological filtration system. Wheaton (1977) points out that there is no general system or set of equations that suitably

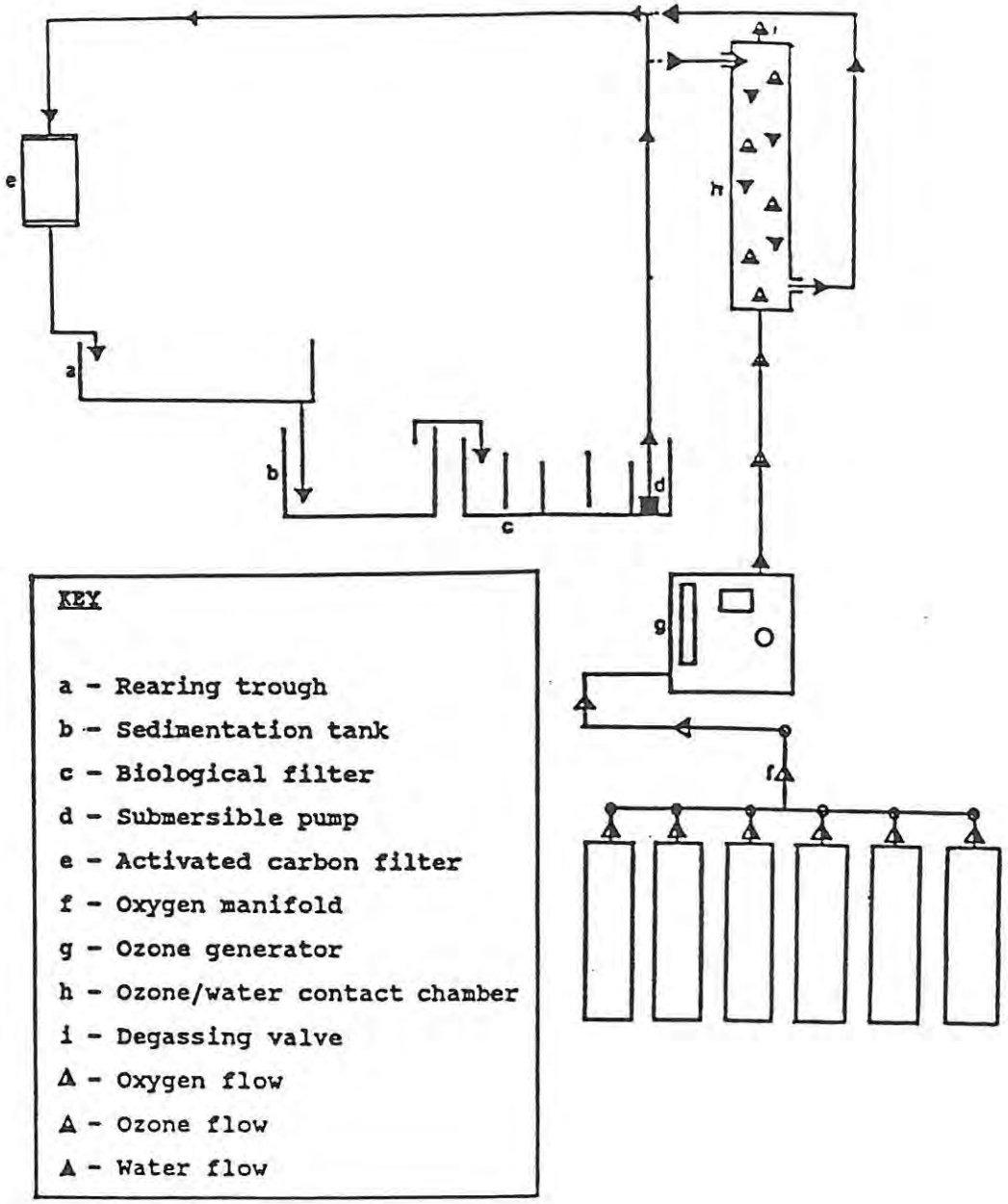

Figure 1: Diagram illustrating the experimental system (not to scale). Components f, g, h and i incorporated only in System B.

apply to and define biological filter design. Variations of certain parameters (i.e. hydrodynamics, dissolved oxygen, pH etc.) can therefore occur between different designs. For each of the identical units, the structure and function of the components were as follows:

i) CULTURE TANKS (Figure 2)

The culture tanks consisted of two rectangular troughs (3 x 0,5 x 0,45 m), constructed of 2 mm moulded steel plate, coated with a non-toxic epoxy paint. A water volume of 450 l was maintained by means of a 0,3 m upstand, outlet pipe (50 mm ID PVC). Water entered the head-end of the tanks through a 0,45 m, horizontal, 32 mm ID perforated pipe. A removable baffle, 0,4 m from the tail-end of the tank, with a series of 45, five mm holes drilled along the bottom edge, allowed for removal of a portion of the waste solids from the troughs which were covered with a 30 % shadecloth net to prevent fish jumping out. The size, and number of holes in the baffle, being greater than those on the inflow pipe, ensured that water flow was not hindered in the tanks. The position of the baffle resulted in an effective culture volume in the troughs of 390 l, with the portion of the tank behind the baffle acting as a primary clarifier where a large proportion of the waste solids accumulated and could be easily removed. Uneaten food and faeces were siphoned from the tanks three times a week. At the head of each tank there was a supplementary air supply delivered through a 10 cm air stone. This whole structure was supported by a 5 cm angle-iron stand, epoxy painted for rust prevention.

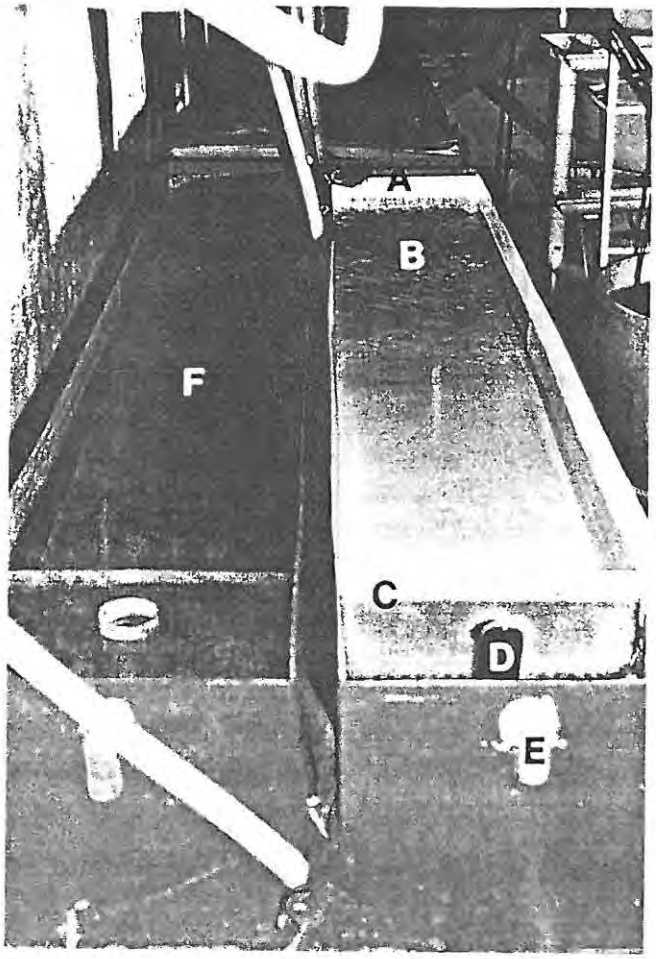

Figure 2a: Photograph of the culture tanks of one system, showing: A) water inflow, B) fish, C) waste removal baffle, D) outflow pipe, E) overflow pipe, F) shade-cloth cover.

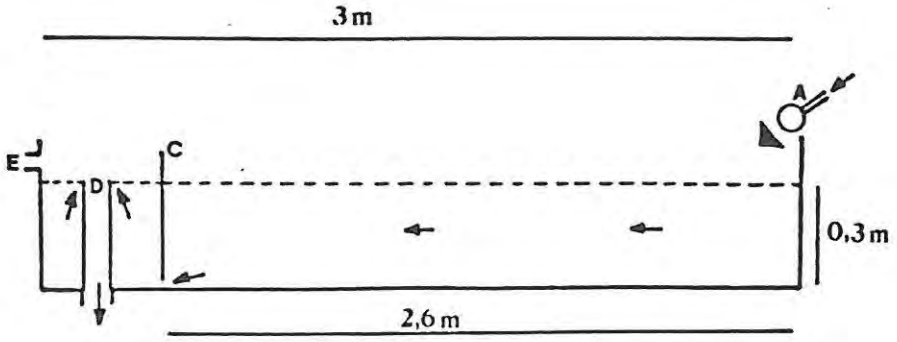

Figure 2b: Cross-section of culture tank with annotation as for Fig. 2a. Arrows indicate water flow.

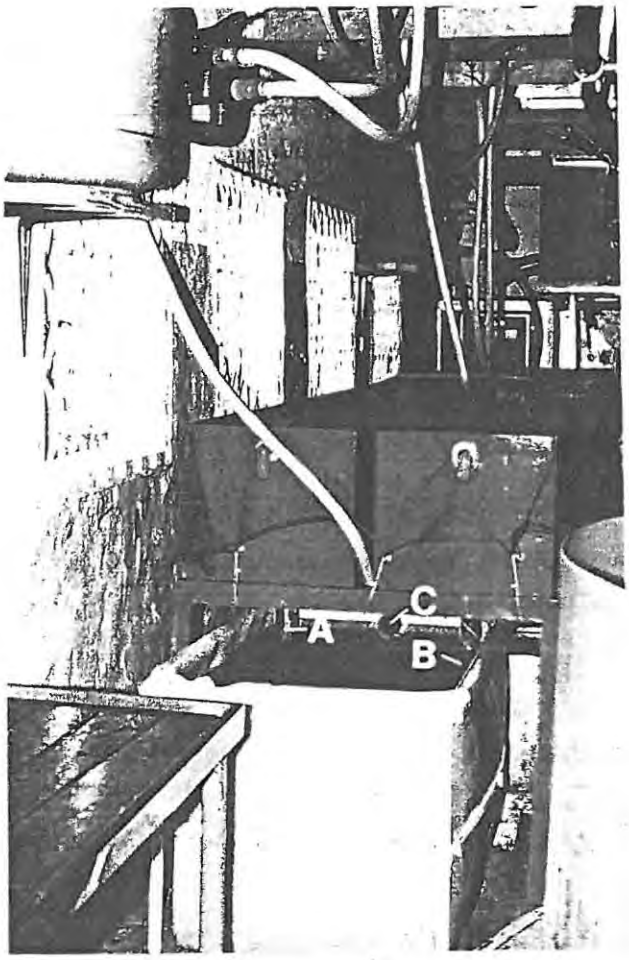

Figure 3a: Photograph showing sedimentation tank, with: A) water inflow pipe from culture tank, B) nylon stocking over inflow pipe (waste solid trap), C) outflow to biological filter.

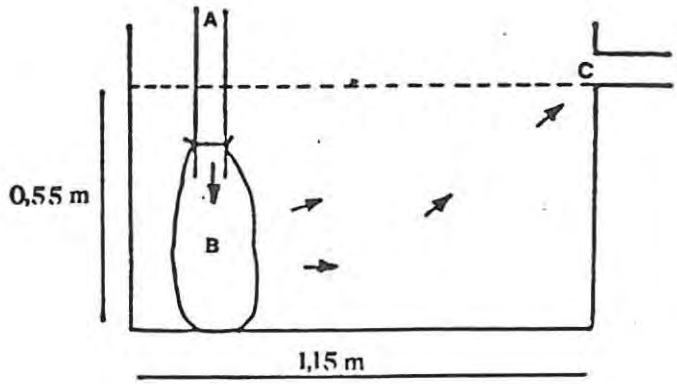

Figure 3b: Cross-section of sedimentation tank with annotation as for Fig. 3a. Arrows indicate water flow.

ii) SEDIMENTATION (Figure 3)

Accumulation of solids is one of the biggest factors limiting fish production in recirculating systems (Alabaster and Lloyd, 1982). A certain amount of detritus however, is also advantageous for increasing the total surface area upon which nitrifying bacteria can exist in the system (Spotte, 1970), but it is important that the majority of these solids are removed from circulation. This will limit blockage of the biological filter which causes channeling and loss of filter efficiency (Spotte, 1970; 1979). Effluent tank water passed from the outlet pipe through a nylon stocking, which acted as an initial mechanical trap for a large proportion of the solids. These were cleaned once a week initially, but when the fish bioload was increased and solid waste production increased, it became necessary to clean the stockings twice a week. Further entrapment of solids occurred in a plastic sedimentation tank (1,15m x 0,65m x 0,55m). Water then passed to the biological filter through a 75mm OD, PVC overflow pipe.

iii) BIOLOGICAL FILTER (Figure 4)

The biological filter was designed as a four-chambered (4 x 0,9m x 0,5m x 0,35m), submerged, serial flow system, and constructed of 10 mm pressed asbestos. This incorporated a combination of upflow and downflow characteristics, with water flowing under and over a series of baffles, from chamber to chamber. Gravel was used as a biofilter medium. The bottom of each chamber was V-shaped with a perforated sludge-drawoff pipe along the base. A false bottom of PVC duckboard was supported 10 cm above the base, to which a 2-m length of 12,5 mm hosing, drilled with 1 mm holes, was attached. When connected to the rotary blower, this hose

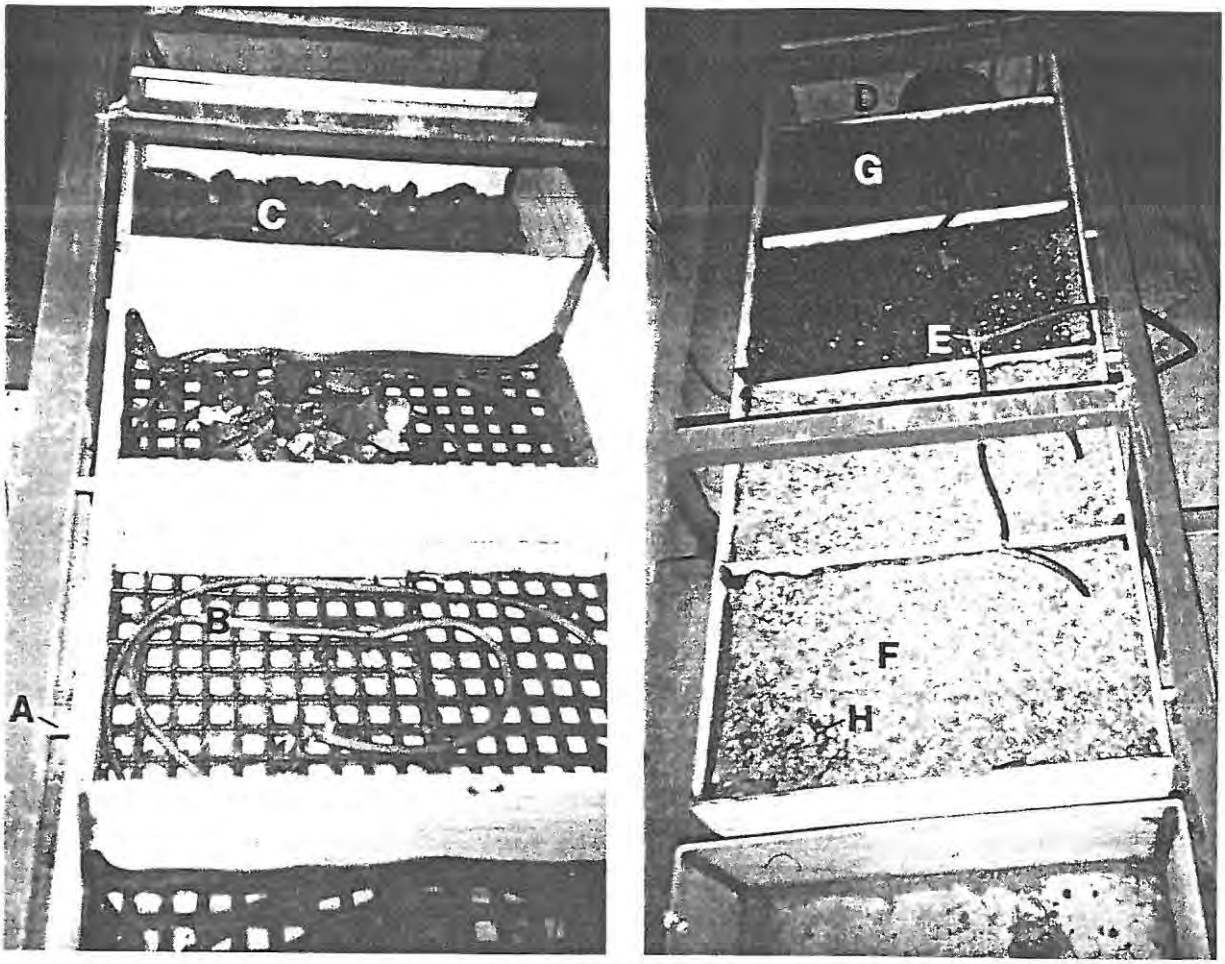

Figure 4a: Photographs of the biological filter, with A) sludge draw-off pipe, B) false bottom with sub-gravel aeration pipe attached, C) large 50 mm gravel, D) pumping chamber, E) sub-gravel aeration attached to air supply, F) 19 mm gravel, G) 10 mm gravel H) shade-cloth between gravel layers.

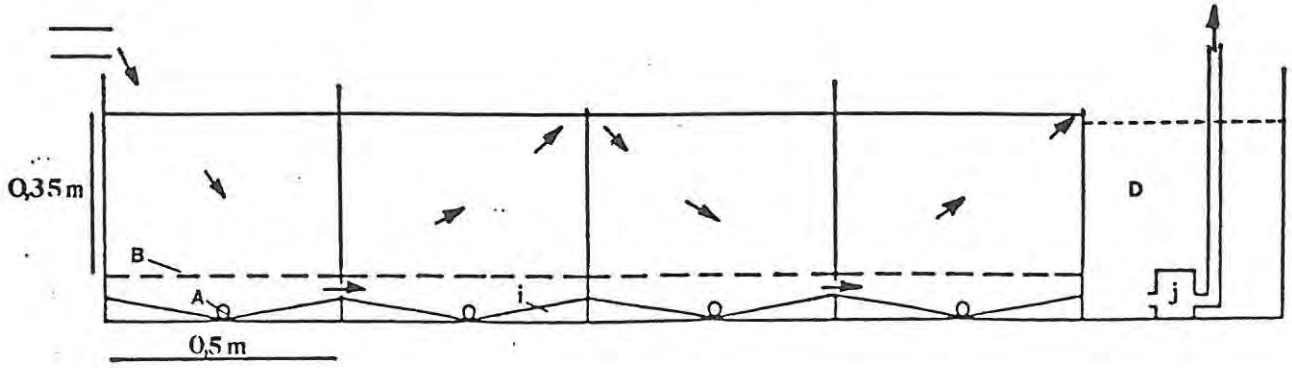

Figure 4b: Cross-section of biological filter, with annotation as for Fig. 4a. (i = V-shaped bottom, j = pump). Arrows indicate water flow.

provided sub-gravel aeration for the filter, which was necessary for the maintenance of healthy populations of aerobic nitrifying bacteria. Each chamber contained a specific quantity of gravel, ranging in size from 50mm to 9mm. This gravel acted as the medium to which the bacteria could attach and colonise. The proportion of each of these gravel sizes in the chambers is shown in Table 1.

Table 1: Amount of gravel used in each biological filter, expressed as percentage by volume of chamber, and real depth of each layer.

GRAVEL SIZE	PERCENT (BY VOLUME) AND REAL DEPTH OF MEDIUM IN EACH CHAMBER: 1	2	3	4
50 mm	50% (130 mm)	40% (100 mm)	30% (80 mm)	10% (30 mm)
25 mm	35% (90 mm)	40% (100 mm)	45% (110 mm)	45% (110 mm)
19 mm	15% (30 mm)	20% (50 mm)	20% (50 mm)	30% (70 mm)
10 mm	--	--	5% (10 mm)	15% (40 mm)

The total surface area of all the gravel in a biofilter containing a definite volume of medium is proportional to the reciprocal of the average grain diameter (Hirayama, 1974). An index of the surface area of a filter was proposed by Hirayama (1966a, in Hirayama, 1974), expressed as the grain-size coefficient G, where:

$$G = 1/R_1 (X_1) + 1/R_2 (X_2) + ... 1/R_n (X_n)$$

R = the mean grain size of each fraction of the gravel bed (mm),

and X = the percentage weight of each fraction.
Using this formula, the grain-size coefficient of the biofilter used in the present study was 3,95.

The quartzite gravel was collected from a quarry, hand-sorted and washed prior to use in the filter. Layers of different-sized stone were separated by 70 % shade cloth to prevent mixing. A fifth chamber (0,9m x 0,5m x 0,4m), acting as a sump, contained a submersible pump (Little Giant 5-MSP), from where water was distributed throughout the system. Once a week it was necessary to top up the pumping chamber of each filter with 25 l of tap water to replace that lost through evaporation, splashing or leakage. This amounted to 1,5 % of the total water volume and was not considered to affect the chemistry of the water.
This unit was accommodated directly below the culture tanks on an expanded-metal base attached to the tank stand.

iv) ACTIVATED CARBON FILTER (Figure 5)

Water was pumped from the biofilter to a pressure filter containing 20 kg of 2,5 mm granular activated carbon (GAC), with a mean residence time of 50 seconds . The primary function of GAC is the removal of dissolved, residual ozone in the water (Sander and Rosenthal, 1975; Stopka, 1975; Fischer ozone generator operating instruction manual, 1989), and was therefore necessary in system B during the ozonation trials. In order to maintain uniformity however, a GAC filter was incorporated into both systems. GAC is elemental carbon, which is a strong reducing agent. On contact with ozone, the carbon is oxidised to carbon monoxide and carbon dioxide, and the ozone molecule is destroyed. Because of this, the inclusion of GAC filtration in

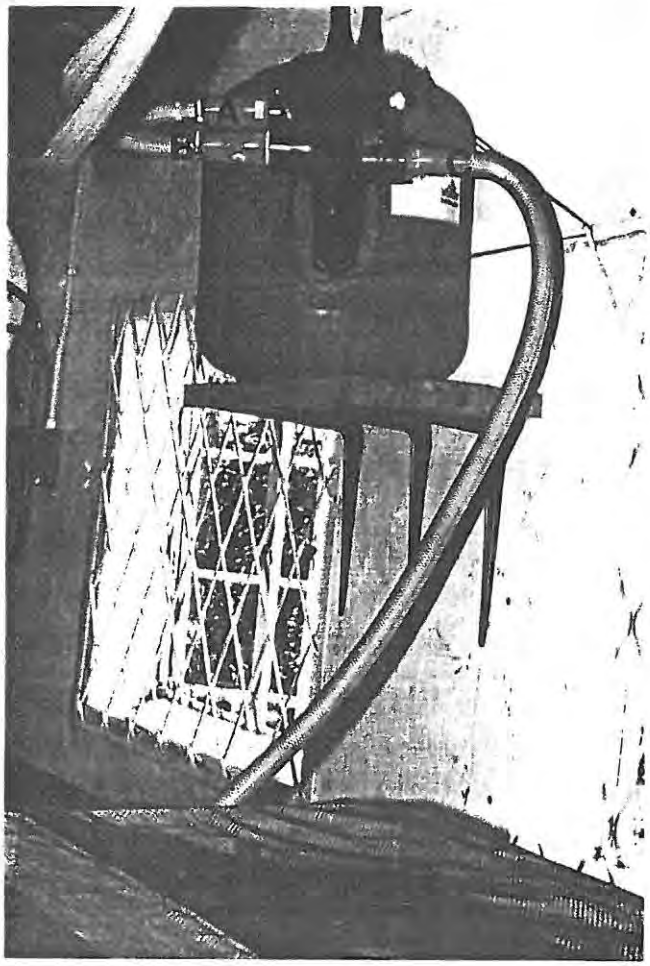

Figure 5a: Photograph showing activated carbon filter, with: A) inflow pipe, B) outflow pipe, C) backflush pipe.

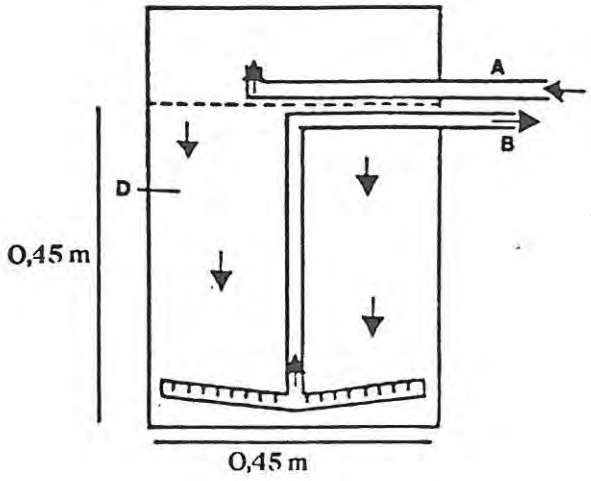

Figure 5b: Cross-section of activated carbon filter, with arrows indicating direction of water flow (D = activated carbon).

the ozonation system was a necessity as a safeguard against toxic levels of residual ozone reaching the fish in the culture tanks. In addition, Spotte (1970) reports activated carbon to be efficient at removing colour and odour in water, while also reducing concentrations of suspended solids, chemical oxygen demand (COD) and total organic carbon (TOC).

It was periodically noticed that water flow entering the culture tanks decreased. This was attributed to compaction of the carbon filter medium. When this occurred, the GAC filters in both systems were backflushed for a period of 15 minutes. This treatment loosened the compressed bed of GAC and also dislodged any excess particulate matter that may have been clogging the filters. After backflushing, the flow of water entering the tanks was normalized.

v) OZONE GENERATION (Figure 6)

Ozone treatment was only applied in system B, while system A acted as an untreated control during the ozonation trials. Therefore, sections I v) and vi) only apply to system B. In system A, the water flowed directly from the pump to the GAC filter.

Ozone was generated using medical grade oxygen with a Fischer 503 silent discharge generator. It was important that the feed gas used was both pure and dry. Moisture, or impurities in the feed gas such as methane and nitrogen result in the formation of carbon dioxide, water (Fischer manual, 1989) and nitric acid (Gargas, 1989) when exposed to the high voltages produced in the ozone generator (34000 V x 0,01 - 3,0 A). While water will decrease the ozone production and ultimately damage the ozone -

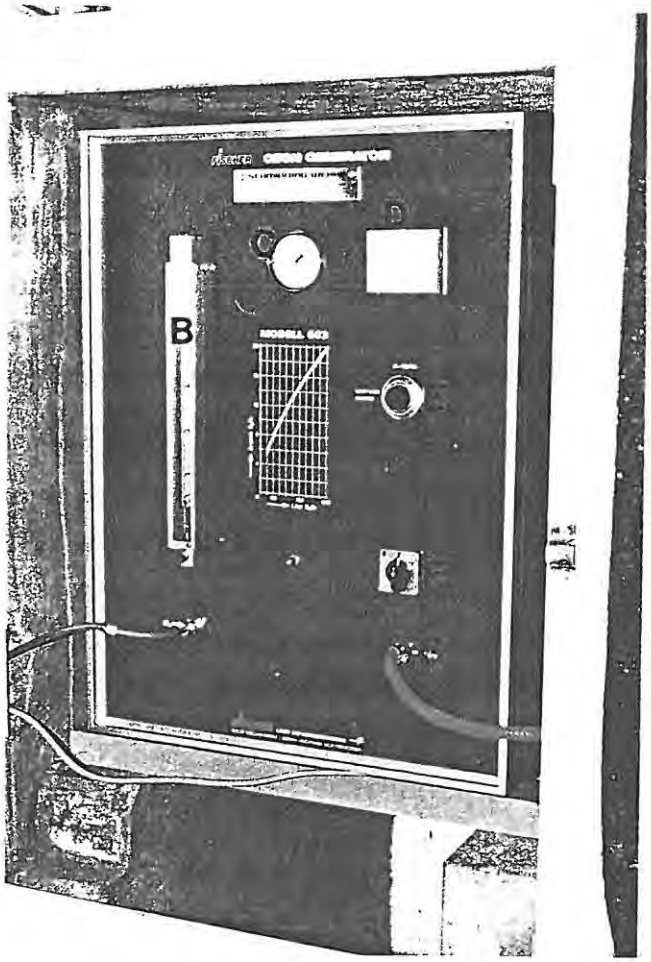

Figure 6: Photograph showing ozone generator, with: A) O_2 inflow, B) O_2 flow meter, C) pressure gauge, D) ammeter, E) power output dial, F) power switch, G) O_3 output.

generating tubes (Fischer manual, 1989), carbon dioxide and nitric acid would have a detrimental effect on the quality of the water to which they were exposed. The Fischer 503 generator can process measurable quantities of oxygen up to 500 l/h and produce up to 20 g O_3/h from this quantity of oxygen.

In order to maintain uniformity throughout the ozonation experiments, oxygen flow rates to the ozone generator were kept at 300 l O_2/h. This flow was considered adequate for the

purposes of this study because the quantities of ozone generated at this rate, with the contact time offered in the contact chamber (see section vi), had been successfully incorporated in previous ozonation studies (Colberg and Lingg, 1978; Paller and Lewis, 1988). The percentage of ozone produced from this volume of feed gas could then be altered by changing the voltage passing across the discharge gap. Amperage control (0 - 3 A in the 503 model, at 0,01 A calibration intervals) can generate up to 17 kV across the discharge gap. Power output was, however, limited by 2 A fuses. The high voltages attained provide enough energy to break the O=O double bond, and the oxygen atoms reform as unstable ozone, O_3 (Wheaton, 1977), which has a half-life of 15 minutes before dissociating back to oxygen.

vi) OZONE/WATER CONTACT CHAMBER (Figure 7)

Design of the vessel in which gas/water contact occurs is of prime importance when considering efficient mass transfer of ozone to the water (Wheaton, 1977). Many different basic contactor designs have been proposed. Rosenthal (1981) stated that while there are several types of gas/liquid designs that could be used in ozonation systems, a contacting system used for one application may not be suitable in another.

For the purposes of this study, a basic system in which water flowed counter-current to a stream of ozone bubbles was proposed. Wheaton (1977) stated that this was the simplest and most widely used design in ozonation systems. It has been either used or suggested as a successful and efficient option by Mangum and McIlhenny (1975), Rosenlund (1975), Sander and Rosenthal (1975), Thurberg (1975), Rosenthal and Otte (1979), Spotte (1979),

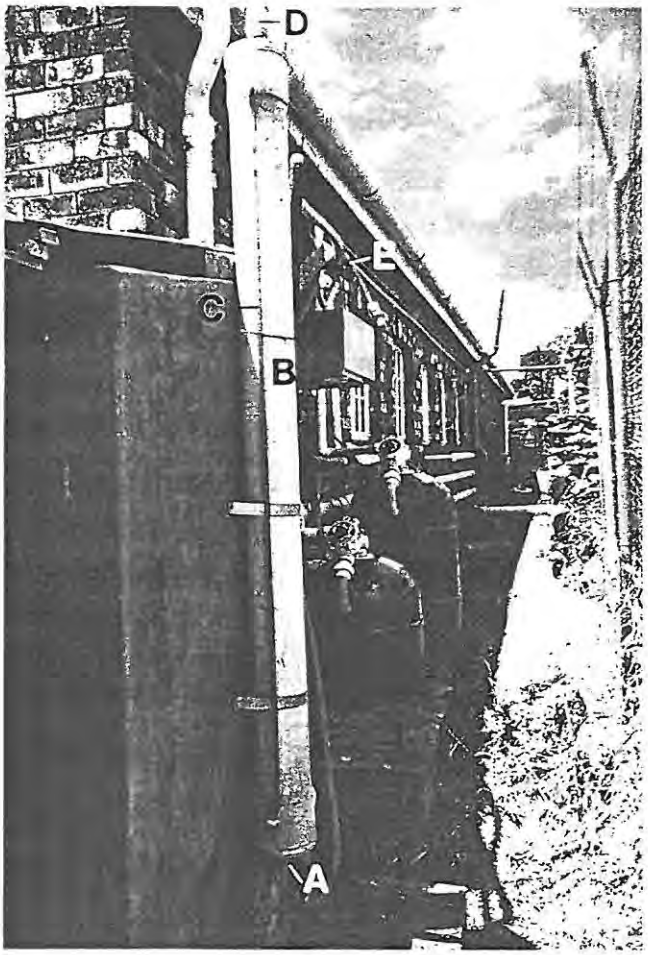

Figure 7a

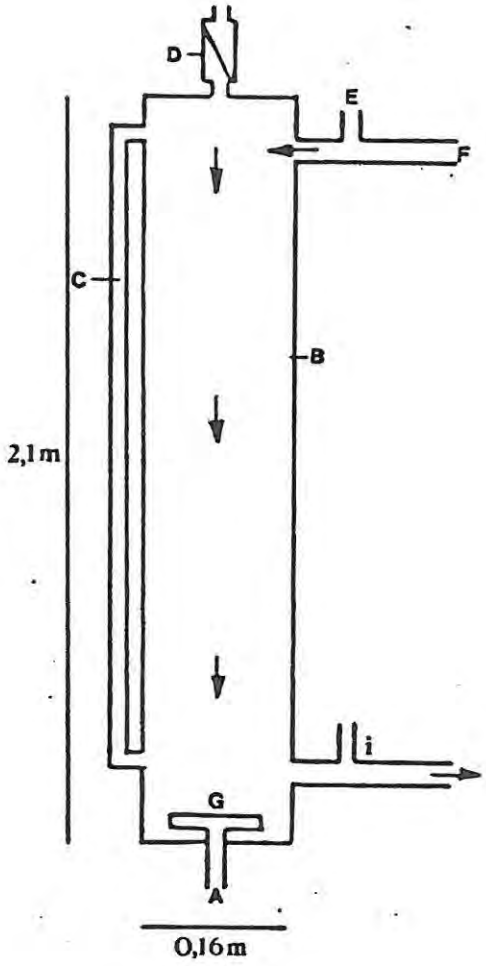

Figure 7b

Figure 7a: Photograph of ozone/water contact chamber, showing: A) O_3 inflow, B) PVC column, C) water level indicator pipe, D) pressure release valve, E) pre-ozonation water-sample point.

Figure 7b: Cross-section of ozone/water contact chamber, with annotation as for Fig. 7a. (F = water inflow, G = O_3 diffuser, i = water outflow and sample point).

Rosenthal (1981) and Paller and Lewis (1988). The systems described all consisted of a body of water flowing down a vertical column against a stream of fine, ozone-rich bubbles. This system maximises gas/liquid contact.

The ozone/oxygen gas mixture was transferred through SABS-approved, 12 mm gas hose from the ozone generator to the gas/water contact chamber. A PVC one-way valve was installed in-line to ensure no backflow of water into the ozone generator, which could have damaged the O_3-generating tubes (Fischer manual, 1989).

The contact chamber consisted of a 2,1-m high, 160 mm PVC pipe. The water in system B flowed into the top of the column and out at the base. Ozone was introduced through a perforated, stainless steel diffuser (70 mm diameter) located at the bottom of the chamber. This produced a stream of fine bubbles which provided a large surface area for gas/water contact. When the column was tested initially it was found that the pressure of gas accumulating in the column was greater than the hydrostatic pressure of the water entering the chamber. As such, the gas pressure pushed all the water out of the column so that water flowed straight into and out of the chamber, while also creating a greatly reduced flow throughout the system, causing a poor gas to water transfer. It was therefore necessary to incorporate a method of releasing excess gas from the chamber. This was achieved by fixing a stainless steel, one-way pressure-release valve (Armstrong, model 11-AV) to the top of the column which was set to maintain a constant water level 10 cm below the top of the column. This ensured that the chamber remained full of water when ozonation was in progress. The water level in the contact chamber was monitored by means of a transparent PVC pipe (10 mm

ID), attached to the outside of the column, which indicated the level of water in the chamber.

The most important factors in producing a large surface area over which the ozone can come in contact with water are the size of the bubble and the contact time (Spotte, 1979; Rosenthal, 1981). The bubble size produced in this system was not accurately measured, but it was estimated by eye to be approximately 2-4 mm in diameter. Rosenthal (1981) states that in most cases the smallest bubble averages about 2 mm, and that bubbles over 5 mm diameter cause excessive ozone waste. Rosenthal (1981) further stated that a bubble of 2-3 mm diameter is preferable.

Contact time varies greatly according to water flow rates and contactor size. In this study, it took approximately 70 seconds for water to pass through the column. Contact times in other studies vary widely. Tipping (1987), investigating the use of ozone in controlling infective pathogens, reported contact times of up to 28 minutes. Rosenthal (1981) quotes contact times of 2,5 - 5 minutes, in bacterial inactivation studies. Rosenthal and Otte (1979) exposed water to ozone for 10 minutes in their intensive culture system. However, Colberg and Lingg (1978) and Paller and Lewis (1988) both showed efficient use of ozone in disinfection and water quality experiments in aquaculture systems using contact times of 60 seconds. This indicates that the contactor design incorporated in the present study was comparable to those described in previous, successful ozonation applications. It was therefore assumed that the contact chamber would provide sufficient gas/water contact for the experimental purposes required.

For safety reasons the ozone contact chamber was located outside

the hatchery building. According to Rosenthal (1981), a threshold ozone exposure concentration of 0,05 - 0,1 mg/l is acceptable in most European countries. An advantage of the gas is that it has a pungent, characteristic odour which is easily smelled at concentrations of 0,05 - 0,1 mg/l, thereby enabling effective detection in the working environment. During the ozone trials, only very slight levels (by smell), coming from the treated water, were detected inside the hatchery. Although measurement of the gaseous O_3 concentrations was not undertaken as the maximum dissolved ozone concentrations produced was only slightly above 0,1 mg/l, it was not considered to be a health hazard.

All pipes and fittings used in the system were stainless steel, PVC, or nylon, due to the highly corrosive nature of ozone. None of the materials used are affected by reactions with the gas.

SYSTEM HYDRODYNAMICS

The hydrodynamic characteristics of each component of the systems are shown in Table 2. Maximum pumping capacity according to the pump specifications were 4870 l/hour. Using the known volumes of each component, and by recording residence times, it was found that mean flow rates varied at different points in the system, and were considerably less than those specified by the pump manufacturer. There are several reasons for this anomaly; i) frictional losses, ii) the gravitational head opposing free flow would have varied at different parts of the system, and iii) wear and tear of the impeller of the pump, reducing maximum pumping capacity as the study proceeded (Mr. H. Mulock-Houwer, Stewarts and Lloyds Trading, Port Elizabeth, pers. comm.). Water-reuse

efficiency of the system, calculated by the amount of water required to top up the system on a weekly basis, was approximately 98 %.

TABLE 2: Dimensions and hydrodynamics of each component of the experimental systems.

SYSTEM COMPONENT	COMPONENT DIMENSIONS	MEAN RESIDENCE TIME
Culture tanks	2 x (2,6 x 0,5 x 0,3 m), vol = 390 l	17,7 min.
Sedimentation tank	1,15 x 0,65 x 0,55 m vol. = 410 l	11,4 min.
Biological filter	4 (in series) 4 x (0,9 x 0,5 x 0,35 m) vol. = 300 l	10 min
Activated carbon filter	0,45 x 0,45 m vol. = 35 l	50 sec.
Ozone contact chamber	2,1 x 0,16 m vol. = 42 l	70 sec.

EXPERIMENTAL ANIMALS

Rainbow trout (Oncorhynchus mykiss) were used to provide the biological load for the system. All fish were of the same original stock. As an initial load for the start-up phase of the biofilter, a bioload of 5 kg m^{-3} in each tank was used. This loading comprised 85 fish (10 cm mean length and 23,5 g mean mass) in each tank. The fish were fed a standard commercial trout diet at a feeding rate given in Stevenson (1987) (see Table 3).

TABLE 3 : Proximate analysis of TRUKA trout pellets. (Information supplied by Truka (Pty) Ltd.)

Nutrient	(g/kg)
Protein	470
Lipid	120
Fibre	40
Moisture	120
Calcium	12
Phosphorus	7

The amount of feed required is expressed as a percentage of the body weight of the fish, and varies with body length and temperature of the water. Fish were taken from their holding pond in the hatchery, and weighed in batches in a bucket of water on a Mettler PE 3000 digital balance, to the nearest 0,1 g. They were then introduced to the experimental tanks. Representative sample batches of fish (30 fish per tank) were subsequently weighed in a similar manner at three week intervals to re-

calculate the required ration and to monitor the total bioload of the systems. A sample number of 30 ensured that the value obtained for the mean mass had a standard error of less than 1 gram (Mrs. E. Hodgen, Dept. of Mathematical Statistics, Rhodes University, pers. comm.). A source of pure oxygen was provided in all temporary holding drums during weighing operations. In addition, this procedure was performed as quickly as possible to minimise handling stress of the trout. Feed rations were also weighed on the digital balance. The fish were fed three times a day, as is the generally accepted rate for fish of this size (Piper, McElwain, Orme, McCraren, Fowler and Leonard, 1982; Stevenson, 1987). Food was introduced at the head of the tanks where it was immediately consumed by the fish.

When mortalities occurred in the tanks, dead fish were removed and replaced with a fish of similar mass ($\pm$ 1 g). Replacement fish were taken from the same stock as the original group, in order to minimise possible differences in performance by fish of differing origins. Before being placed in an experimental tank, replacement fish were given a half-hour bath in a solution of 0,5 ppm malachite green and 250 ppm formalin, as a precaution against external parasites. Because the system had been constructed entirely of new equipment, it was assumed that the experimental system was free of disease organisms associated with established systems that have held fish for some time. All nets and cleaning equipment used in the experimental systems that may have been contaminated from other sources were dipped in a 10 % formalin bath before and after use.

EXPERIMENT I : WATER QUALITY SUCCESSION IN A NEW RECIRCULATION AQUACULTURE SYSTEM

This aspect of the study involved daily monitoring of the changes that occurred in the water quality of the systems described above, from the time that they were stocked with fish to when the carrying capacity of the systems was exceeded and became unsuitable for further culture. The purpose of this was to provide an indication of the dynamics and interrelationships of various water chemistry parameters that are considered important in aquaculture. Furthermore, it was necessary to determine what changes in water quality might indicate that culture conditions were becoming unsuitable. The duration of the study was 92 days.

The water quality parameters monitored during the study were: total and un-ionized ammonia, nitrite, nitrate, alkalinity, carbon dioxide, calcium, turbidity, total dissolved solids, pH, redox potential, dissolved oxygen, temperature and ozone (during the ozonation trials). A study of the literature indicated that these variables were among the majority of those that had been monitored in previous ozonation studies. In addition, apart from dissolved solids, ozone and redox potential, which had special relevance to the present project, the above parameters were designated either "intermediate" or "highly significant" importance status in aquaculture systems by Walmsley *et al.* (1986).

Methodology and analysis techniques for the colorimetric and titrimetric tests were obtained from the Hach water analysis handbook (1986 edition) for the DR (Direct Reading) range of spectrophotometers. Unless stated otherwise, the procedures in

this handbook are based on the methods described in APHA's (American Public Health Association) "Standard Methods for the Examination of Water and Wastewater.

Total ammonia (± 0,01 mg/l) was determined using the Nessler reagent method.
Un-ionized ammonia was calculated using an equation given in Alabaster and Lloyd (1982);

$$\% \text{ un-ionized ammonia} = 100/1 + \text{antilog } (pK_a - pH).$$

Values for pK_a at different temperatures between 0-30 degrees Celsius were taken from Emerson, Russo, Lund and Thurston (1975).
Low-range nitrite (± 0,0005 mg/l) concentrations were measured by the diazotization method, high-range levels (± 1,0 mg/l) by the ferrous sulphate method (Prescott and Johnson's Qualitative Chemical Analysis. New York, 1933, in Hach manual).
Nitrates (± 0,1 mg/l) were determined by the cadmium reduction technique, and turbidity (± 5 FTU, Formazin Turbidity Unit) by the absorptometric method.
Ozone (± 0,005 mg/l) was measured using the DPD (N,N-Diethyl-p-phenylenediamine) total chlorine method (Palin, 1967, in Hach manual). Because of the rapid dissociation rate of ozone, it was important that this test was performed as quickly as possible after the water sample had been collected, and as such, the test was performed within a minute of sample collection. Ozone having a half-life of 15 minutes (Colberg and Lingg, 1978), this sampling procedure would result in only a 3% loss of ozone.
The above are all colorimetric tests and were performed using a Hach DR-EL 4 Spectrophotometer.

Alkalinity, carbon dioxide and calcium were all determined by

titrimetric methods using the Hach digital titrator system (± 0,5 mg/l). For these manually performed tests, a mean value from three titrations was used for each reading.

Tap water was used to fill the systems. Before the trials began, regular tests were made of the chlorine content of the water, using the DPD total chlorine method. This was to ensure that the chlorine concentration was below toxic levels before the fish were introduced to the tanks. Total dissolved solids (± 0,5 mg/l), pH (± 0,05 pH unit) and redox potential (± 1 mV) values were recorded with Hanna digital tester pens. Dissolved oxygen measurements were taken with a Hanna HI 8043 dissolved oxygen meter (± 0,05 mg/l), and water temperature was recorded with a Brannan, standard alcohol thermometer (0 - 50 degrees C, ± 0,1 degree).

Readings of the above-mentioned variables were taken each day, in the mornings. The analyses were routinely performed between feeding applications to eliminate possible effects of the feed on water quality. All glassware and other laboratory equipment used in the analytical procedures was washed using Sodosil RM 01 alkaline cleaning concentrate, and rinsed using demineralized water. A number of the analysis procedures called for the use of demineralized water as well. This was provided by passing tap water through a Keil Demin Quick DL 2 demineralizer, incorporating a mixed bed cation/anion resin. The levels of accuracy offered by the various techniques were considered suitable for the purposes of this study, both in terms of comparison with previous studies, and with regard to the actual levels recorded in this study.

EXPERIMENT II : THE EFFECT OF OZONE ON WATER QUALITY IN A RECIRCULATION AQUACULTURE SYSTEM

Trials to establish the effect of ozone on water quality were initiated only once the systems had become fully conditioned, as defined by Spotte (1970), i.e. the situation in which the filter bacteria are in dynamic equilibrium with the routine formation of their energy source (ammonia and nitrite). This situation is reached when total ammonia remains below 0,1 mg/l and nitrite below 1,0 mg/l. There were two reasons for this:

i) it was decided to use the initial conditioning phase of the biofilters as a yardstick by which to compare the similarity in performance of systems A and B. If, and once this similarity had been established, it was presumed that any differences observed between the two systems once ozonation experiments had started could be attributed to this treatment, or the lack of it.

ii) it was important that the bacterial populations in the biological filters became established and operating as efficient nitrification units. This is because the removal of toxic nitrogenous metabolites by bacteria is a vital factor in any functioning recirculating system. Any effects of ozone on this process, whether beneficial or not, would have eliminated the relevance of i) above, and/or affected the efficiency of the nitrification process.

Ozonation trials were therefore performed in system B, superimposed on the water quality experiment, for which daily sets of data were still obtained from both systems. System A acted as the untreated control system during the ozone trials, which were initiated on day 59 of the study.

Initially, the effect of a one-hour per day ozone treatment at a

low dose was examined. Eighteen (18) replicates of this trial were performed. Oxygen flow was set at 300 liters/hour, with a power setting of 15% (0,2 amps). This oxygen flow corresponds to an ozone output of 15 grams/hour (Fischer manual). Values for ammonia, nitrite, nitrate, calcium, TDS, redox potential, dissolved oxygen (DO) and ozone concentrations in the culture tanks were recorded immediately prior to ozonation. At ten minute intervals during the one hour trial, samples of water were collected from the culture tanks and tested for ozone, DO, TDS and redox potential. Water samples were also taken from two sampling points, immediately before entering, and after leaving the contact chamber (pre- and post-treatment), to give an indication of the quantity of ozone being transferred to the water in the contact chamber. Ozone content of the water from these points was also tested at ten minute intervals through the hour. After one hour, the ozone generator was switched off, and all pre-trial water tests repeated. These readings were then also recorded one and three hours after each trial. During this and subsequent experiments, analysis of water quality in system A continued as before. The results from these data acted as an indicator to the changes occurring in the duplicate system without ozonation, under identical culture conditions.

The next set of experiments consisted of three six hour trials, using the same ozone dose as before. These extended trials were performed to determine if prolonged exposure to ozone produced any markedly different effects in either water quality or the culture animals. While continuous ozonation is not recommended (Sander and Rosenthal, 1975; Edmonds, 1988), Rosenthal (1981) states that exposure to ozone for up to 6 hours will not affect fish, hence the time period for the present trials The same water quality parameters were monitored, but samples were only

taken at hourly intervals through the trial, and one hour after the ozone treatment had been terminated.

Having investigated the effect of low ozone dosages, it was necessary to determine the effects of higher ozone concentrations on water quality. A set of three, one-hour trials were conducted, using the same oxygen flow as previously (300 l/hr), and the ozone generator operating on a 50 % power setting (1,5 amps). At these settings, the ozone generator functioned properly for only approximately 10 minutes before the amperage started fluctuating from 0,2 to over 2 A. As the machine is fitted with 2 amp fuses, it became necessary to manually operate the power dial in order that the 2 ampere output was not exceeded for any prolonged time. It was presumed that the electric current was arcing out at some point on the circuit, producing this effect. This situation continued for the duration of the trial, producing an erratic ozone supply as a result of the fluctuating voltage across the discharge gap. While the ozone concentrations that were attained were slightly higher than in the previous one hour trials, they were not high enough to produce significantly different results than before (see results section).

A final experiment was carried out to determine the effect of pure oxygen alone on water quality. This was done to exclude the possibility that the excess oxygen, and not ozone, was producing any observed effects within the system. The procedure followed was as before, with oxygen flow through the ozone generator set at 300 l/hr, but the generator was not switched on. This experiment was repeated three times.

The experiments that were performed during this study are

summarised below.

TABLE 4 : Type, and duration of experiments performed in each system during the study.

EXPT. NO.	TYPE OF EXPT.	SYSTEMS USED	DURATION (DAYS)
EXPT I	WATER QUALITY ANALYSIS	SYSTEMS A & B (replicates)	0-92
EXPT II	OZONE ON WATER QUALITY	SYST. A (control) SYST. B (ozonated)	59-83
II a)	LOW-DOSE, 1 HR/DAY.		59-77
II b)	LOW-DOSE, 6 HR/DAY.		78-80
II c)	HIGH-DOSE, 1 HR/DAY.		81-83
II d)	OXYGEN, 1 HR.		85-87

RESULTS

EXPERIMENT I : WATER QUALITY SUCCESSION IN A NEW RECIRCULATION AQUACULTURE SYSTEM (DAYS 0 - 92)

The following water quality parameters were measured and recorded on a daily basis in order to establish the variations and inter-relationships of the different criteria that occur in a functioning, recirculation aquaculture unit.

i) TOTAL AMMONIA, (NH_4 - N)

Ammonia exists in aqueous solution in two forms :- ionized (NH_4^+) and un-ionized (NH_3) (Spotte, 1970; 1979; Stickney, 1979; Alabaster and Lloyd, 1982). They are usually measured together as total ammonia (NH_4).

Changes in total ammonia concentrations over the 92 day study are shown in Figure 8. Initially, ammonia levels were negligible as there was little biological activity within the systems. With the introduction of fish at 5 kg/m^3 on day 4, and the commencement of feeding, the ammonia concentration increased to approximately 0,8 mg/l by day 20, as a result of metabolic production by the fish. At this point, the substrate concentration (ammonia) exceeded the capacity of the nitrifying bacterial population to utilize it sufficiently. The drop in ammonia on days 25 - 30 corresponds with the establishment in the biological filter of a population of autotrophic bacteria. Identification of the bacteria in the biofilters were not undertaken, but it was assumed that the same genera of bacteria,

Nitrosomonas, that have previously been accredited to performing nitrification were also responsible for the same process in this study. These organisms utilize ammonia as an energy source, and convert it by oxidation reactions to nitrite (Spotte, 1970; 1979). This reaction, as shown by Haug and McCarty (1972), is as follows:

$$55\ NH_4^+ + 5\ CO_2 + 76\ O_2 \rightarrow C_2H_7O_2N + 54\ NO_2^- + 52\ H_2O + 109\ H^+$$

This is the first step in the nitrification process, as defined by Spotte (1970). Points of importance arising from this equation are:

i) the large quantities of oxygen needed for the reaction to proceed efficiently, illustrating the necessity for supplementary aeration in the biological filter.

ii) the amount of hydrogen ions (H^+) generated by the oxidation of ammonia, which is of great relevance when considering changes in pH and alkalinity in the same system (see this chapter, pp. 43 & 45 respectively).

Once the bacterial population became established within the systems, and was in equilibrium with substrate input, ammonia levels stabilized at levels similar to pre-stocking (0,03-0,04 mg/l). This situation was maintained until day 70 when ammonia concentrations once again rose rapidly, to the end of the study on day 92, reaching peak levels of 1,23 mg/l and 1,39 mg/l in systems A and B respectively. Day 70 corresponds to the date when the stocking density in the tanks was increased from 10 kg/m^3 to 15 kg/m^3 by adding extra fish to the system.

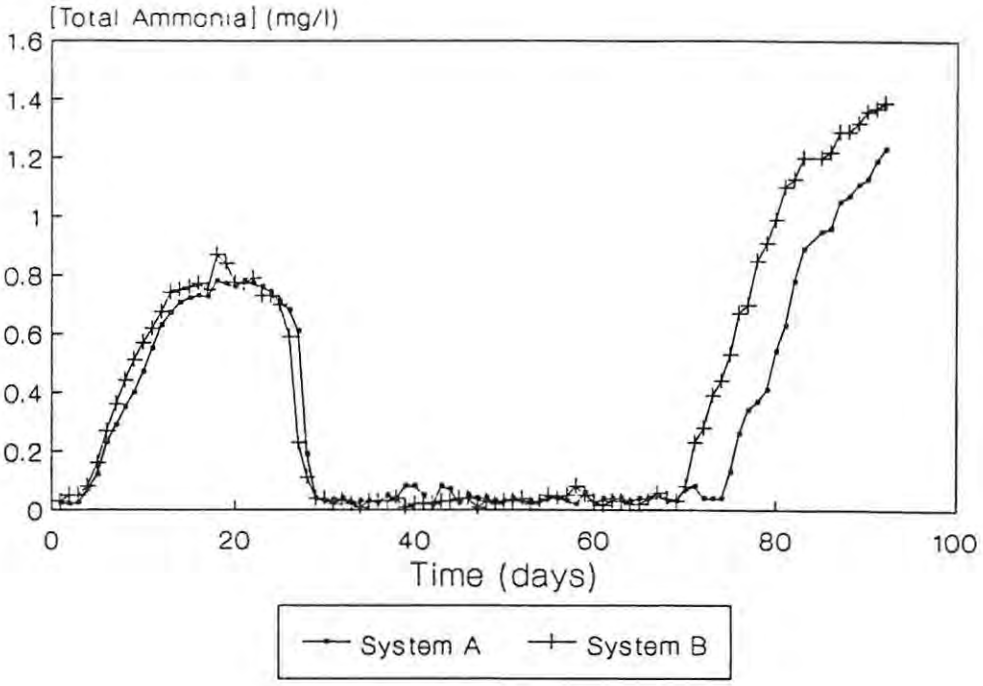

Figure 8: Changes in total ammonia (NH_4-N) concentrations in systems A and B during the study.

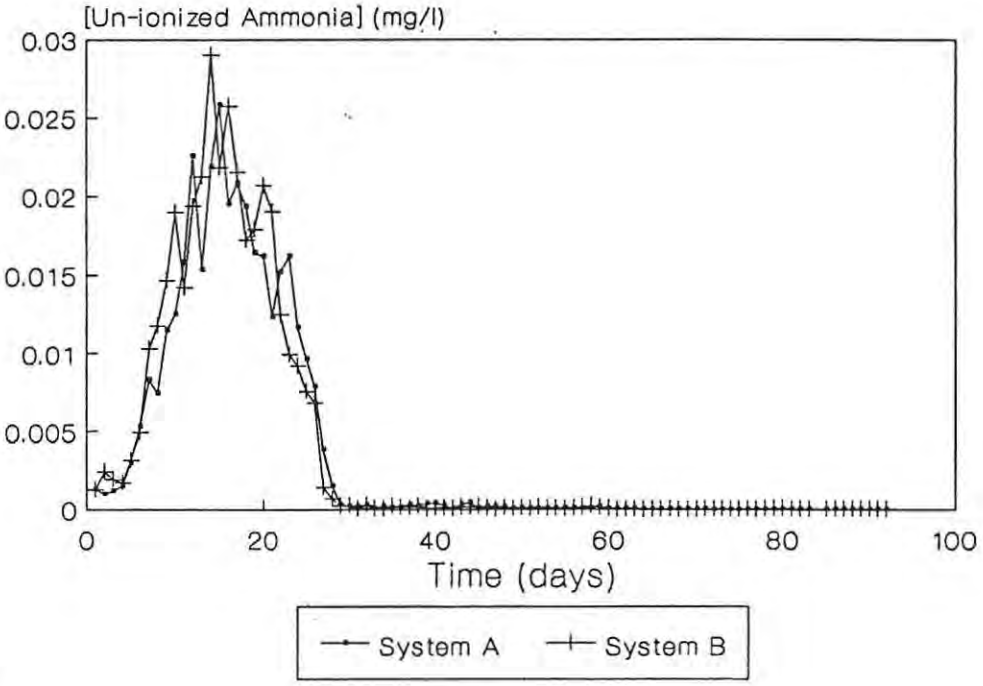

Figure 9: Changes in un-ionised ammonia (NH_3) concentrations in systems A and B during the study.

ii) UN-IONIZED AMMONIA, (NH_3)

Changes in un-ionized ammonia during the study are shown in Figure 9. The initial peaks in total and un-ionized ammonia between days 15 - 20 coincided directly. Once the Nitrosomonas population became established, the NH_3 and NH_4 - N concentrations were maintained at low levels until day 70 when the stocking density in the tanks was increased. Total ammonia concentrations then rose sharply (Figure 8), although the un-ionized fraction remained at low levels. This can be ascribed to the low pH (<6, Figure 12), on which NH_3 is highly dependent (Liao and Mayo, 1972; Emerson et al., 1975; Alabaster and Lloyd, 1982). The percentage of NH_3 at pH 6 is below 0,03, hence minute concentrations of this known fish toxin were present during the latter stages of the study.

iii) NITRITE, (NO_2 - N)

Nitrite concentrations show a similar, yet delayed, trend as the total ammonia curve (Figure 10a). Levels began to rise rapidly on day 15, as ammonia-oxidising bacteria populations became established. The observed time lag between decreased ammonia levels and nitrite oxidation is because ammonia inhibits the growth of Nitrobacter sp. (Lees, 1952). Spotte (1970) adds that a reduction in nitrite levels does not occur until the majority of the ammonia has been oxidised by Nitrosomonas sp.. In turn, the presence of nitrite encourages the growth of Nitrobacter sp. bacteria in the system (Spotte, 1970; Wheaton, 1977: Heales, 1985). Nitrites reached a peak on day 30 in system B at 35 mg/l, and approximately 10 days later in system A at 43 mg/l, before falling dramatically in both systems to 0,06-0,08 mg/l within 10

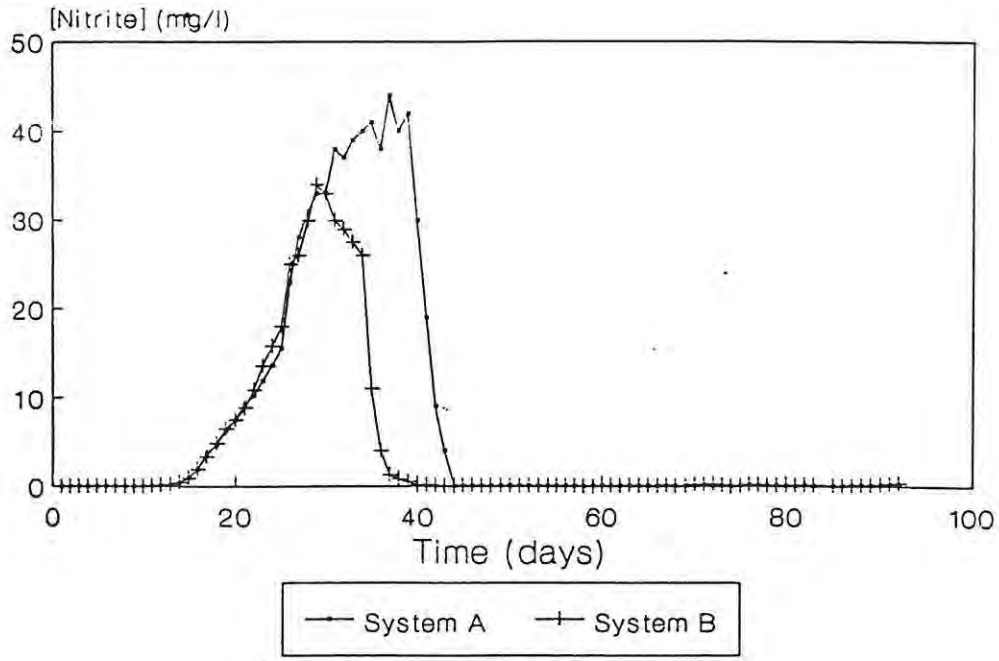

Figure 10a: Changes in nitrite (NO_2-N) concentrations in systems A and B during the study.

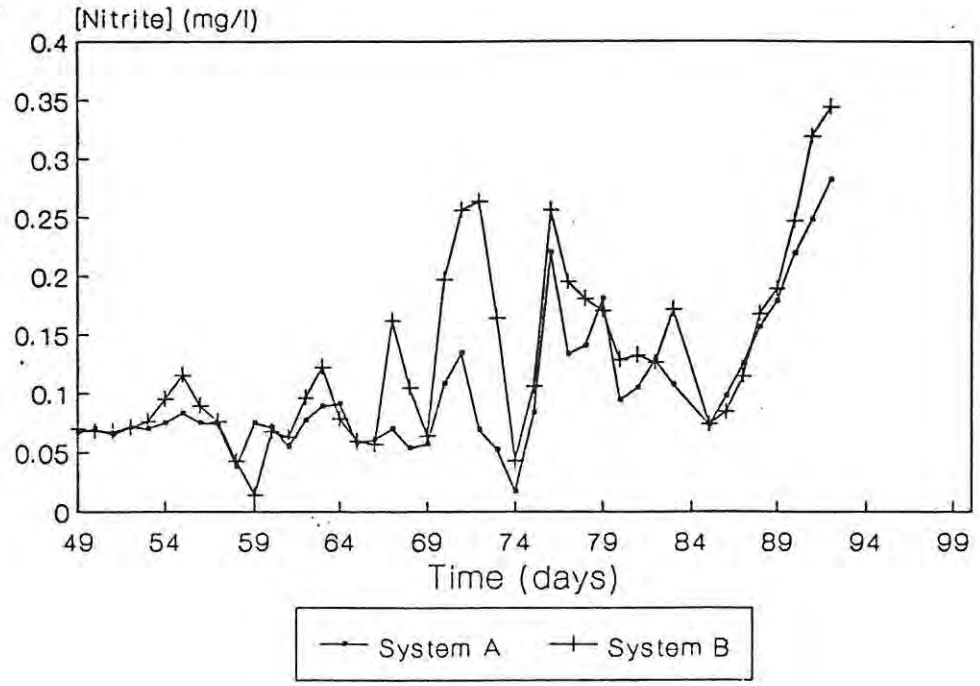

Figure 10b: Changes in nitrite (NO_2-N) concentrations in systems A and B from days 49 - 92 of the study.

days. As with ammonia and Nitrosomonas sp., this corresponds with the stabilization of Nitrobacter sp., and the second stage of nitrification (Spotte, 1970). Haug and McCarty (1972) illustrated the process by the equation:

$$400\ NO_2^- + 5\ CO_2 + NH_4^+ + 195\ O_2 \rightarrow C_5H_2O_2N + 400\ NO_3^- + H^+$$

As with the oxidation of ammonia, the need for large quantities of oxygen for the process to occur is evident, hence the air supply in the biological filter.

The levels of nitrite recorded between days 16 and 43 were well in excess of those reported as lethal to rainbow trout by previous authors (Smith and Williams, 1974; Russo et al., 1974; Westin, 1974; Smith and Russo, 1975). Although widespread mortality of the fish was noted during this period (20 % of total population in system A, 16 % in system B), the concentrations of nitrite attained (43 mg/l and 35 mg/l in systems A and B respectively) suggest that much greater, if not total mortality, would have occurred. It appears therefore that there was some form of protective effect being offered to the trout against nitrite toxicity, possibly by either calcium (Crawford and Allen, 1977) or chloride (Perrone and Meade, 1977) in the water. This factor is dealt with in greater detail in the discussion. The possibility that poor analytical procedure was responsible for the seemingly high nitrite levels obtained is negated by the fact that the procedure was followed exactly from the spectrophotometer manual using pre-packaged chemicals in the same manner as the other spectrophotometric tests which did not exhibit the same problem of unusually high values.
Nitrites also show an increase from day 70 onwards, rising to 0,282 mg/l in system A and 0,344 mg/l in system B by the end of

the study. This trend is shown in Figure 10b. It is assumed that this increase can also be attributed to the fact that the rate of metabolite production by the fish was exceeding the oxidizing capacity of the biofilter. As was the case with rising ammonia levels, this suggests that the carrying capacity of the system had been surpassed (Spotte 1970).

iv) NITRATE, (NO_3 - N)

Nitrate is the final product of nitrification (Spotte, 1970). It is produced by the oxidation of nitrites by Nitrobacter. Levels of this compound began to rise once these bacteria started to accumulate in the biofilter. Although there are some bacteria that may convert nitrate to gaseous nitrogen (Stickney, 1979), nitrates generally accumulate within recirculating systems (Spotte, 1970; 1979; Collins et al., 1975; Wheaton, 1977; Boyd, 1979; Heales, 1985). This was also shown in the current study (Figure 11). Significant increases in concentrations of nitrate began on day 20, in conjunction with the increasing Nitrobacter population. On days 35-40, and 40-45 in system B and system A respectively, nitrate levels drop sharply before continuing to rise again. This corresponds with the rapid decrease in nitrite levels, or establishment and stabilization of Nitrobacter sp. population. It is suggested that there is an "overshoot" period during which nitrate production adjusts from the rapid oxidation of nitrite by insufficient bacteria, to a more stable oxidation rate once the bacteria population has reached a size capable of processing all the available energy source. Throughout the rest of the study, nitrate levels steadily increased, reaching 49 mg/l in system A and 46 mg/l in system B.

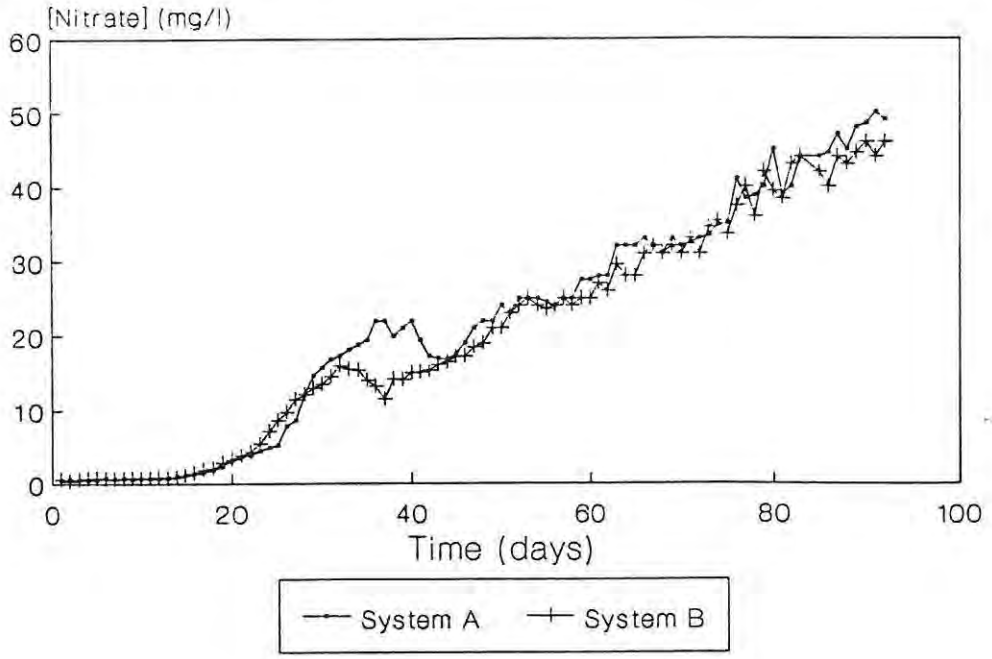

Figure 11: Changes in nitrate (NO_3-N) concentrations in systems A and B during the study.

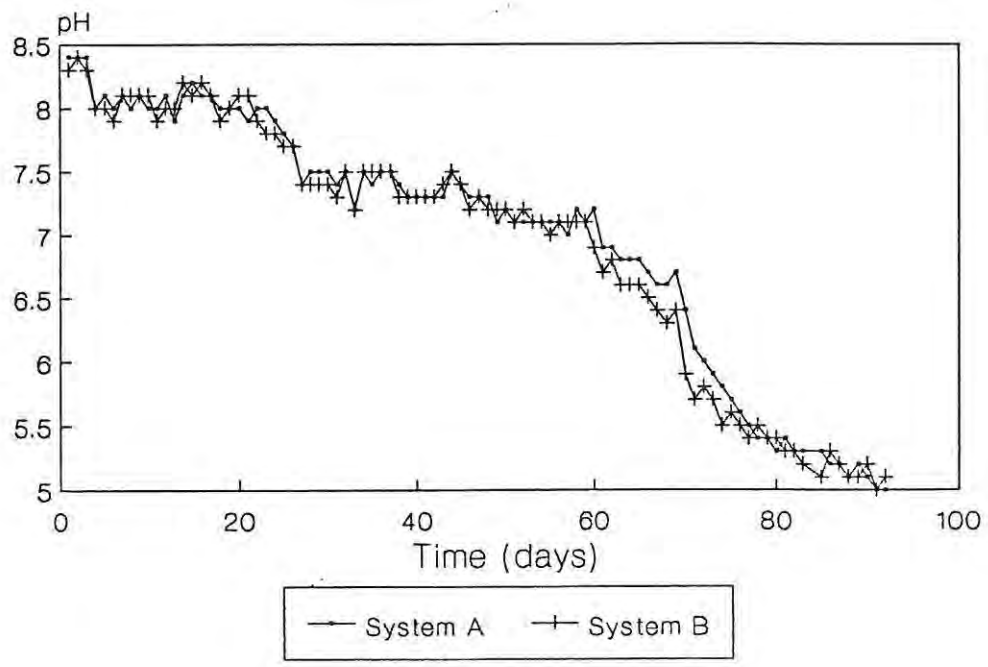

Figure 12: Changes in pH in systems A and B during the study.

v) pH

The pH of the water in both systems decreased during the study (Figure 12). There was an initial drop from approximately 8,35 to 8,0 when the fish were introduced to the systems on day 4. Spotte (1970) and Wheaton (1977) state that all oxidation reactions, such as nitrification, produce hydrogen ions (see equations on pages 37 and 41), and result in the formation of acids. Thus pH tends to drop during nitrification. The hydrogen ions react with bicarbonates, producing carbon dioxide. Reduction of bicarbonate ions and the increase in carbon dioxide (CO_2) also lower the pH. In addition, CO_2 is produced by the fish, and combines readily with water to produce carbonic acid (Spotte, 1970; 1979). This further reduces the pH in a system. The initial fall in pH can therefore be attributed to the stocking of fish into the systems, the consequent production of ammonia, and the beginning of nitrification. The pH stabilized at this level (8,0) until another drop to approximately 7,4 on day 28, which corresponds with the increase in nitrite concentrations, and increased nitrifying activity.

From day 60 - 80 the pH fell rapidly from 7,1 to 5,3, finally reaching approximately 5,0 at the end of the study. The decreases in pH observed between days 20-30, 40-50 and 60-70 all coincide approximately with increases in the amount of feed provided to each tank. It seems probable that increasing feed rations result in an increase in fish and bacterial metabolism due to the more abundant energy source for the two different organisms: pellets for the fish and nitrogenous waste for the bacteria. Carbon dioxide production would increase due to more rapid respiration, and the greater metabolite level would give rise to more acidic nitrogenous components. Both these factors

result in a drop in pH. Increased respiratory rates of both fish and bacteria would occur during the latter stages of the study when the poor water quality, in particular the low dissolved oxygen levels, would stress the animals, increasing CO_2 production and lowering the pH.

vi) CARBON DIOXIDE, (CO_2)

During the initial conditioning phase of the systems (days 0-35), carbon dioxide concentrations ranged between 15-25 mg/l (Figure 13). Apparent peaks on days 5, 16 and 28 correspond with the initial increases in ammonia, nitrite and nitrate concentrations. These peaks may be related to increases in aerobic bacteria respiration as they respond positively to the increased energy supply. This is confirmed by Spotte (1970; 1979) who states that bacterial oxidation processes increase concentrations of carbon dioxide. Furthermore, carbon dioxide is produced by the reaction of bicarbonates with the hydrogen ions produced by nitrification (Wheaton, 1977).

In the fully conditioned system, CO_2 stabilized between 10-15 mg/l. On day 70, when additional fish were added to the tanks to increase the bioload, carbon dioxide levels began to increase substantially, reaching over 50 mg/l on day 92. This could be directly associated with increased respiration of the fish in the tanks, because of both the larger number of fish, and stress related to the increasingly sub-standard water quality resulting from a system overload. In addition, the respiratory rate of aerobic bacteria throughout the system would increase as they attempt to process the excess supply of nitrogenous waste from the fish. This rise in the CO_2 concentrations throughout the

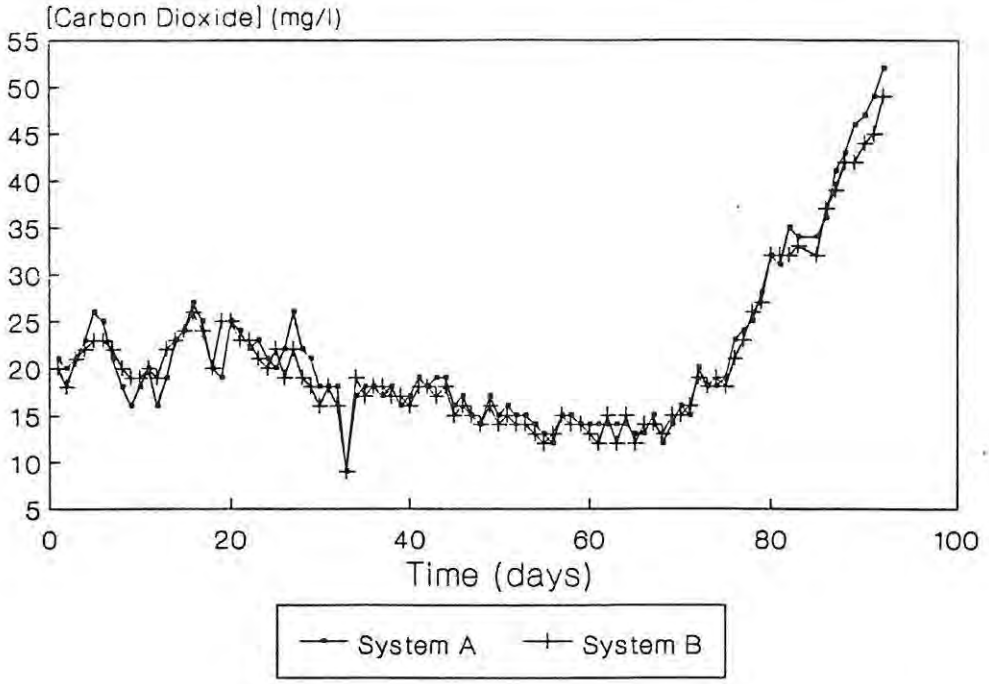

Figure 13: Changes in carbon dioxide concentrations in systems A and B during the study.

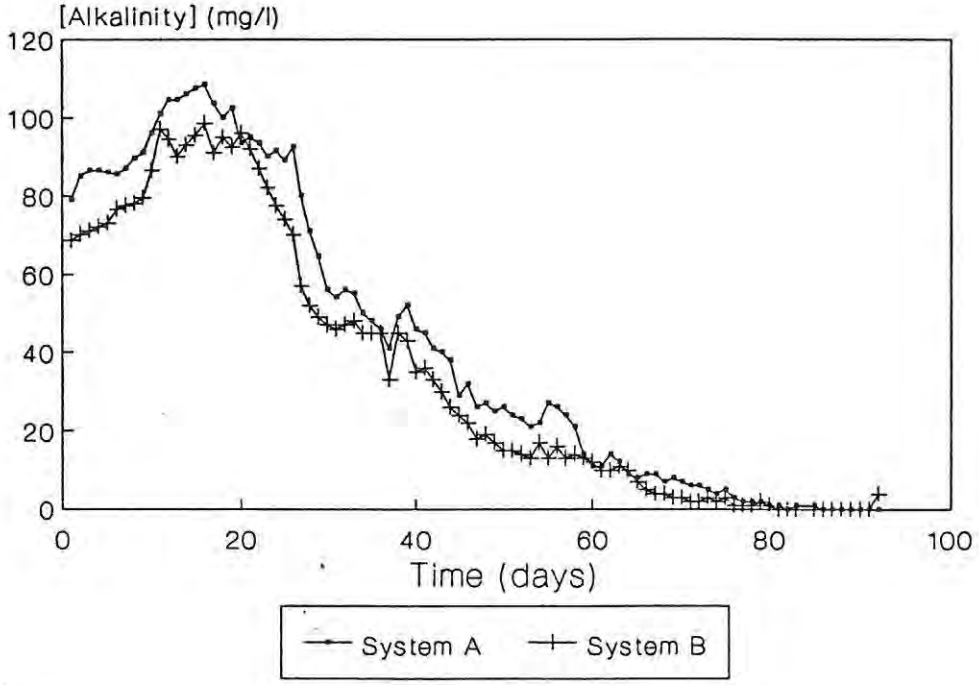

Figure 14: Changes in alkalinity concentrations in systems A and B during the study.

systems was probably the major cause of the rapid drop in pH during the same period.

vii) ALKALINITY

Alkalinity has been defined by Alabaster and Lloyd (1982) as "the acid-neutralizing capacity of a water; the abundance of compounds in a water which shift the pH to the alkaline side of neutrality". Piper et al. (1982) simply states that alkalinity refers to the ability of dissolved chemicals to accept hydrogen ions (H^+). The alkalinity value is also a measure of the ability of that system to resist changes in pH (i.e. the buffering capacity of the system) (Stickney, 1979). The cause of alkalinity (measured in mg/l of calcium carbonate, $CaCO_3$), is generally carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-).

Changes in alkalinity during the study are shown in Figure 14. In the first 15 days, alkalinity increased by approximately 25 mg/l in each system, before steadily dropping to zero by day 80. The initial increase corresponded with the rise in ammonia levels (Figure 8), which predominated in the system at this stage, and being basic in nature, is also a source of alkalinity (Hirayama, 1974; Boyd, 1979; Stickney, 1986). Once the nitrification process began, the major sources of alkalinity, carbonates and bicarbonates, readily reacted with hydrogen ions produced by nitrification to form carbonic acid (H_2CO_3), thereby reducing the concentration of the latter (H^+). When there is a high concentration of hydrogen ions (low pH), the concomitant removal of carbonate and bicarbonate result in a decrease in alkalinity (Stickney, 1979). Kaiser and Wheaton (1983) also state that for every 1 mg of ammonia that is oxidized, 7,14 mg alkalinity is

destroyed as a result of reaction with the H^+ ions generated by the oxidation of NH_4. Continual generation of hydrogen ions by nitrification processes throughout the study ultimately resulted in all the sources of alkalinity being removed from the system, to the extent where this value fell to zero by day 80.

viii) CALCIUM, (Ca^{++})

The tap water used to fill the system contained levels of 100-110 mg/l calcium (as $CaCO_3$) (Figure 15). In the first 15 days after the fish were introduced to the tanks, this level rose by approximately 20 mg/l. For the first 4-5 days after being introduced to the tanks, the fish were acclimating to the new conditions, and did not consume all the food that entered the systems. This uneaten food would have leached its nutrients, including calcium, into the water. It is therefore assumed that the initial rise in calcium was associated with this factor. Calcium concentrations in both systems then declined over the next two weeks. This loss of calcium from each system may have been due to assimilation of the mineral by the fish and populations of bacteria that were establishing themselves in the biofilter during this time. From day 60 to the end of the study, calcium concentrations approximately doubled, reaching 320 and 270 mg/l in systems A and B respectively. This increase coincides with a drop in pH and an increase in carbon dioxide (Figures 12 and 13).

From day 70 to the end of the study, calcium levels in system B were significantly lower than in system A, $F = 5,019$ ($p < 0,0302$) df (1;44).

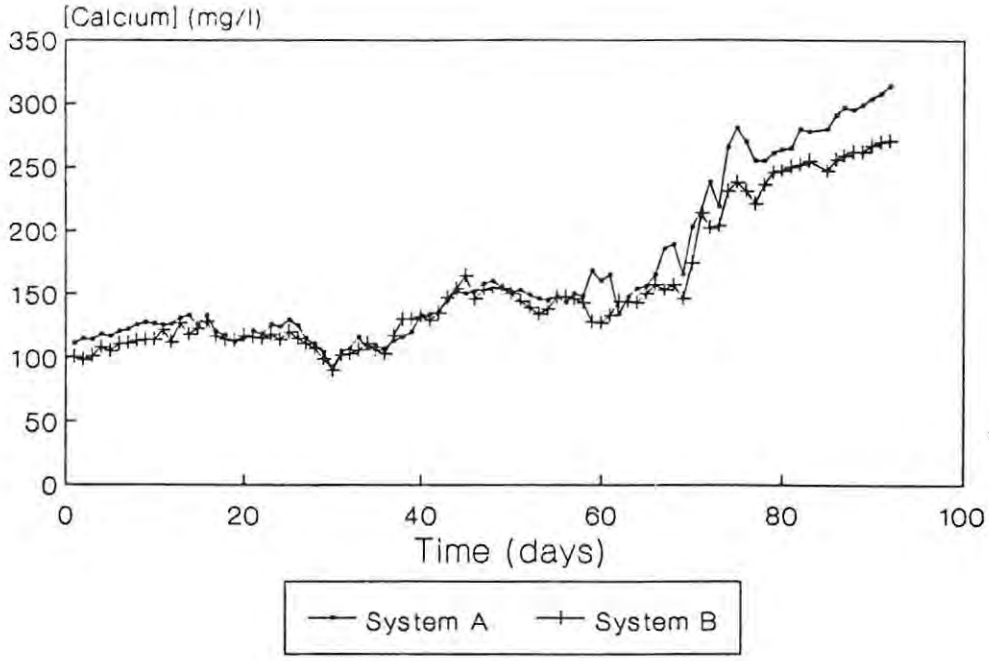

Figure 15: Changes in calcium concentrations in systems A and B during the study.

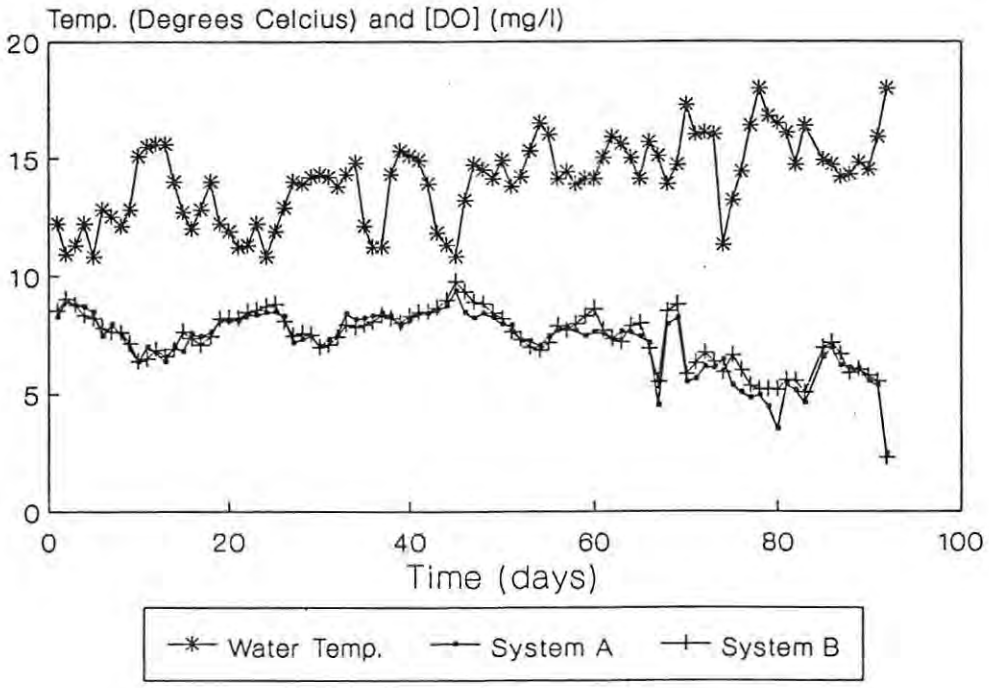

Figure 16: Water temperature, and changes in dissolved oxygen (DO) concentrations in systems A and B during the study.

ix) WATER TEMPERATURE AND DISSOLVED OXYGEN, (DO)

Water temperature and dissolved oxygen concentrations recorded in each system are shown together in Figure 16. A close inverse relationship exists between these two parameters (Spotte, 1970; Piper et al., 1982). This trend was also clearly shown in this study. Water temperature in the tanks fluctuated according to the ambient air temperature inside the hatchery, increasing slightly during the second half of the study. For the majority of the study, dissolved oxygen concentrations fluctuated between 5,5-9 mg/l. Towards the end of the study, when the systems were heavily stocked and the oxygen demand of the systems was high, dissolved oxygen measurements showed a gradual decrease in concentration.

On two occasions DO levels fell well below 5 mg/l, both times resulting in mortalities among the fish. The first case, on day 74, was caused by a power failure followed by an emergency generator failure during the night which put all the pumps, and compressor supplying the systems with air, out of order. This drop in DO is not shown on Figure 16 because oxygen levels in the systems had recovered within two hours, when the fault was discovered. The second time mortalities occurred, the compressor supplying supplementary air to the tanks and biofilter was disconnected for maintenance purposes after a breakdown. The oxygen demand being exerted by the biological filter organisms and the fish was so great that within half an hour 11% (52 out of 463) of the fish had died. Because of the overall poor state of the water quality at this point it was decided that all surviving fish be removed to a cleaner system outside the hatchery, and the experiment was terminated.

x) REDOX POTENTIAL

The redox (reduction-oxidation) potential of a solution is a measure of the proportion of oxidised to reduced substances in that solution (Boyd, 1979). It is a useful parameter to monitor because it gives an indication as to the level of biological activity within a system (Sander and Rosenthal, 1975).

It should be noted that the redox potential of aerated water (such as that used in this study) ranges from 450 - 520 mV, decreasing with DO (Boyd, 1979). The values recorded in the present study (0 - 100 mV) should only occur in non-oxygenated water with a high hydrogen sulphide concentration. The fact that DO never fell below 3 mg/l during this study suggests that the redox potential of the water should have been much greater than was actually recorded. The only explanation for this is that the digital tester pen used to record redox potential was faulty, but there was no way of checking this as it was pre-calibrated by the overseas manufacturers.

In view of the afore-mentioned problem, it may have been possible that even though the values obtained were incorrect, the general trends shown in redox potential during the study were subject to interpretation. The redox potentials in both systems exhibited similar trends (Figure 17), decreasing for the first 20 days before showing a general increase to the end of the study. During the first three weeks when the bacteria had not yet established themselves, there was a low proportion of oxidised material in the systems compared with levels of reducing agents present in the water (Brady and Humiston, 1975). Once the bacterial populations established and oxidation of ammonia and

nitrite commenced, the proportion of oxidised to reduced substances increased, as shown by the rise in redox potential for the remainder of the study. The drop in redox potential that was recorded in both systems over the last 10 days of the study may have been due to reduced bacterial activity in the biofilter. Because of the relatively poor quality of the water, in particular the low pH, the efficiency with which the bacterial populations oxidised the available substrate may have been impaired. This would explain the decrease in the redox potential of the water.

xi) TOTAL DISSOLVED SOLIDS, (TDS)

Total dissolved solids (TDS) accumulated slowly in both systems until approximately day 65, after which levels began to rise more rapidly as the study progressed (Figure 18). Ozonation trials began on day 59. There was an immediate decrease in TDS values recorded in the ozone system. This supports previous reports by Spotte (1970) that ozone can remove dissolved solids from water. There was still, however, an overall increase in TDS in both systems up to the end of the study. The concentration in system A showed a steady increase, while in system B the pattern consisted of sharp declines followed by equally marked rises. In the last five days of the study, when no ozonation trials were conducted, the TDS value in system B appeared to stabilize again. During the ozonation trials, it appears that TDS values only dropped when levels exceeded approximately 40 mg/l. This suggests that there is a threshold level below which ozone has no effect on dissolved solids. Further research would be necessary to substantiate this claim. TDS values recorded during the study did not constitute in any way to being a limiting factor or

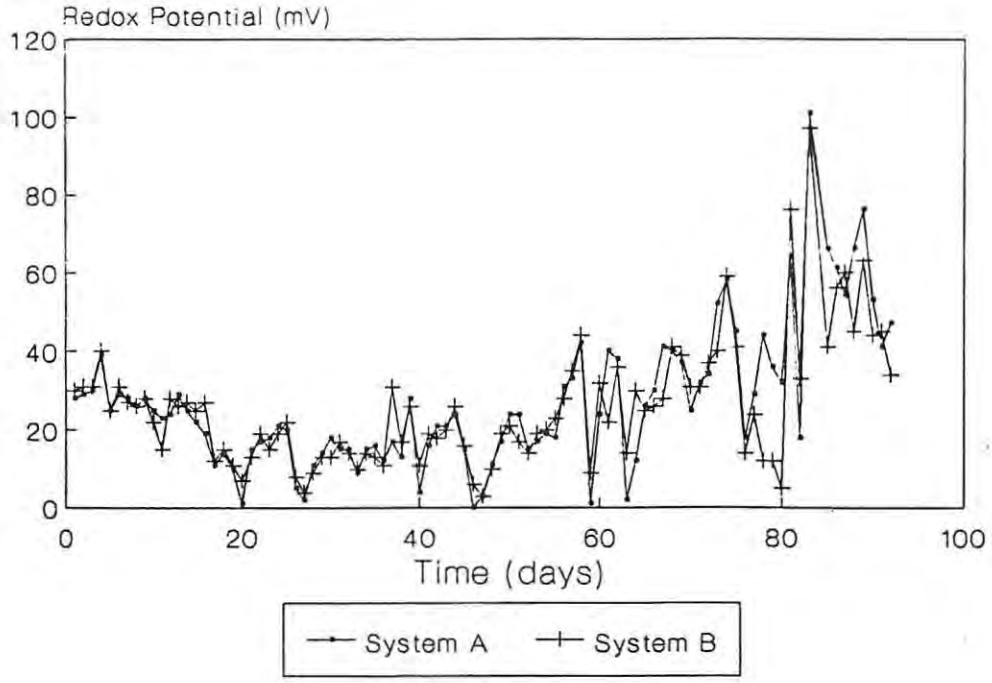

Figure 17: Changes in redox potential of the water in systems A and B during the study.

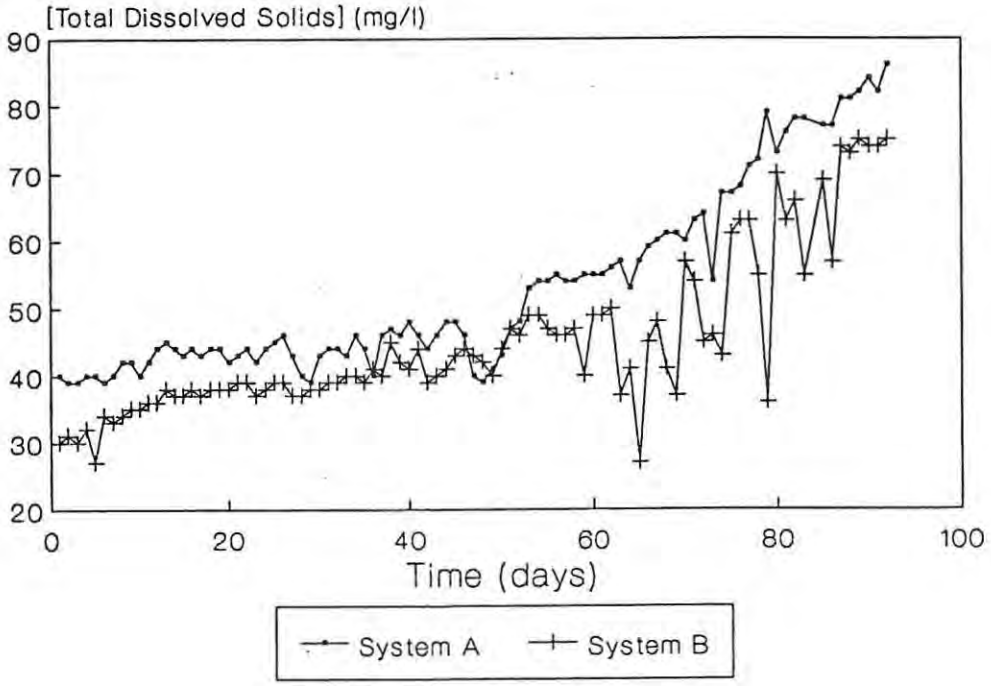

Figure 18: Changes in total dissolved solids (TDS) concentrations in systems A and B during the study.

health hazard to the culture animals. Meade (1989) states that fish can tolerate up to 400 mg/l dissolved solids.

xii) TURBIDITY

Turbidities, or suspended solids recorded in systems A and B through the study are illustrated in Figure 19. Low, stable values occurred throughout the study, with the exception of the last 10 days when turbidity in system A increased substantially. This rise could have been due to greater waste production by the densely stocked fish, and increased detritus formation by the faecal matter and dead bacteria from the biological filter. In addition, the water in system A was more discoloured than in system B. Colour also constitutes turbidity (Boyd, 1979).

During the last 20 days of the study, turbidities recorded in system B were significantly lower than those in system A, $F = 8{,}182$ ($p < 0{,}0064$) df (1;44). It is well documented that ozone reduces turbidity by bleaching coloured substances in water, and breaking down large particulate matter to a size that bacteria are able to process (Ciambrone, 1975; Murphy, 1975; Spotte, 1979; Williams *et al.*, 1982; Sutterlin *et al.*, 1984; Lohr and Gratzek, 1986). The levels of suspended solids, as with total dissolved solids, during this study did not attain concentrations great enough to be considered harmful to the fish. Alabaster and Lloyd (1982) state that there is no evidence that suspended solid concentrations less than 25 mg/l have any harmful effects on fish. Turbidity can be caused by any matter suspended in the water column, and may be biotic or abiotic (Boyd, 1979). Turbidity caused by phytoplankton is not harmful to fish, but Wallen (1951, *in* Boyd, 1979) demonstrated that turbidity caused

by clay colloids resulted in fish mortalities only at concentrations above 175000 mg/l. A problem arose in that turbidity in this study was recorded in Formazin Turbidity Units (FTU), and it was not possible to convert this standard measurement of turbidity to an equivalent value in mg/l. It appeared, however, that the turbidities attaained during the present study did not affect the fish in any way.

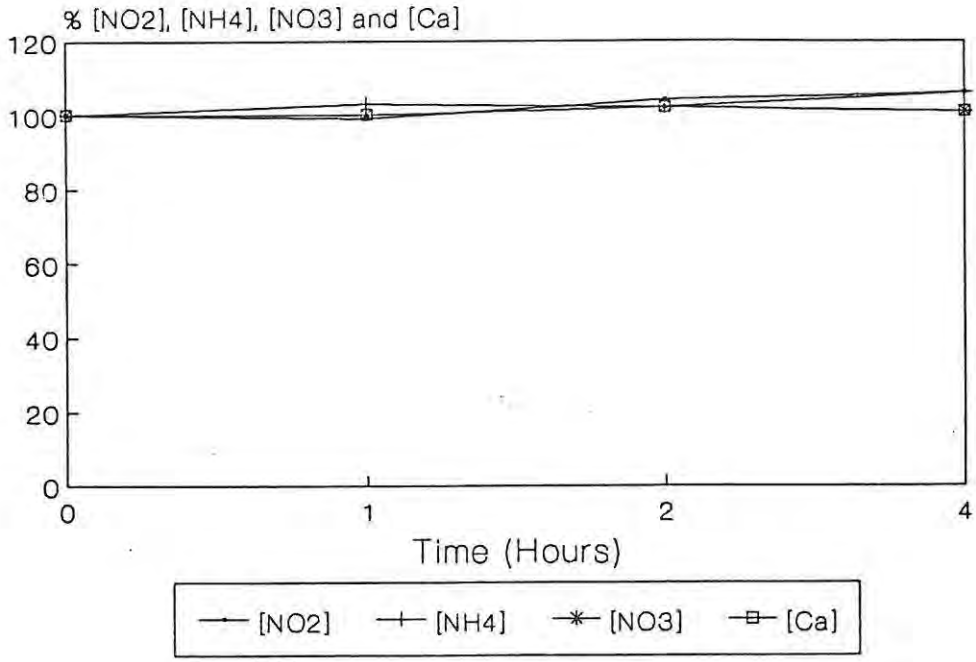

Figure 19: Changes in turbidity in systems A and B during the study.

EXPERIMENT II : THE EFFECT OF OZONE ON WATER QUALITY IN A RECIRCULATION AQUACULTURE SYSTEM

This experiment was performed to determine whether ozone treatment would enhance water quality and subsequently improve the production capacity of that system.

a) ONE-HOUR "LOW-DOSE" TRIALS, (DAYS 59 - 77)

Ozone concentrations measured in the tanks, immediately before entering and after leaving the contact chamber during the one hour ozonation trials (n = 18) are shown in Figure 20. Initial values at all three points began at approximately 0,01 mg/l. The manual for the Hach spectrophotometer used for this study states that any chlorine, bromine, iodine, chromate or oxidized manganese present in the water will react with the DPD (N,N-Diethyl-p-phenylenediamine) Total Chlorine reagent used in this test and read as ozone. To check the validity of this, samples were taken from the control system and subjected to the same test. Similar values of 0,008-0,01 mg/l were recorded in the absence of any ozone treatment, indicating that other chemical components were contributing to this basic value.

Within five minutes of treatment, a concentration of 0,05 mg/l dissolved O_3 was measured in the water leaving the contact chamber. With a total system residence time of approximately 40 minutes, it took a proportionate amount of time for all the water in the system to receive this initial pulse of treated water, although there would be considerable mixing of treated and untreated water as it passes through the system. During the second half of the trial, ozone concentrations continued to

increase after having stabilized for the previous 30 minutes. Its concentration then doubled over the next 25 minutes before falling once the treatment ceased.

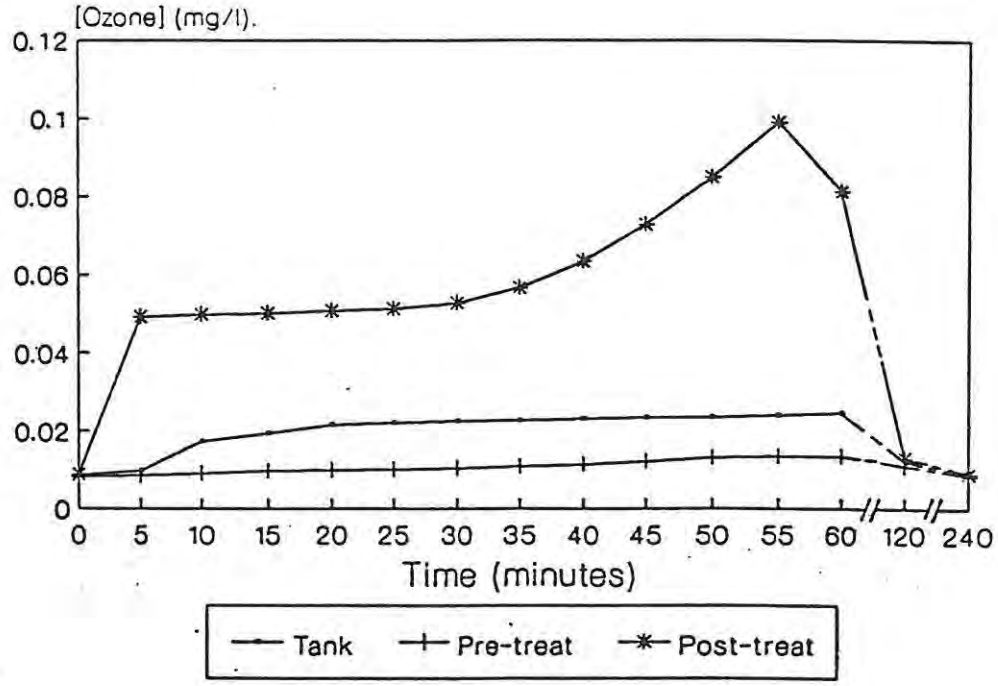

Figure 20: Ozone concentrations measured in the culture tanks, and immediately pre- and post-treatment in the contact chamber, during and after the one-hour, low-dose trials.

Throughout the hour-long treatment, there was no substantial increase in ozone levels in the water entering the contact chamber. It appeared therefore, that greater mass transfer of ozone occurred in the water that had previously been exposed to the gas, than in untreated water. A probable explanation for this is that in the initial pass, ozone was acting primarily on suspended solids in the water, because they are bigger and therefore more prone to the action of ozone. As such, suspended solids assert an ozone demand on the system, with ozone acting on larger particles in the water before dissolving and contacting

the smaller chemical components. In the second pass of water through the contact chamber, there is subsequently less extraneous matter to use up the ozone, so more is dissolved in the water. This accounts for the two-phase uptake of ozone by the water during the trial period.

Concentrations of residual dissolved ozone recorded in the culture tanks during the one hour trials increased by approximately 0,01 mg/l within ten minutes after which levels remained constant for the remainder of the trial. The reduction in ozone content of the water from post-treatment to the culture tanks indicated the efficiency of the activated carbon filter at removing residual dissolved ozone from the water. Ozone has a half-life of approximately 15 minutes (Colberg and Lingg, 1978; Gargas, 1989). At the ambient flow rate, it took less than five minutes for water to reach the tanks from the contact chamber. This was not long enough for appreciable dissociation of ozone to oxygen to occur. It was therefore assumed that the activated carbon filter was responsible for the removal of the majority of residual ozone. The rapid dissociation of ozone was evidenced by the decrease in dissolved concentrations in the water one and three hours after the trial finished. The concentrations of ozone to which the fish were directly exposed were well within the safe level of 0,1 mg/l suggested for salmonid fishes by Benoit and Matlin (1966).

During the one hour ozone experiments, dissolved oxygen (DO) concentrations measured in the tanks increased rapidly with the onset of ozone generation. Within 20 minutes, DO levels exceeded 20 mg/l, the maximum range of the probe used. Oxygen content of the water returned to normal levels within two hours of the trial being completed. During the time that the DO concentrations of

the water were above saturation levels, the fish did not exhibit any sign that they were suffering from oxygen supersaturation, or "gas bubble disease" as it is otherwise known. This has been reported as a major cause of mortality among fish involved in past ozonation research (Rosenlund, 1975; Spotte, 1979; Mr. M. Davies, pers. comm.).

Percentage changes in total ammonia, nitrite and nitrate concentrations during the one hour ozone experiments are shown in Figure 21a. A decrease in excess of 60 % of the nitrite in the ozone system occurred within one hour of ozonation. There was a slight increase in both ammonia and nitrate concentrations. In the control system (Figure 21b), all three parameters increased marginally over the trial period. In the absence of any other differing factor between the two systems, the decrease in nitrite can be attributed to oxidation by ozone, as previously reported (Honn and Chavin, 1976; Colberg and Lingg, 1978; Otte and Rosenthal, 1979; Sutterlin et al., 1984; Lohr and Gratzek, 1986). One-way analysis of variance showed that there was a significant difference between the level of nitrite and those of ammonia, nitrate and calcium after the one hour treatment, $F = 814,745$ ($p < 0,0001$) df (2;30). After one and three hours of ceasing ozone treatment, nitrite levels had increased to 65 % and 97 % of the original value respectively. There was no significant change in either ammonia, nitrate or calcium concentrations over this period, $F = 6,981$ ($p < 0,05$) df (2;30). There was also no change in total ammonia, nitrite nitrate or calcium concentrations measured in the control system, $F = 6,034$ ($p < 0,05$) df (2;30).

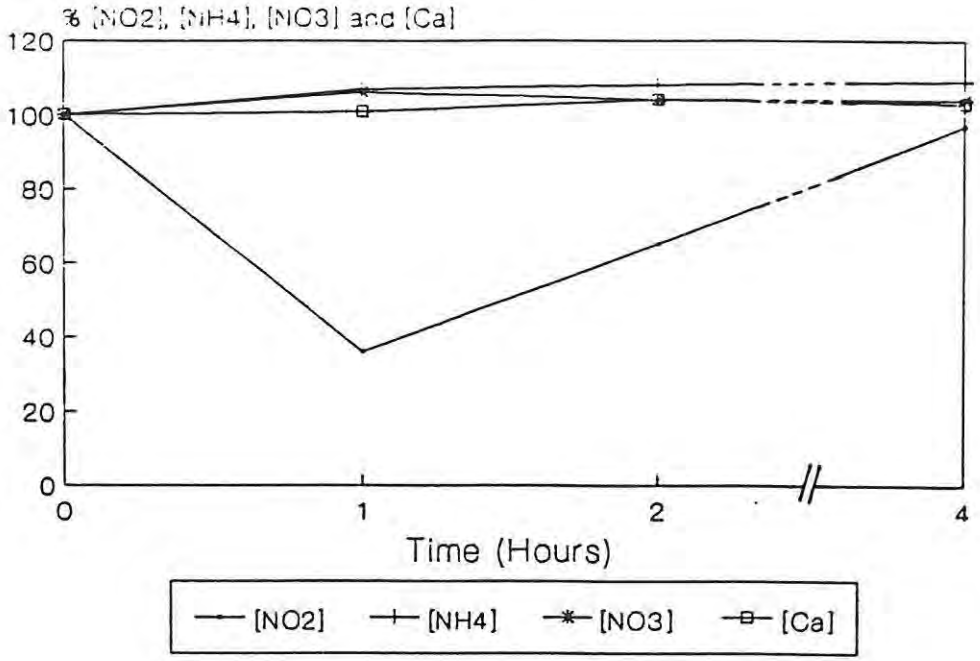

Figure 21a: Percentage changes in nitrite, total ammonia, nitrate and calcium concentrations during and after the one-hour, low-dose ozone trials in the experimental system B.

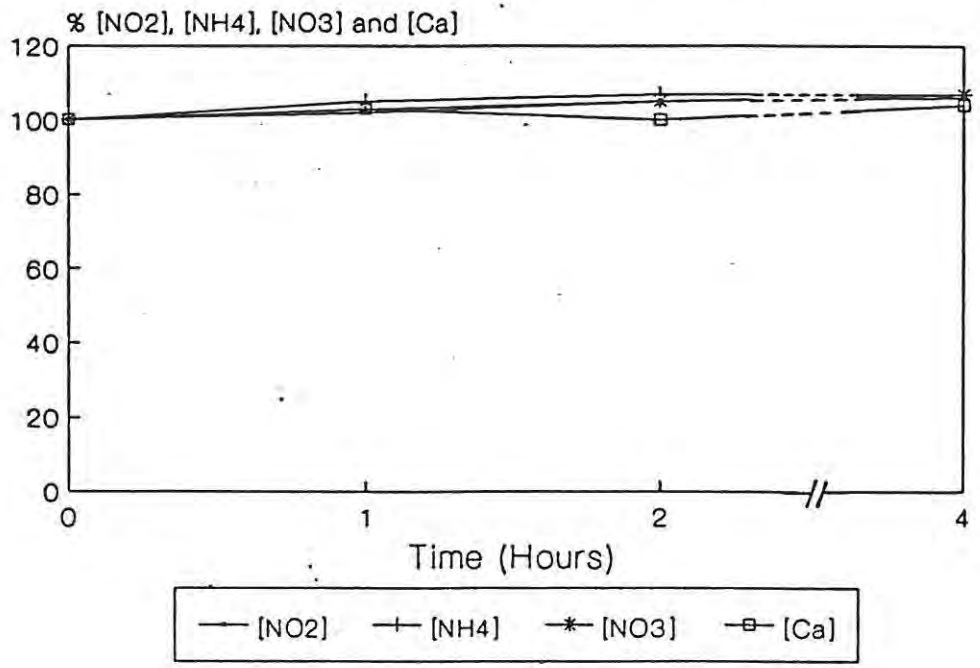

Figure 21b: Percentage changes in nitrite, total ammonia, nitrate and calcium during and after the one-hour, low-dose trials in the control system A.

During the first 20 minutes of the treatment, there was a rapid rise in the redox potential of the water (Figure 22). Thereafter the level remained constant. The general increase in redox potential supports the claims by Mangum and McIlhenny (1975), Sander and Rosenthal (1975) and Rosenthal (1981) that ozonation increases the redox potential in treated water. The higher the value for redox potential, the greater the rate of cell respiration (Sander and Rosenthal, 1975). This would suggest that both the fish and the bacteria in the filter would perform better, giving greater growth and higher yields per unit volume of water. Total dissolved solids concentrations decreased marginally during the trials (Figure 22), providing supporting evidence for the claims of Spotte (1979) and Rosenthal (1981) that ozone can reduce levels of TDS.

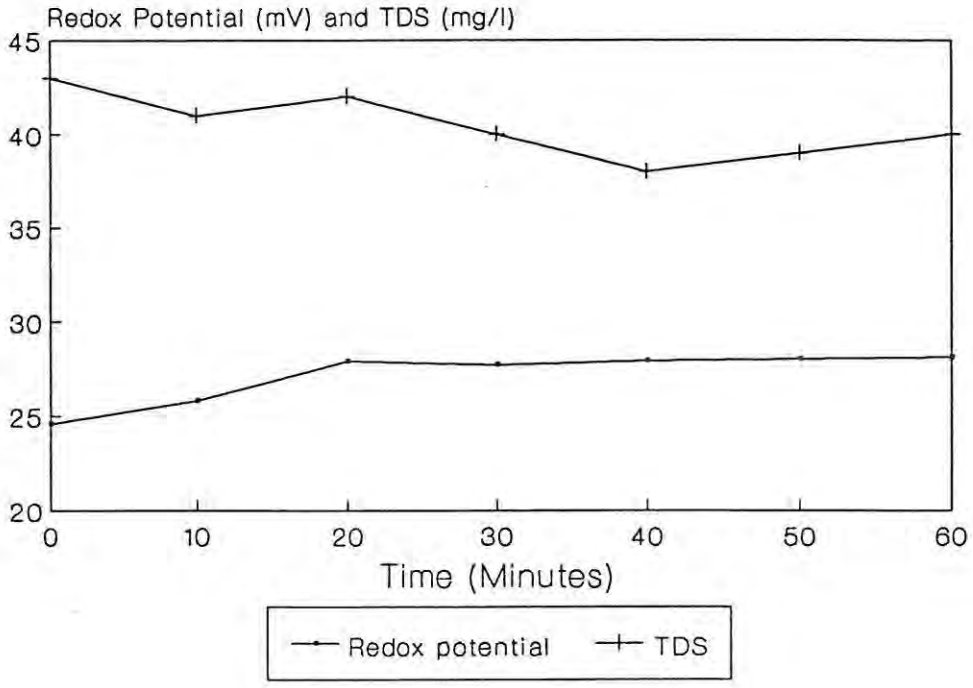

Figure 22: Changes in redox potential and total dissolved solids (TDS) during the one-hour, low-dose trials, in the experimental system B.

b) SIX-HOUR, "LOW-DOSE" TRIALS, (DAYS 78 - 80)

A similar trend to the initial one-hour experiments was observed in the results of the six hour ozone trials (n = 3). There was no significant difference in the trends exhibited by three replicates of this experiment, so it was not considered necessary to perform further replicates F = 0,197 ($p < 0,8150$) df (2), (Mrs. S. Radloff, Department of Mathematical Statistics, Rhodes University, pers. comm.).

Post-treatment ozone concentrations increased throughout the trial to 0,12 mg/l after six hours (Figure 23). In the previous experiments the levels of ozone in the water leaving the contact chamber after one hour contained approximately 0,1 mg/l O_3. In the six hour trials, this value was only obtained after 4 hours.

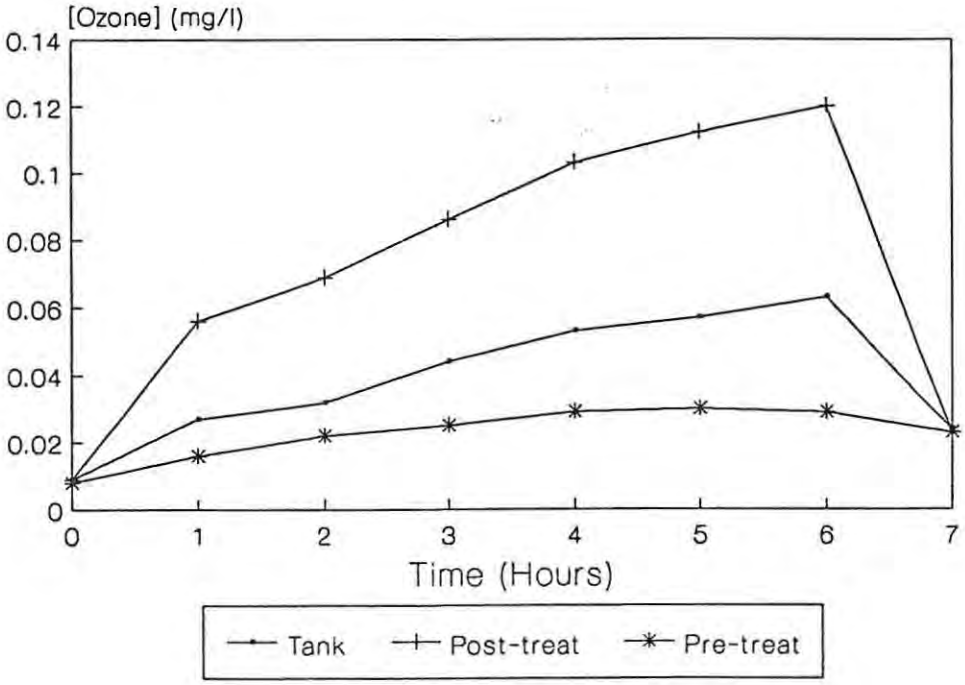

Figure 23: Ozone concentrations measured in the culture tanks, and immediately pre- and post-treatment in the contact chamber, during and after the six-hour, low-dose trials.

This anomaly can only be explained by a reduction in the production of ozone by the generator, because all other factors were the same in the two sets of trials. Residual dissolved ozone reaching the tanks by the end of the trial measured approximately 0,06 mg/l. This was still within safe suggested limits for rainbow trout. Confirmation of this was that no irritation, averse reaction or other of the effects described by Hubbs (1930), were observed among any of the fish in the ozonated system.

The difference between ozone concentrations recorded in the contact chamber and entering the tanks in this as with the previous trial, demonstrated the efficiency of activated carbon at removing residual ozone from the water. Pre-treatment ozone levels did not exceed 0,03 mg/l, indicating that in one pass through the system, the majority of excess ozone either dissociated to oxygen, was lost to the air or as stated, was removed by the activated carbon filter. One hour after turning the generator off, ozone concentrations throughout the system had almost returned to pre-trial levels.

Six hour ozonation treatments resulted in substantial removal of nitrite from the experimental system (Figure 24a). After five hours, only 14 % of the initial concentration was present. There was no effect on total ammonia, nitrate or calcium, $F = 1,216$ ($p < 0,05$) df (3;12). As before, nitrite levels increased after cessation of the six-hour ozone treatment, although in this case, concentrations remained reduced for up to 24 hours.

In the control system, total ammonia, nitrite, nitrate and calcium concentrations all increased slightly over the six hour trial, and through the following 24 hour period (Figure 24b).

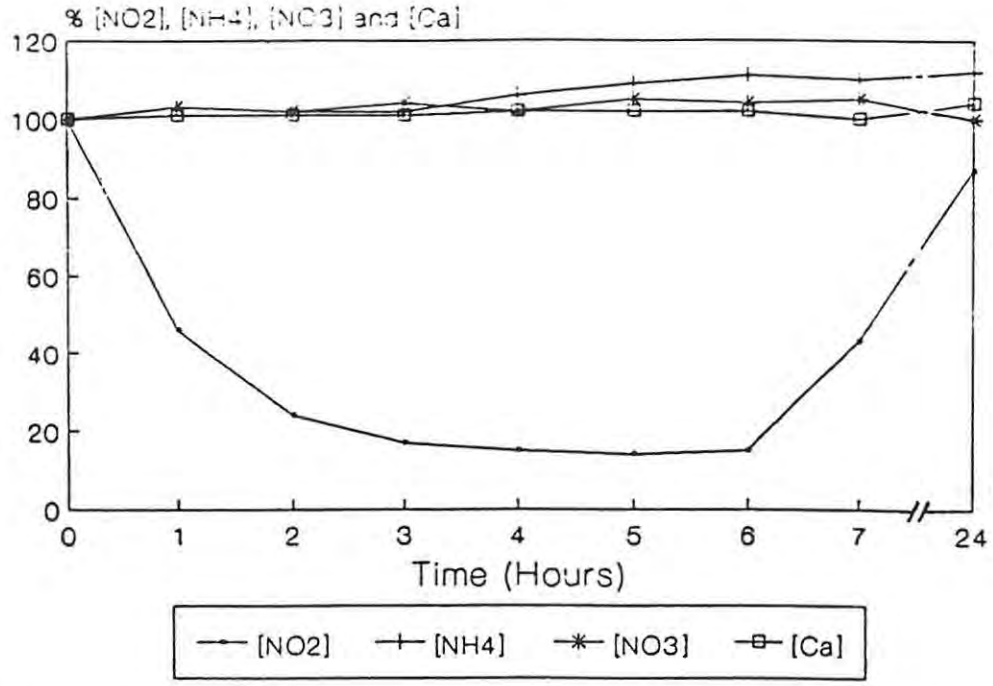

Figure 24a: Percentage changes in nitrite, total ammonia, nitrate and calcium concentrations during the six-hour, low-dose trials in the experimental system B.

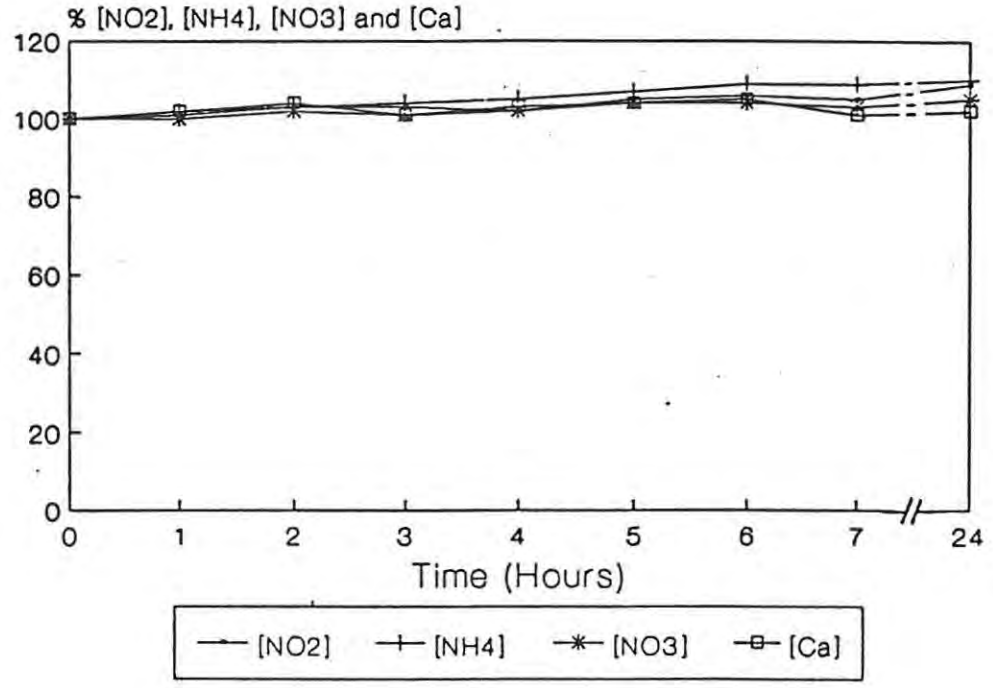

Figure 24b: Percentage changes in nitrite, total ammonia, nitrate and calcium concentrations during the six-hour, low-dose trials in the control system A.

These increases were not, however, significant, F = 3,885 (p < 0,05) df (3;12). As with the ozone system, such increases represent natural accumulations of these substances due to fish metabolism.

Redox potential and Total Dissolved Solids (TDS) in the experimental system show respective increasing and decreasing trends during the six hour trials (Figure 25). Being a powerful oxidising agent, ozone increases the proportion of oxidised to reduced substances within the ozonation system, hence the increase in redox potential. TDS levels decreased because of the ability of ozone to crack long-chain dissolved organics in water into their constituent components (Spotte, 1979; Rosenthal, 1981).

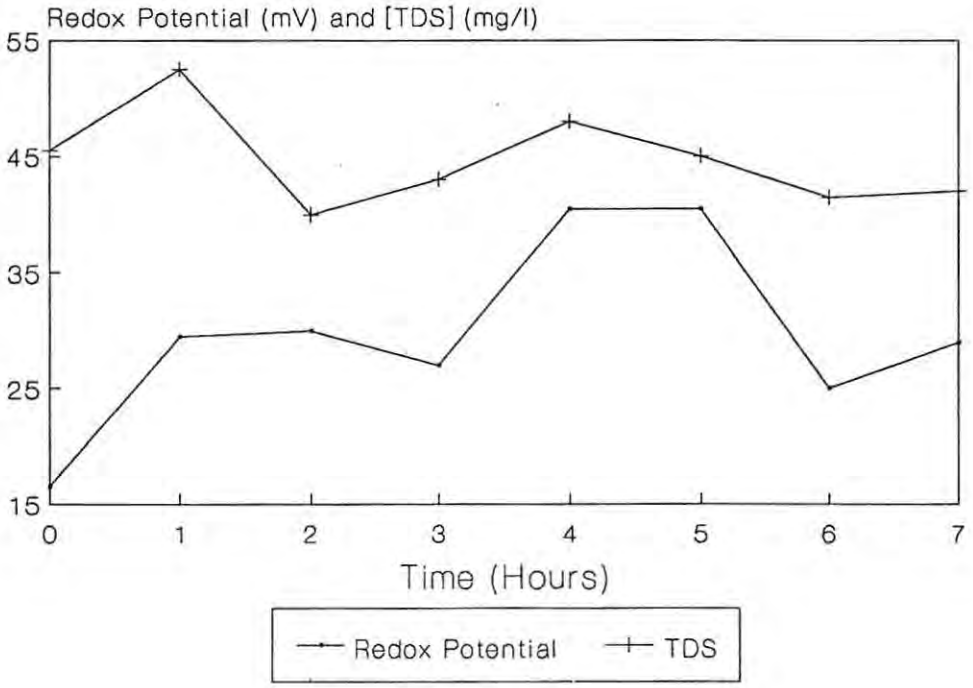

Figure 25: Changes in redox potential and total dissolved solids (TDS) during the six-hour, low-dose trials in the experimental system B.

c) ONE-HOUR, "HIGH-DOSE" TRIALS, (DAYS 81 - 83)

As mentioned in the materials and methods, it was not possible to increase ozone production due to a fault in the generator. Nevertheless, "high-dose" trials were performed, and the results shown here.

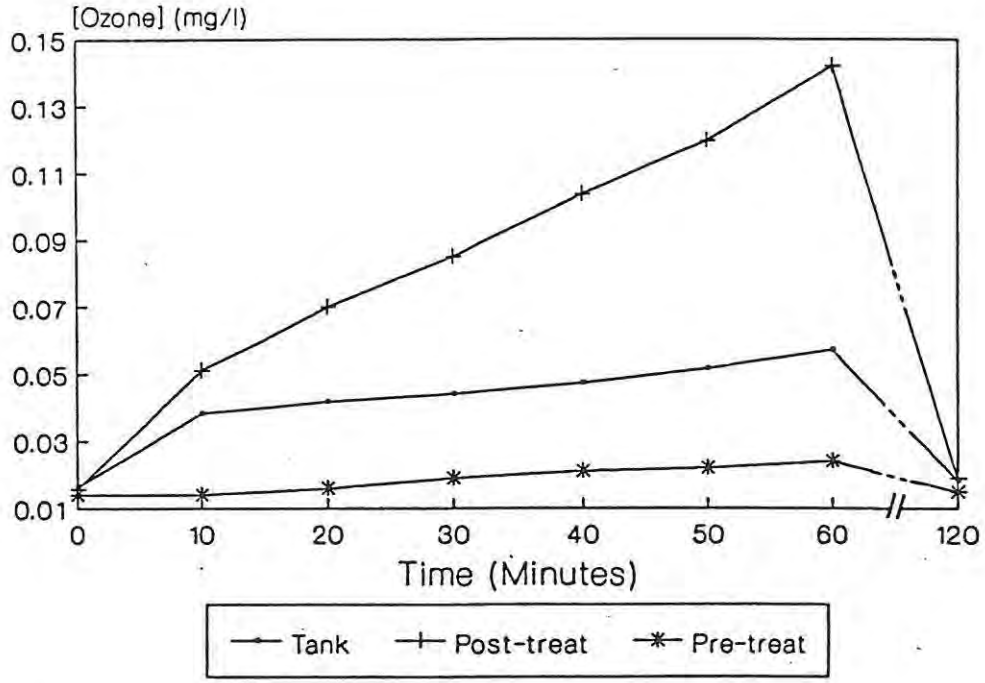

Figure 26: Ozone concentrations measured in the culture tanks, and immediately pre- and post-treatment in the contact chamber, during and after the one-hour, high-dose trials.

The results of the high-dose, one hour ozone trials were similar to the one-hour, "low dose" experiments. Ozone concentrations measured in water leaving the contact chamber attained levels only slightly higher than in the "low-dose" trials (0,14 mg/l as opposed to 0,10 mg/l) (Figure 26). These concentrations were not, however, significantly different in the two sets of experiments, according to a Student's t-test performed on ozone measured leaving the contact chamber in the two trials (t = -

2,031; $p < 0,0818$). Levels of residual ozone entering the tanks were much lower than post-treatment values, indicating the removal of excess gas by the activated carbon filter. Although still within safe limits for the fish, these concentrations were double those recorded in the first trials. This possibly indicates that the activated carbon was losing its capacity for ozone reduction, and would need replacing or regenerating if further ozonation trials were conducted. Pre-treatment ozone levels in these, as in the original set of trials, indicated that the majority of excess ozone was expelled in the first pass through the system. One hour after finishing the treatment, ozone concentrations throughout the system had almost returned to pre-experimentation levels.

In terms of the effects of ozone applications used in these trials on total ammonia, nitrite, nitrate and calcium, the results were once again similar to the first ozonation experiments. While total ammonia, nitrate and calcium were not significantly affected, nitrite concentrations decreased to 26% of the original level after one hour of ozonation (Figure 27a). This result was significantly different from the other three measured parameters, $F = 267,893$ ($p < 0,0001$) df (3;12). In the three hours following the treatment, nitrite gradually increased to 97% of the pre-trial concentration.

The control system showed no significant changes in levels of total ammonia, nitrite, nitrate or calcium monitored during the same period (Figure 27b), $F = 0,872$ ($p < 0,05$) df (3;12).

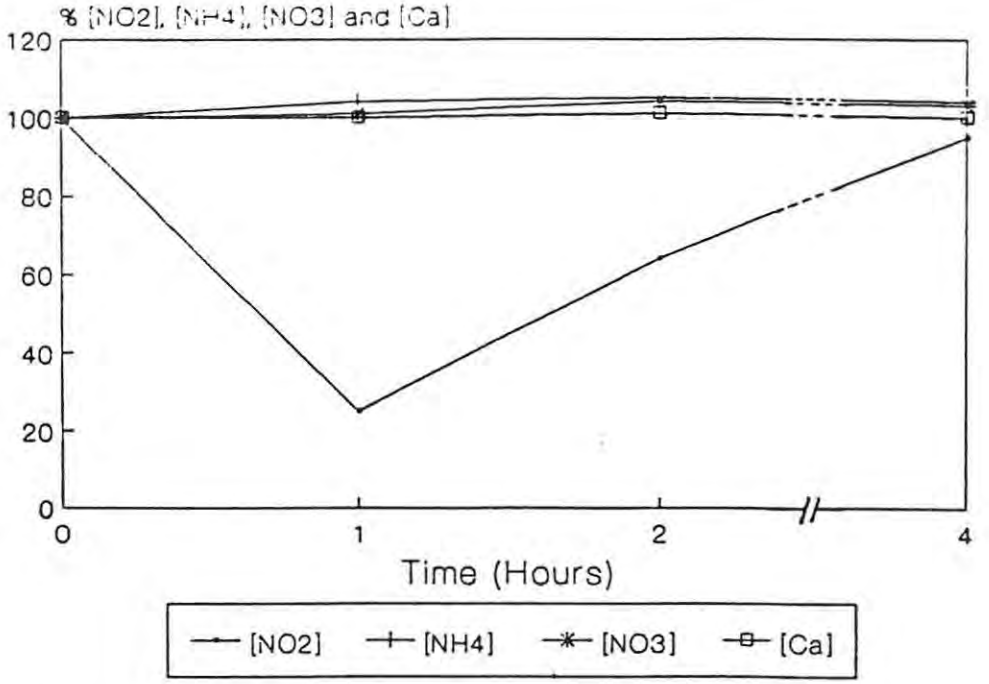

Figure 27a: Percentage changes in nitrite, total ammonia, nitrate and calcium concentrations during and after the one-hour, high-dose trials in the experimental system B.

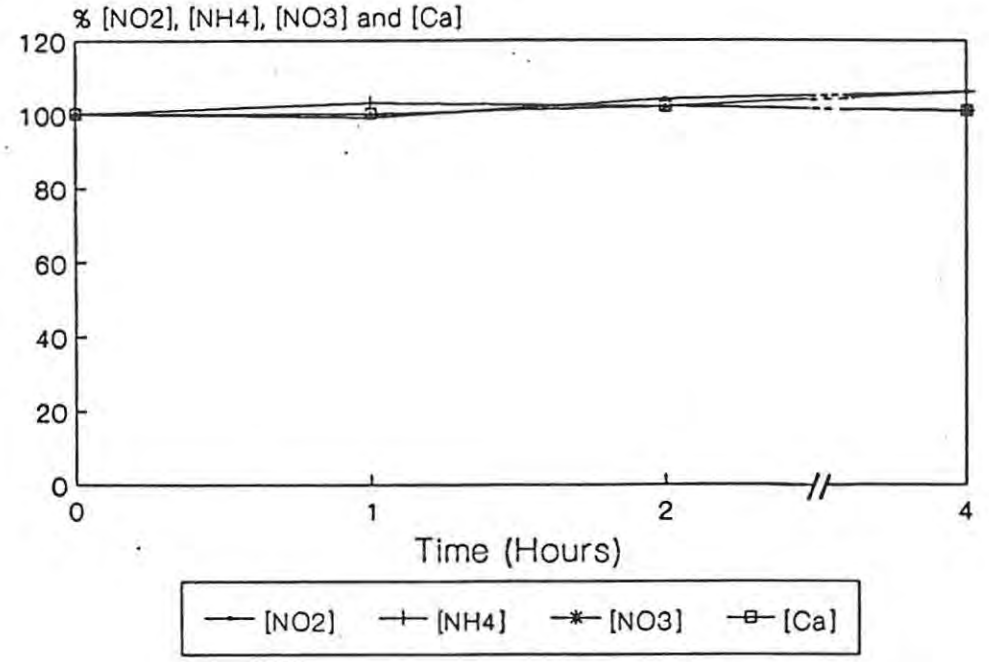

Figure 27b: Percentage changes in nitrite, total ammonia, nitrate and calcium concentrations during and after the one-hour, high-dose trials in the control system A.

Figure 28 shows the changes in redox potential and total dissolved solids (TDS) during the "high-dose" trials. The redox potential of the water dropped markedly during this period, which, according to previous reports, is not expected. TDS showed a slight decrease during the experimental period.

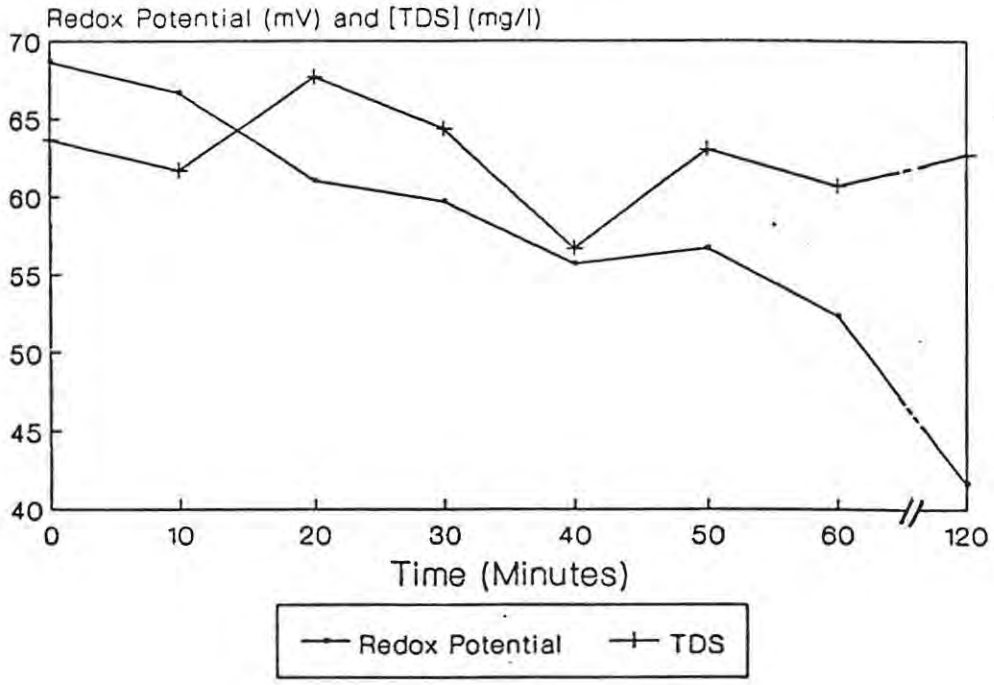

Figure 28: Changes in redox potential and total dissolved solids (TDS) during the one-hour, high-dose trials in the experimental system B.

d) ONE-HOUR, OXYGEN TRIALS, (DAYS 85 - 87)

Figure 29 shows total ammonia, nitrite, nitrate and calcium levels measured in the ozone system before and after one hour of oxygen application, at the same rate as was used in all of the ozonation trials. In the absence of ozone, there was no significant change in nitrite concentration during the treatment, compared to the other parameters measured, $F = 5,489$ ($p <$

0,05) df (3;12). This result eliminates the chance that excess oxygen, and not ozone causes the variations in nitrite concentration observed in the previous trials. There was no significant difference in the trends observed in three replicates, indicating that further trials were not required. F = 1,663 (p < 0,2663) df (2) (Mrs. S. Radloff, pers. comm.).

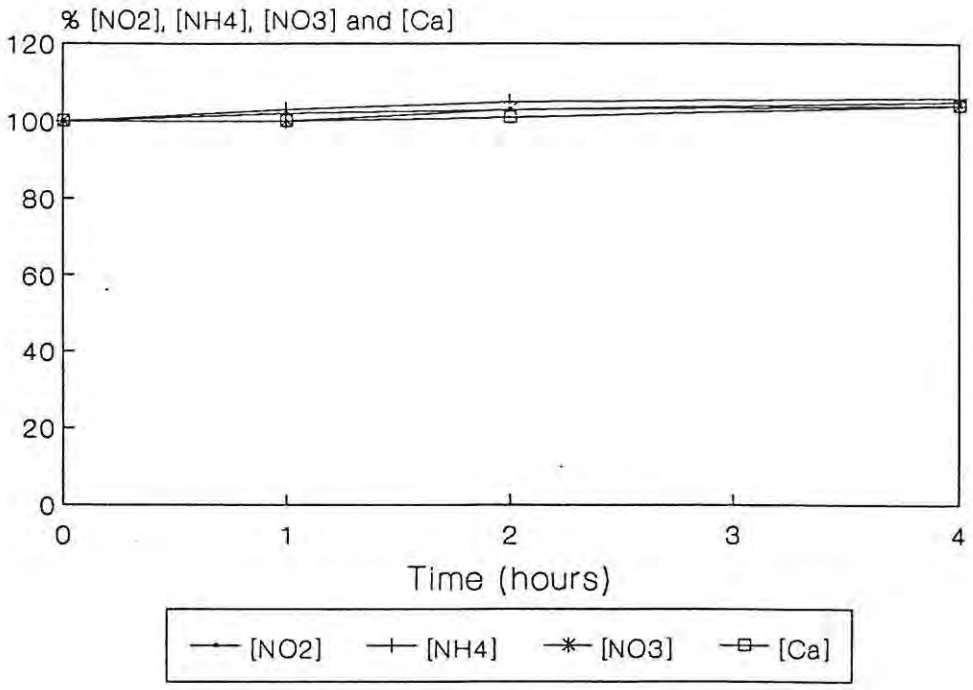

Figure 29: Percentage changes in nitrite, total ammonia, nitrate and calcium concentrations during the one-hour oxygen trials in the experimental system B.

FISH GROWTH AND SURVIVAL

At the end of the study, the fish in both systems were removed from the tanks and weighed. The mean initial and final mass of the fish in each tank are shown in Table 5. A one-way analysis of variance, in conjunction with Scheffe's multiple range test was performed on the individual masses of the fish in each tank. This indicated that there was no significant difference in mean mass of the fish held in the control and ozone systems at the completion of the experimental period, $F = 2,628$ ($p < 0.05$) df (3;458).

TABLE 5: Mean mass, growth rates, and mortalities of fish in each tank, during the study.

TANK NO.	SYSTEM A1	SYSTEM A2	SYSTEM B1	SYSTEM B2
DAY				
0 : Mean mass (g)	23,4	23,6	23,1	23,9
No. of fish	85	85	85	85
S.D.	3,1	3,5	3,2	2,9
92 : Mean mass (g)	71,4	72,8	65,0	70,0
No. of fish	110	112	121	119
S.D.	19,7	21,5	16,8	19,6
Total mass gain (g)	48,0	49,2	41,9	46,1
Growth rate (g/day)	0,52	0,53	0,46	0,50
Natural mortality	13	15	12	7
Mortality by equipment failure	17	24	38	27

DISCUSSION

EXPERIMENT I : WATER QUALITY SUCCESSION IN A RECIRCULATION AQUACULTURE SYSTEM

The operating efficiency of a water-recirculation system depends on the main function of the biological filter; that is, to facilitate the conversion of ammonia (NH_4-N) to nitrite (NO_2-N) by Nitrosomonas sp. and then to nitrate (NO_3-N) by Nitrobacter sp. bacteria (Carmignani and Bennett, 1977). The reason for this is that both ammonia and nitrite are toxic to aquatic animals (Liao and Mayo, 1972; Wickins, 1976; Heales, 1985). Hirayama (1974) claims that purification of water by microorganisms is the most important way of eliminating polluting substances. The process by which this biological detoxification of ammonia to nitrate occurs has been defined by Spotte (1970) as nitrification. Achieving the aims of this study relied primarily on the construction of systems that could operate and be maintained as efficient nitrification units.

The means by which the performance of the systems as nitrification units were adjudged, was by direct comparison with previous studies investigating this process. Changes in total ammonia, nitrite and nitrate concentrations in both systems (Figs. 8, 10a and 11) during the initial stages of operation showed similar trends to those in earlier nitrification studies (Liao and Mayo, 1972; Forster, 1974; Hirayama, 1974; Collins et al., 1975; Carmignani and Bennett, 1977; Mevel and Chamroux, 1981; Kaiser and Wheaton, 1983; van Rijn, Diab and Shilo, 1984; Heales, 1985).

The changes in water quality that occur in a new, recirculating culture system follow a sequence of events: initially the biological filter does not support enough bacteria to purify the polluting metabolic substances (ammonia) produced by the fish, and so they (the pollutants) accumulate within the system (days 4-20, Fig. 8). Populations of the nitrifying bacteria Nitrosomonas build up, using the ammonia as an energy source, until a point is reached where the bacterial population is in equilibrium with the substrate input, and ammonia levels decrease rapidly to negligible levels (days 20-30, Fig. 8). Nitrite, produced by the biological oxidation of ammonia, then begins to accumulate (days 15-40, Fig. 10a) in the absence of nitrite-oxidising bacteria, Nitrobacter, until this population is established and in equilibrium with nitrite production. Consequently, the levels of this substance fall (days 35-45, Fig. 10a), and are replaced by rising nitrate concentrations (Fig. 11).

It took 40 - 44 days for the total ammonia and nitrite concentrations to be stabilized at relatively low levels (below 0,1 mg/l). This indicated that the systems were fully conditioned and established for extensive culture purposes (Spotte, 1970; Hirayama, 1974; Heales, 1985). This pre-conditioning period corresponds with the lengths of pre-conditioning phases reported in similar circumstances: at least 28 days (Forster, 1974), 40 - 60 days (Hirayama, 1974), 28 - 56 days (Liao and Mayo, 1974) and 33 days (Collins et al., 1975). The rate of establishment of bacterial populations responsible for nitrification increases with temperature (Spotte, 1970; 1979; Wheaton, 1977; Kaiser and Wheaton, 1983). The conditioning times reported by Forster (1974), Hirayama (1974) and Collins et al. (1975) are slightly shorter than in this present study because

the temperatures at which their research was undertaken were considerably higher (20 - 26 degrees Celsius vs. 11 - 15 degrees C).

As stated previously the need for removing ammonia and nitrite from culture water results from their known toxicity to aquatic animals.

Only the un-ionized form (NH_3) of total ammonia is poisonous (Spotte, 1970; Smith and Piper, 1975; Boyd, 1979; Stickney, 1979; Alabaster and Lloyd, 1982; Piper et al., 1982; van Rijn et al., 1984; Lucchetti and Gray, 1988; Tidwell and Webster, 1991). The concentration of NH_3 is highly dependant on, and increases with water temperature and pH (Liao and Mayo, 1972; Emerson et al., 1975; Wickins, 1976; Tomasso, Goudie, Simco and Davis, 1980; Alabaster and Lloyd, 1982). Elevated levels of total ammonia are most commonly recorded during the initial stages of nitrification in a recirculating system. It is therefore during this period that culture animals are most susceptible to poisoning by high un-ionized ammonia concentrations.

The 96-hour LC_{50} for rainbow trout ranges from 0,16 - 1,1 mg/l NH_3 for fry (<0,1 g) to adults (2.6 kg) respectively (Thurston and Russo, 1983). Alabaster and Lloyd (1982) reports that the lowest lethal concentration shown for salmonids is 0,2 mg/l, although other adverse effects caused by prolonged exposure are absent only at levels below 0,025 mg NH_3/l. Un-ionized ammonia concentrations recorded in this study exceeded this level for a maximum of only two days in either system (days 15-17, Fig. 9), which cannot be considered prolonged exposure. It can be said therefore that ammonia would not have been toxic during this study. This is borne out by the fact that no morbidity or

mortality among the fish was observed during the period that unionized ammonia levels were at their peak.

Conversely, nitrite concentrations reached levels (43 mg/l and 35 mg/l in systems A and B respectively) well in excess of those described as safe. Liao and Mayo (1972) state that nitrite (NO_2-N) levels in excess of 0,2 mg/l are toxic to salmonid fishes. In 1974 the same authors showed mortalities of rainbow trout at 0,15 mg/l NO_2-N. Russo, Smith and Thurston (1974) report that 96-hour LC_{50} nitrite values for rainbow trout range from 0,19 - 0,39 mg/l. Smith and Williams (1974) noted that 0,55 mg/l nitrite caused 55% mortality within 24 hours among yearling rainbow trout, and Brown and McLeay (1975) observed similar mortalities at 0,23 mg/l nitrite in 96 hours for fingerlings of the same species. 96-hour LC_{50} nitrite values for salmonids have also been obtained by Thurston et al. (1978) (0,5-0,6 mg/l), Wedemeyer and Yasutake (1978) (0,5-0,9mg/l), and Russo, Thurston and Emerson (1981) (0,19-0,41 mg/l). Peak concentrations attained in the present study were 215 and 175 times higher than a safe recommended value of 0,2 mg/l NO_2-N, in systems A and B respectively. It was therefore not surprising that regular mortalities were recorded in both systems during the time that NO_2-N concentrations were above this level (0,2 mg/l). Of the total natural mortality (Table 5) recorded in systems A and B throughout the study, 60 % (17 fish) and 74 % (14 fish) respectively occurred during this period of elevated nitrite concentrations (days 16 - 43). Because the nitrite levels were so high above LC_{50} values for such an extended period, it might be expected that mortalities would have been far greater. A probable reason for this is the protective effect that high calcium levels have on nitrite toxicity, as shown by Crawford and Allen (1977). The calcium content of water varies greatly

depending on its source (Boyd, 1979). Compared to other examples, the calcium concentration in the water used in the present study seems relatively high (100 mg/l). Furthermore, this value increased as the study progressed, reaching between 250-300 mg/l by the end of the project. Crawford and Allen (1977) showed that a calcium concentration of 125 mg/l eliminated the acutely toxic effects of 100 mg/l nitrite on chinook salmon fingerlings. Calcium levels ranged from between 100 - 140 mg/l during the initial period of elevated nitrite concentrations, therefore it is possible that even though the LC_{50} value for nitrite on rainbow trout were exceeded during this period, mortality due to nitrite toxicity was greatly reduced due to the protective effect of calcium.

Wedemeyer and Yasutake (1978) suggest that the variation in LC_{50} values for nitrite obtained by different researchers might be caused by differences in size and species of salmonid used, as well as by water chemistry effects, such as the protective effect of the chloride ion on nitrite toxicity, as shown by Perrone and Meade (1977). Other factors such as increasing total hardness or pH (Wedemeyer and Yasutake, 1978) have also been shown to reduce nitrite toxicity, and may have influenced the results of previous nitrite toxicity studies. None of these conditions prevailed during the initial period of high NO_2-N concentrations in this study and therefore would have had no effect on the level at which nitrite became toxic to the fish.

During the period when nitrite concentrations were above 0,2 mg/l, symptomatic indications of death of a fish followed a regular sequence of events. The fish became darker in colour and swam close to the surface. These weaker fish also moved to the tail-end of the tank where turbulence and general physical

competition with other fish was not as strong as where the water entered. Rapid opercular movement indicated that the fish were suffering respiratory stress, even though oxygen levels in the tanks was sufficient (>5 mg/l, Fig. 16). The fish finally died with their mouths and operculae wide open. Some of the dead fish were examined and it was found that the blood of these animals was a darker, brown shade compared to that of a healthy fish. The symptoms shown by the fish that died in this study (laboured breathing, asphyxia, swimming near the surface and chocolate-coloured blood) are similar to those described by Smith and Williams (1974) and Collins et al. (1975). Methaemoglobin is produced by the oxidation of the iron in haemoglobin, from the divalent (Fe^{++}) to the trivalent (Fe^{+++}) form, by nitrite (Smith and Williams, 1974; Collins et al., 1975; Stickney, 1979). It is chocolate-brown in colour, and is incapable of transporting oxygen (Crawford and Allen, 1977; Colt, Ludwig, Tchobanoglous and Cech Jr., 1981). Stickney (1979) observed that catfish that died of nitrite poisoning did so with open mouths. Both Westin (1974) and Bohl (1977) observed that fish exposed to high nitrite levels became dark in colour. If all these factors are considered, it may be assumed that the mortalities observed in the two systems between days 16 and 43 be attributed to nitrite poisoning.

During the latter stages of the study (days 70 - 92), nitrite levels again exceeded 0,2 mg/l (Fig. 10b), but there were no mortalities among the fish that could be attributed to nitrite poisoning. This period coincided with levels of calcium (180->280 mg/l, Fig. 15) that are considered high for freshwater systems (Boyd, 1979). Crawford and Allen (1977) showed that the toxicity of nitrite to chinook salmon fingerlings was inhibited when calcium was added to freshwater. In addition, they showed that mortality due to nitrite was greater in calcium-free

artificial seawater than in normal seawater, and that adding calcium to the former decreased toxic effects. The elevated calcium levels in the final phase of this study could be the reason why no nitrite-initiated mortalities were recorded.

The increase in nitrate (NO_3-N) levels in recirculating systems is another indication that nitrification is occurring normally (Spotte, 1970; 1979; Collins et al., 1975; Wheaton, 1977; Boyd, 1979). This trend was clearly shown in both systems (Fig. 11). There are certain bacteria that convert nitrate to gaseous nitrogen which then escapes to the atmosphere (Stickney, 1979). In larger recirculating systems, nitrates may be assimilated by algae in holding tanks associated with the biological filter. Despite these measures nitrate concentrations still accumulate, but because it is relatively non-toxic (Hirayama, 1974; Spotte, 1970; 1979; Wheaton, 1977; Brown and Gratzek, 1980), it does not pose a threat to aquatic animals in recirculating systems. Meade (1985) stated that nitrate concentrations in excess of 400 mg/l are not lethal to rainbow trout.

A decline in pH and alkalinity has also been shown to occur during the conditioning of a new recirculation system (Hirayama, 1974; Collins et al., 1975). The fall in pH is mainly due to the production of hydrogen ions during the biological oxidation of ammonia to nitrite, shown by Haug and McCarty (1972) (see results). Although the alkalinity rose initially (Fig. 14), the trend after days 15 - 20 was one of general decrease. The preliminary rise was due to the predominance of ammonium ions and associated dissociation products of ammonia in water, which constitute a source of alkalinity (Hirayama, 1974; Boyd, 1979; Stickney, 1986). As nitrification proceeded however, the hydrogen ions would react with the major source of alkalinity,

carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) (Spotte, 1970; Piper et al., 1982), to produce carbonic acid and ultimately carbon dioxide. The reaction by which this occurs is:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-}$$

This both uses up the sources of alkalinity, if they are not replaced, and further accelerates the decline in pH, due to the presence of increased carbonic acid and CO_2 levels. If required, carbonate and bicarbonate levels can be restored by adding a source of calcium carbonate ($CaCO_3$) to the water (Spotte, 1970; Boyd, 1979; Brown and Gratzek, 1980). Although maintaining the pH and alkalinity of the water within recommended ranges (pH 6,5-8; alkalinity 10-400 mg/l, Meade, 1989) by this method is recommended for aquaculture purposes, this was not done in the present study because it would have interfered with the natural progression of changes in water quality that were being monitored.

Addition of calcium carbonate, for example, would have altered concentrations of calcium (measured as $CaCO_3$) recorded during the study, and the natural trends in variation of calcium levels in a recirculating system would not have occurred. Calcium is a very important dietary component of both bacteria (Kaiser and Wheaton, 1983) and fish (Wheaton, 1977; Tucker and Boyd, 1985; Lovell, 1989). Bacteria use calcium primarily for cell wall formation while fish require it for tissue and bone formation and maintaining osmotic balance. While the fish receive some of their calcium requirements directly from their feed, they are able to absorb most of their dietary calcium needs directly from the water, through their gills (Lovell, 1978; 1989; Brown and Gratzek, 1980).

The initial rise in calcium (days 0 - 15, Fig. 15) occurred while the fish were still acclimating to the new systems, and it was noticed that not all the food ration was consumed. Uneaten food would have leached its nutrients (including calcium), (see Table 3), into the water. It is therefore assumed that the initial rise in calcium was associated with this factor. The subsequent decrease in calcium concentrations over the next two weeks may have been due to assimilation of the mineral by the fish, and populations of bacteria that were establishing themselves in the biofilter during this time. The rapid rise in calcium levels from day 60 to the end of the study coincides with a drop in pH (Fig. 12), an increase in carbon dioxide (Fig. 13) and a very low alkalinity value (Fig. 14).

McDonald and Wood (1981) showed in laboratory studies that in salmonid fish exposed to low pH, urinary excretion of calcium and loss of surface bound calcium at the gills increases. In addition, by reacting with free carbon dioxide and water as acidity increases due to biological oxidation processes, calcium and other mineral carbonates are brought into solution (Spotte, 1970). The dissociation products of this reaction are bicarbonate ions and free calcium. Both these factors may explain the increase in calcium measured during this period. Furthermore, Lovell (1977b, in Stickney, 1979) showed that channel catfish appeared to lose the ability to take calcium from the water when the alkalinity was extremely low, although the actual values were not given. When calcium levels started to rise in the present study on day 70, the alkalinity in each system was less than 10 mg/l (Fig. 14). It is possible that like channel catfish, rainbow trout are unable to absorb calcium from water at low alkalinities, consequently resulting in a rise in calcium levels in the water.

High calcium levels would also have a beneficial effect on the water because it reduces the toxic effects of ammonia (Tomasso et al., 1980), nitrite (Crawford and Allen, 1977; Stickney, 1986) and pH (Alabaster and Lloyd, 1982). As discussed earlier, ammonia toxicity was not considered a problem during the latter stages of the study, because of the minimal un-ionized ammonia concentrations that exist at the low pH which prevailed at the time. Nitrite concentrations were, however, rising above safe recommended levels of 0,2 mg/l. The high calcium content of the water may have played an important role in countering the lethal effects of this toxin. It may have also reduced any harmful effect that the low pH might have been having on the fish.

The difference in calcium concentrations recorded in the two systems from day 70 onwards (Fig. 15) was shown to be significant. Although this occurred during the ozonation experiments, the ozone treatment would have no effect on the calcium levels, because as a divalent ion, Ca^{++} is in its highest oxidation state (Dr. R.B. English, Chemistry Department, Rhodes University, pers. comm.). After a review of the available literature, and personal communication with professional chemists, no explanation for this difference could be found, and as yet remains unexplained.

Artificially increasing the carbonate concentrations in the water would also have directly affected carbon dioxide concentrations (see reaction on page 77, this chapter). By adding carbonate ions to the system the reaction would have been pushed further to the left, resulting in increased carbon dioxide formation. The three peaks in CO_2 levels on days 5, 16 and 28 (Fig. 13) correspond with the initial increases in total ammonia, nitrite

and nitrate concentration. These peaks may be related to increases in aerobic bacteria respiration as they respond positively to the increased energy supply. This is confirmed by Spotte (1970; 1979) who states that bacterial oxidation processes increase carbon dioxide production. The single low value recorded on day 33 must be ascribed to a sampling error because there was no apparent reason why the CO_2 level should drop for just one day before returning to previous levels.

Once the systems were fully conditioned (approximately day 50-55), the culture animals and bacteria were in a state of equilibrium in their environment, carbon dioxide concentrations stabilized (days 30 - 70). On day 70, when additional fish were added to the tanks to increase the bioload, carbon dioxide levels began to increase substantially, reaching over 50 mg/l on day 92. This could be directly associated with increased respiration of the fish in the tanks, due to the larger number of fish and stress among them, resulting from the increasingly substandard water quality as a consequemce of the system overload. In addition, the respiratory rate of the aerobic bacteria throughout the system would increase as they attempt to process the excess supply of nitrogenous waste from the fish. This rise in CO_2 concentrations in both systems was probably the major cause of the rapid drop in pH during the same period, due to the formation of carbonic acid and hydrogen ions in water.

Carbon dioxide is known to be toxic to fish. The exact levels at which CO_2 affects fish does not seem clear however. Piper et al. (1982) put the safe minimum level at 10 mg/l. Brown and Gratzek (1980) state that carbon dioxide can reach 30 mg/l in heavily stocked aquaria without harming the fish if oxygen levels remain suitable. As long as dissolved oxygen levels are high, Boyd

(1979) claims that fish can only tolerate up to 10 mg/l, although most species can survive in water containing up to 60 mg/l CO_2. In well aerated water, carbon dioxide levels can reach 100 mg/l before becoming toxic to rainbow trout (Alabaster and Lloyd, 1982). High carbon dioxide levels have, however, been shown to reduce survival times of fish in waters with pH values not normally considered lethal. In the present study, CO_2 concentrations of up to 50 mg/l (Fig. 13) did not appear to affect the fish directly in any way. With the low pH, and other harmful chemical factors reaching known toxic proportions in both systems, the observed levels of CO_2 may however have contributed to stress and susceptibility of fish to these factors. A common factor in previous carbon dioxide toxicity studies has been the need for water to be well aerated if CO_2 is not to harm the fish. When the air supply to the tanks and filters was cut off on day 92, and 52 of the fish died from lack of oxygen, the elevated carbon dioxide concentrations will have reduced the ability of fish to consume oxygen (Spotte, 1979). This would result in fish dying even faster at the low DO levels than under normal circumstances.

The pH of water has itself been shown to kill fish outside a specific range. This range has been quoted as 5,0 - 9,0 (Alabaster and Lloyd, 1982). No mortalities that could be directly attributed to pH were recorded in this study (pH range 5,0 - 8,4; Fig. 12). It is possible however, that if the experiments had continued and the pH had fallen any further, toxicity due to this parameter may have become a factor. High concentrations of calcium recorded in the latter stages of the study may have reduced the likelihood of pH becoming lethal to the fish. It has been stated by Alabaster and Lloyd (1982) that elevated calcium levels (actual values not given) does reduce the

toxicity of pH. In addition, Brown and Gratzek (1980) state that the general productivity of a system, in terms of fish growth, is considerably reduced below a pH of 5,0.

There have been reports that, in a reuse system, low pH harms bacterial populations and consequently affects the process of nitrification in the biological filter, causing it to cease below pH 6,0 (Collins et al., 1975) or 5,5 (Forster, 1974). Conversely, Hirayama (1974) maintained that even if pH and alkalinity reached very low levels (actual values not given), the nitrifying capacity of the biofilter was not affected. The present study showed that even though the pH fell to 5,0, nitrification was not seriously affected, supporting the claims of Hirayama (op cit.). This was proved by the continued production of nitrite and nitrate at pH 5,0. If nitrification had been affected, Nitrosomonas sp. and Nitrobacter sp. bacteria would lose the ability to oxidise the substrate (ammonia) being produced by the fish. If this occurred, nitrite and subsequently nitrate levels would not increase. It is possible that nitrification was slowed down, but because the actual rate of the process was not measured, this cannot be substantiated.

The water temperatures recorded in this study (11 - 18 degrees Celsius; Fig. 16) correspond directly with the range of temperatures regarded as optimal for rainbow trout (Crawshaw, 1977; Beitinger and Fitzpatrick, 1979; McCauley and Casselman, 1981). The inverse relationship between temperature and dissolved oxygen (DO) was clear. For most of the study, water temperatures fluctuated between 11 and 16 degrees, with corresponding DO values between 6 and 9 mg/l, above the recommended minimum safe DO level for fish culture of 5 mg/l (Boyd, 1979; Piper et al., 1982). From day 50 to the end of the

study, temperatures showed a general increase, with a corresponding decrease in DO levels. This period coincided with an increase in bioload and maximum biofilter activity. Both of these factors would have placed a very high oxygen demand on the system. On the two days when the water temperature reached 18 degrees Celsius, DO levels fell below 5 mg/l. While this water temperature would not directly limit DO under normal conditions, it appears that in conjunction with the high bioloads that were being held in the systems at the time, oxygen levels were reduced. This effect was compounded on the second of these days when the supplementary air supply to the systems was undergoing maintenance following a breakdown, and the DO concentration dropped below 3 mg/l. This resulted in widespread mortality among the fish in both systems and the study was subsequently terminated.

The redox potential is a relatively uncommon water quality parameter to be measured in aquaculture. Sander and Rosenthal (1975) claim, however, that it can be used as a measure of the amount of biological activity in a system, and as such can be useful in providing an indication of the condition of the biological filter and the operating efficiency of the system in general. A high redox potential in water is beneficial because it promotes cell respiration (Sander and Rosenthal, 1975), and should therefore improve the nitrifying performance of the bacteria in the system. Data relating to what value constitutes a "high" redox potential value was not given by the above authors, although ranges they recorded in a public aquarium were approximately 250 - 450 mV, as opposed to 0 - 100 mV in the present study (Fig. 17). Boyd (1979), however, states that the redox potential of normal oxygenated freshwater ranges from 450 - 520 mV. As mentioned in the results chapter, it would appear

that there was a fault with the redox potential test probe that was used during the study, on account of the low values obtained. It would not be possible, therefore, to draw any conclusions from the redox potential data obtained in this study, although the increasing or decreasing trends observed in this parameter during the experiments might still give an indication of how redox potential varies in such a system.

In a new recirculating system, the redox potential of the water decreases during the initial conditioning phase because of the low proportion of oxidised to reduced substances in solution (days 0-30, Fig. 17). This is due to the absence of bacteria which oxidise the substrate produced by the fish. Once these bacterial populations become established, and they attain full oxidising capacity, the ratio of oxidised to reduced substances increases, and the redox potential begins to rise (days 30-85, Fig. 17). A point may be reached, however, where denitrification may occur and nitrate and nitrite in the water is biologically reduced by either heterotrophic or autotrophic bacteria to nitrous oxide or free nitrogen (Spotte, 1970; Wheaton, 1977). This situation generally arises in old culture water where nitrate levels are relatively high, and occurs best under low dissolved oxygen (< 1,0 mg/l), or anoxic conditions. Under this scenario, the redox potential of water will decrease because of the greater proportion of reduced compared to oxidised substances that would exist. This may have been the case during the last 8 days of the study when redox potential values obtained in both systems decreased slightly. Oxygen levels were dropping, ammonia concentrations were increasing and the pH was at levels (5,0 - 5,3) which have been suggested reduce the operating efficiency of nitrifying bacteria (Spotte, 1970; Forster, 1974; Collins et al., 1975; Wheaton, 1977). All these factors should

contribute towards an increase in the amount of reduced substances in the water, thus decreasing the redox potential.

While control of waste solids, both dissolved and suspended, is known to be a major problem in recirculating systems (Alabaster and Lloyd, 1982; Boersen and Westers, 1986), these parameters did not attain levels that could be considered harmful in the culture of fish (Figs. 18 and 19). Increases in solids are expected with time in recirculation systems, due to the accumulation of faecal and detrital matter. In this study, the increase in both dissolved and suspended solids only became clearly apparent once the carrying capacity of the systems had been exceeded (day 70). Before this point levels of waste solids were contained at relatively low, stable concentrations, well within the recommended levels of 400 mg/l and 80 mg/l for dissolved and suspended solids (Meade, 1989).

Once the ozonation trials had been completed, both systems were left to run without interference, to monitor further changes in water quality. Judging by the state of the water in system A, it was suspected that the biofilters would collapse soon afterwards due to the heavy loading in the tanks. The water was highly discoloured, with a strong odour. On day 74, Mr. M. Davies noted an increase in both the colour and odour of the water in system A. Wheaton (1977) states that fish farmers can use colour as an indication of the general quality of culture water, and that knowledge of the conditions suggested by changes in water colour and tranparency is a valuable management tool for the prediction of maximum loading rates in an aquaculture system.

The visual quality of the water in system B deteriorated rapidly after the ozone trials ceased. Within three days it appeared

similar to that in system A. In both systems, total ammonia, nitrite and nitrate concentrations were rising on a daily basis, with a concommitant increase in carbon dioxide levels. Turbidity and dissolved solids were also increasing, while there was a general drop in dissolved oxygen levels.

The steadily rising concentrations of total ammonia and nitrite recorded from day 70, when additional fish were placed in the systems to increase the bioload, indicated that both systems had exceeded their carrying capacity. According to Spotte (1970), the extent to which ammonia and nitrite increases when fish are added to a system is dependent on how much the additional load stresses the carrying capacity of the system. If an increase in bioload is still below the maximum carrying capacity, equilibrium with the new conditions is reached within 3 days in warm water systems and only slightly longer in cold water systems. If the increase in bioload exceeds the maximum carrying capacity of the system, this is indicated by permanently elevated ammonia and nitrite concentrations. The fact that levels of these metabolites were still rising after 22 days suggests that the system could not accommodate any more bacteria to process the substrate, and therefore the carrying capacity of the system had been exceeded.

It was not possible to determine exactly how long the fish could remain healthy under the prevailing conditions because of the loss of the supplementary oxygen supply to the biofilters and tanks on day 92. As discussed previously, this resulted in the rapid death of 11% of the fish, primarily due to asphyxiation, although the relatively high total ammonia, nitrite and CO_2 concentrations, the relatively high temperature of the water and the low pH and alkalinity may have increased susceptibility of

the fish to stress and mortality by low DO concentrations. Having achieved the aim of the experiment (i.e. to monitor the water quality of a system from a virgin state until the carrying capacity of the system was exceeded), it was decided to move the fish to a more favourable system on the fish farm, and terminate the study.

Monitoring the water quality in systems A and B provided detailed and valuable information as to the changes that occur, and the close relationships and inter-dependency of various water quality criteria considered important in the culture of aquatic animals. The rapid flow-rate, serial biofilters exhibited normal and efficient nitrification characteristics during the initial conditioning phase of the systems. Regular water quality analysis clearly indicated when the carrying capacity of the systems had been exceeded, even though the fish still appeared healthy. This indicates that monitoring the condition of the culture animals does not necessarily provide an accurate assessment of the state of the system in general. The toxicity of nitrite to rainbow trout at elevated concentrations was shown during the initial conditioning phase of the biofilter, as was the protective effect of calcium on nitrite toxicity, and the ability of bacteria to continue nitrification at low pH (5,0) and alkalinity (0 mg/l) was also shown in this study.

EXPERIMENT II : THE EFFECT OF OZONE ON WATER QUALITY IN A RECIRCULATION AQUACULTURE SYSTEM

Having established that nitrification was efficiently taking place in both systems, it was decided that ozonation experiments could be initiated. The similarity in performance of systems A and B during the initial conditioning period suggested that once ozonation experiments started, differing results between the two systems could be directly attributed to the presence or absence of ozonation treatment. System B was used for the ozonation trials while system A, without ozone treatment, acted as the control.

The cost of ozone production is, at present, one of the biggest factors limiting its use in aquaculture (Williams et al., 1982; Lohr and Gratzek, 1984; 1986). It was therefore decided that the application of ozone be initiated as a 1 hour/day low-dose treatment, followed by extended treatments and high-dose experiments. The continual use of ozone is not recommended (Sander and Rosenthal, 1975; Edmonds, 1988) as this results in high residual ozone concentrations which can have toxic effects on fish. This point was also made by Sutterlin et al. (1984) when Atlantic salmon died due to the ozone generator being accidentally left on for 15 hours as opposed to the usual 2-3 hour treatment. Rosenthal (1981) maintains that periods of continuous ozonation up to 6 hours will not affect fish. It was decided that, for any single period, this would be the maximum time that fish would be exposed to ozonated water in this study.

The results obtained in all three sets of ozonation trials were similar enough for them to be considered together. In general, it appeared that ozone had limited effect on long-term water

quality. The only marked changes in each experiment were a rapid increase in dissolved oxygen (DO) concentrations in the water, and a significant reduction in the amount of nitrite present in system B. The increase in DO was expected as pure oxygen was used as a feed gas for the ozone generator. Only 5% of the oxygen is converted to ozone, but the remaining 95% is also passed through the contact chamber, resulting in DO levels rapidly exceeding saturation levels. This is in itself a potential problem as there have been reports in the literature of fish suffering from "gas-bubble disease", and dying, as a result of being exposed to oxygen-supersaturated water during ozonation experiments (Rosenlund, 1975; Spotte, 1979).

The symptoms of this "disease" include small gas bubbles appearing under the skin of the fish as the supersaturated oxygen comes out of solution in the blood (Brown and Gratzek, 1980). In addition, haemorrhaging in the fins may occur due to occlusion of small blood vessels by gas bubbles. This situation did not manifest itself during the present study, even though DO levels reached in excess of 20 mg/l (supersaturation) within 20 minutes of starting ozone treatments. Oxygen concentrations returned to normal levels within two hours of halting the ozone application, both in the one- and six hour trials. At no stage did the fish appear in any way adversely affected by the high oxygen levels. In fact, during the six hour trials, when especially prolonged exposure to oxygen-supersaturated water occurred, the fish in system B appeared far more active and responsive at feeding time than the fish in system A. Similarly, Murphy (1975) noted that dolphin held at Sea World Oceanarium in Ohio appeared to thrive rather than show signs of stress due to oxygen supersaturation in an ozonated system.

The most significant effect of ozonation was the rapid and significant reduction of nitrite from system B (Figs. 21a, 24a and 27a). In the "low"- and "high"- dose trials respectively, there was a 64% and 74% decrease in nitrite after one hour. In the six-hour trials, there was an 86% reduction in nitrite levels after 5 hours. The majority of previous reports have stated that nitrite is oxidised directly to nitrate (Honn and Chavin, 1976; Colberg and Lingg, 1978; Rosenthal and Otte, 1979; Rosenthal, 1981; Sutterlin et al., 1984; Paller and Lewis, 1988). In chemical terms, this is the most logical pathway for the reaction to occur (Dr. R.B. English, Chemistry Department, Rhodes University, pers. comm.).

The removal of nitrite from the system was however, only temporary. Within three hours of ending the ozone treatment in the one-hour trials, and twenty four hours in the six-hour trial, nitrite levels had been restored to 90% or more of the pre-ozonation nitrite concentration. There was not, however, any increase in nitrate levels during the actual period of ozone application. If NO_2-N was in fact being oxidised to NO_3-N, then this would be expected. It is possible that the amount of nitrite existing in the system (0,07 - 0,26 mg/l) during the trial period, even if it were all oxidised, would not significantly increase existing nitrate levels (25 - 44mg/l) measured during the same period. It is also possible however, that due to ozonation, the nitrite was being converted to some other product that reverted to nitrite once ozonation ceased. Hydrogen peroxide (H_2O_2) is formed as a result of the dissociation of ozone in water (Spotte, 1979; Lohr and Gratzek, 1986). Greenwood and Earnshaw (1984) state that in the presence of H_2O_2, peroxonitrous acid (HOONO), an isomer of nitric acid, is formed as an unstable intermediate during the oxidation of

aqueous nitrite to nitrate. The reaction is especially prevalent in acidic conditions, such as those that prevailed during the ozonation trials. While some complete oxidation of nitrite to nitrate may have occurred, it is suggested that the scenario presented above predominates in the system. Once ozonation stops, the peroxonitrous acid reverts to nitrite, resulting in the observed increase in this parameter in the hours following ozone application. This reaction of ozone on nitrite has not been made in previous studies of the process. It follows that if an acid was formed from the reaction of ozone on nitrite, a drop in pH would occur. The pH of the water in system B was not monitored closely enough during the ozonation trials to substantiate this claim, and it therefore requires further and more specific studies to observe a drop in pH and prove the formation of peroxonitrous acid.

There was no decrease in the levels of either ammonia, nitrate or calcium during any of the ozone trials. The ability of ozone to oxidise ammonia to nitrite has been the subject of much debate. While some authors state that this does not occur (Murphy, 1975; Wheaton, 1977; Lohr and Gratzek, 1986; Paller and Lewis, 1988), others claim that ammonia oxidation by ozone is achieved (Honn and Chavin, 1976; Colberg and Lingg, 1978; Rosenthal and Otte, 1979; Rosenthal, 1981; Hsieh et al., 1990). Only the un-ionised fraction (NH_3) can be oxidised (Hoigne and Bader, 1978), and this only exists as a large proportion of the total ammonia at high pH. Ionised ammonia (NH_4^+) cannot be oxidised (Spotte, 1979). It is therefore improbable that any ammonia oxidation by ozone will occur in natural freshwater systems where the pH generally does not exceed 8,5 and un-ionised ammonia concentrations are relatively low. In seawater however, where the pH is higher, ammonia will exhibit a greater tendency to be oxidised by ozone

due to the higher percentage of NH_3. When ozonation trials began, the pH of the water was 7,0 and beginning to drop steadily. Un-ionized ammonia concentrations were minimal (Fig. 9), and on account of this, and the evidence presented, little ammonia oxidation by ozone would be expected.

Nitrate (NO_3-N) cannot be oxidised further (Dr. R.B. English, pers. comm.). It was therefore not expected that nitrate concentrations would decrease during ozonation. Due to the effect of ozone on nitrite however, it was anticipated that there might be an increase in nitrate, as this is the supposed oxidation product of nitrite. As mentioned before, no significant increase in nitrate levels were observed in any of the three sets of ozone trials. The process and final products of nitrite oxidation by ozone has already been discussed. To reiterate however, even if nitrate was formed by the process, the amount produced from the levels of NO_2-N available in the experimental system would not add significantly to the already present nitrate concentrations of 25 - 44 mg/l.

The lack of any significant change in calcium levels recorded in any of the experiments is explained by the fact that Ca^{++} is already in its highest oxidation state (Dr. R.B. English, pers. comm). Even an oxidising agent as powerful as ozone is not capable of elevating it to the Ca^{+++} state. The lower calcium levels recorded in the ozone system in the latter stages of the study can therefore not be attributed to an effect of ozonation. The reason for this discrepancy cannot be explained at present.

This study provided inconclusive evidence that ozonation increases the redox potential of water, as stated by Mangum and McIlhenny (1975), Sander and Rosenthal (1975) and Rosenthal

(1981). The first two sets of trials indicated that there was a slight rise in redox potential over the one- and six-hour periods (Figs. 22 and 25). However, the final one -hour experiments showed a drop in redox potential through the trial period far more substantial than the observed increases during the previous experiments (Fig. 28). As such we are unable to conclude that ozone increases the redox potential of water, or promotes greater cell respiration, thereby increasing the productivity and general efficiency of a system. On the contrary, the fact that there was no noticeable difference in performance of systems A and B and the biomass that each system supported suggests that ozonation does not benefit biological systems in the aforementioned ways.

A visible change that took place in system B during ozonation treatments was an improvement in water clarity compared with the control system. This trend was not particularly apparent in the turbidity measurements shown in Fig. 19. There was however, a problem in reading low turbidities on a scale with calibrations of 20 FTU (Formazin Turbidity Units). The only time when any significant trend became apparent in turbidity was in the last 20 days of the study, when system A was more turbid than system B. This can be attributed to the turbidity-reducing effect of ozone, which both bleaches coloured substances in the water and breaks down large particulate matter a smaller size which bacteria are able to process, and in doing so, reducing the concentration of turbidity-causing compounds.

In the last 10 days of the study, there was a visible increase in the turbidity of the water in both systems. Ozonation trials by this time had been completed, and this rise can be attributed to increased solids production in the systems due to the high

bioload, and the inability of the biological filter to handle the waste. As Meade (1989) suggests that turbidities less than 80 mg/l are safe for fish culture, levels attained in this study were not considered a threat to the performance of the system or the fish. The reduction of turbidity by ozone can only be considered a supplementary result however, as there are cheaper and more efficient methods of turbidity control, such as mechanical filtration (Spotte, 1979). The turbidity-reducing effect of ozone has nonetheless been widely documented (Ciambrone, 1975; Spotte, 1979; Williams et al., 1982; Lohr and Gratzek, 1986; Gargas, 1989; Hsieh et al., 1990). The ability of ozone to decrease colour in water results from the formation of peroxide (H_2O_2) as one of its dissociation compounds. Peroxide acts as a bleach, and as such may "remove" coloured substances from the water by breaking down chromophoric compounds (Spotte, 1979; Lohr and Gratzek, 1986).

In addition to the changes in the colour and turbidity, there was a noticeable reduction in the smell of the water in system B. This odour is characteristic of old culture water, and was particularly strong in the control system. As with colour, the removal of odour in aquaculture systems by ozone is more of an aesthetic effect than one which will increase production in a system (Spotte, 1979). This effect of ozone has however, also been noted previously (Sander and Rosenthal, 1975; Rosenthal, 1981; Gargas, 1989).

The pH of the water is an important factor in ozonation studies. Not only is it important for determination of the un-ionised ammonia concentration, and hence the amount of total ammonia that might be oxidised by ozone, but it also directly affects the dissociation characteristics of ozone. The pH range through

which these experiments occurred was 7,0 to 5,2 (Fig. 12). While ozone would not react as quickly at this pH as opposed to a higher value (Colberg and Lingg, 1978; Gargas, 1989), it would take longer to dissociate (Spotte, 1979; Rosenthal, 1981). This means that contact time with the water was effectively increased. Hence, once having left the chamber, the contact and reaction of ozone with the water would continue until reaching the activated carbon filter where residual dissolved ozone was removed. Wheaton (1977) maintains that at a pH of 6, ozone has a more significant effect than at pH 8. This might suggest that during these experiments, ozone was being applied in its most effective operating range.

Without ozone treatment, there was no significant change in any water quality parameter in the control system (Figs. 21b, 24b and 27b). Generally, total ammonia, nitrite, nitrate and calcium concentrations showed slight increases over trial periods. These were not however significant, and were probably due to the natural accumulation of the substances in the system. These results did prove however, that the changes in nitrite concentration observed in the experimental system during the trials were due to ozonation.

One point that could not be discounted was that the unconverted oxygen in the ozone/oxygen mixture, which was brought into contact with the water, was oxidising the nitrite, in addition to increasing the dissolved oxygen content of the water. In order to ascertain the effect of oxygen alone, a similar trial to previous experiments was performed (see Materials and Methods, Table 4, Experiment II d). The results from this set of trials showed that apart from increasing DO levels, oxygen alone had no effect on either total ammonia, nitrite, nitrate or calcium (Fig.

29). This proved that it was the ozone portion of the gas mixture that was reducing nitrite concentrations in the previous trials.

Effects of ozone toxicity on the fish were not evident at any stage of the trials. The symptoms of ozone poisoning in fish have been described in detail by Hubbs (1930). These follow a sequence of events, starting with locomotory and respiratory irritation. This is typical of sub-lethal doses of ozone. At higher doses, loss of equilibrium indicates that poisoning is irreversible and the fish will die. The loss of balance worsens until the fish lies on its side for prolonged periods, interspersed by rapid dashes. Just prior to death, the fish lie belly-up, gasping, colour fades and intense tetanus (spasmic muscular contraction) sets in. Gill movement is slight, and the skin on the ventral surface of the fish becomes oedematous (swollen). The pathological effects of ozone poisoning include epithelial damage to gill tissue, gill aneurysms and hyperplasia and oedema (Rosenlund, 1975). Lohr and Gratzek (1986) reported impaired respiratory, excretory and osmoregulatory capability in channel catfish exposed to ozone. Storage of glycogen in livers of exposed fish was claimed to be less than in control fish, and mucosal cell turnover was increased.

As mentioned previously, fish in system B appeared more active and responsive to feeding both during and after ozonation compared to the control fish. Ozone concentrations reaching the culture tanks never approached the lethal level of 0,1 mg/l suggested for salmonids by Benoit and Matlin (1966). The highest level attained during the six-hour trials was approximately 0,6 mg/l, even though water leaving the contact chamber contained an average of 0,12 mg/l O_3. This indicates the efficiency of the

granular activated carbon (GAC) filter at removing residual dissolved ozone from the water. In addition, it suggests that without altering the design of the existing system, substantially larger doses of ozone could be applied to the water without a threat of toxic levels passing through to the tanks. The effects of high doses (approximately 5 mg/l) of ozone on water quality remain to be investigated. The inability of the ozone generator to produce greater than 1,5 mg/l O_3 due to the generator fault prevented this aspect being included in the present study. If the generation of such levels of ozone resulted in more than toxic residual concentrations reaching the tanks, the inclusion of a degassing/retention unit between the contact chamber and the tanks might be necessary. This would allow excess ozone to be bubbled off to the atmosphere, and the prolonged retention time between contact and exposure to fish would mean a greater chance of the gas dissociating to oxygen.

That there was no significant difference in mass of the fish held in the two systems is not consistant with previous findings. Tipping (1987; 1988) showed that steelhead trout (Oncorhynchus mykiss) grew substantially better in an ozonated system as opposed to an identical control unit. Sutterlin et al. (1984) also showed growth of Atlantic salmon to be better in an ozonated system. In addition, due to the various enhancements in water quality that have been attributed to ozone, it might be assumed that such a treatment would induce improved growth of culture animals. The fact that there were no appreciable improvement in the standard of the water in this study negates this assumption. However, duration of the ozone trials was probably not long enough to augment any significant growth differences between the fish in systems A and B.

Although specific analyses to determine the condition of the fish were not performed, it appeared at the end of the study that they were in good condition, even though the carrying capacity of the system had been exceeded. This assumption was based on the facts that the fish still fed actively and general appearance and body conformation was uniform throughout the test population. Because culture conditions vary widely from system to system, it is impossible to prescribe a standard growth rate for any fish species against which performance of both fish and system may be measured. McEwan (1988) achieved growth rates of approximately 1 g/day for rainbow trout during feeding trials performed at the Rhodes University experimental fish farm. The mean growth rate of approximately 0,5 g/day (Table 5) obtained in the present study does not therefore seem particularly good. However, this relatively slow growth rate can almost certainly be explained by the fact that for 22 of the 92 study days the fish were living at high densities in systems in which the carrying capacity had been exceeded, and culture conditions were far from optimal. By monitoring the water quality, it was shown that the maximum load that either system could hold, without exceeding safe recommended levels of various system pollutants and creating an unfavourable culture environment, was 15 kg/m^3 of yearling rainbow trout in each tank. Above this stocking density, the water quality deteriorated rapidly and growth was most probably slowed. Qualifying the size and species of fish in stating the carrying capacity of the system is necessary because it can vary according to these factors (Piper et al., 1982). The number of fish that a system can support is also a function of the size of the fish themselves, ranging from 25 kg/m^3 to 5-10 kg/m^3 for adult broodstock (Stevenson, 1987). The maximum bioload attained in this study (15 kg/m^3) is considered good for a system holding juvenile rainbow trout.

CONCLUSIONS

This study illustrates the potential of recirculating systems in intensive aquaculture, in terms of water-reuse efficiency, maintenance of water quality and the high bioload per unit volume of water that a system can support . Experiment I illustrated the need for monitoring the quality of the water, particularly during the initial conditioning stages, and when the bioloads are high. Regular water analysis provides a knowledge of when chemical components in the water are reaching concentrations which are either toxic or will reduce the growth of the culture animals. There are however, visible changes that occur in the water that can warn of impending system overload and collapse. Monitoring the water quality has shown that several chemical components are closely related and affect the dynamics of the system accordingly. Small variations in one or more of these can cause rapid and marked changes in the culture environment, resulting in overall performance loss in the system.

Experiment II showed that ozone does not have any long-term beneficial effects on the culture environment or system performance, by way of improving water quality. This was shown by the lack of difference in the standard of water in system B after application of ozone compared to the untreated, control system. A significant reduction in nitrite concentration occurs, but this is only temporary, as nitrite returns to pre-ozonation levels within hours after ceasing the treatment. Ozone has no effect on ammonia at low pH (< 7), but does reduce levels of dissolved solids and generally improves the aesthetic appearance of culture water by reducing colour and odour.

Although the experimental period may not have been long enough to

show any effect, it appears that ozonation of the water does not enhance growth of fish. Furthermore, ozone does not have the ability to significantly enhance water quality once optimal culture conditions are exceeded, or to improve the efficiency of the biological filter. As such, a system incorporating ozonation cannot support a greater bioload than an identical system without the treatment. The toxicity of ozone to culture animals can be circumvented by efficient system design, by incorporating post-treatment activated carbon filters to remove residual ozone from the water before it reaches the culture tanks.

From the evidence gathered in this study, it may be concluded that ozone is not an effective means of improving water quality, and subsequently, production capacity in freshwater aquaculture systems. However, its proven potential as a sterilizing agent and for disease control purposes, although not considered in this work, suggests that ozone will still play an important role in the development of the aquaculture industry worldwide.

RECOMMENDATIONS FOR FURTHER RESEARCH

i) Determine the nature of the ozone-nitrite reaction (i.e. is nitrite completely oxidised to nitrate or is the intermediate product, peroxonitrous acid, formed).

ii) Investigate the effects of high doses of ozone (1 - 10 mg/l) on water quality, and the effects of water temperature on the dissociation characteristics and effectiveness of ozone.

iii) Establish more accurately at what concentrations ozone becomes toxic to fish. Furthermore, are there intra-specific (fry vs. adult) or inter-specific (e.g. trout vs. catfish) differences in tolerance of fish to ozone?

iv) Examine the biocidal effects of ozone in aquaculture.

v) Repeat all research in seawater, as the different chemistries of fresh- and salt-water produce different reactions with ozone.

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