THE EVALUATION OF A SHORT-TERM HOLDING SYSTEM FOR THE NORTH AMERICAN LOBSTER, HOMARUS AMERICANUS

by

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ABSTRACT

The water quality parameters for use in the design of lobster (Homarus americanus) holding facilities were reviewed from published literature. The review indicated that several parameters (temperature, pH, salinity, dissolved oxygen, oxygen demand, suspended solids, ammonia and nitrite nitrogen) could be of major importance in the design of commercial short-term holding facilities where partial or complete water recycle is required. Of major interest were the changes in water quality immediately following the introduction of lobsters into the system.

Monitoring of the changes in water quality for different biomass loading rates and temperatures was carried out at a commercial holding facility. The facility typically holds approximately 1000 kg of lobster in 13 tanks with a system water volume of 17500 l. Normally the water is completely recycled with treatment consisting of drop aeration, sand filtration and UV sterilization.

Results indicated that water temperature is the most important factor in the maintenance of water quality and design of a lobster holding facility. It has an impact on lobster

metabolism, the dissolved oxygen concentration and biochemical oxygen demand of the holding water, as well as affecting the rate of nitrification of ammonia. It was demonstrated that during the first 0.5 h after lobster introduction at the high experimental temperatures, water quality often deteriorated to a deleterious level, particularly with respect to dissolved oxygen concentration. Acceptable levels were regained gradually after the critical period. At a normal operating temperature of 7°C and a lobster load of 1100 kg, oxygen demand was reduced, as dissolved oxygen dropped from 10.5 to 8.5 $mg \cdot l^{-1}$ compared to a drop from 9.0 to 5.5 at 13°C and from 8.2 to 3.3 $mg \cdot l^{-1}$ at 19°C. An attempt to detect nitrification in the sand filters indicated that very limited activity was present at 12 and 17°C. The most important regulating factors were possibly the residence time of the holding water in the filter units and competition from heterotrophic bacteria. Results from experiments designed to establish the effectiveness of the UV sterilizers at controlling bacterial levels in the holding water showed that the units were effective in controlling bacteria at the three test temperatures.

General recommendations regarding the design and maintenance of a short-term lobster holding system include: temperature control as an essential design feature; TOC control

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1.0 INTRODUCTION

The American Lobster (Homarus americanus) has long been and continues to be a very popular food item in North America and around the globe. This popularity ensures that the lobster fishery will continue to be one of the most economically important on the east coast of North America. A summary of statistics from 1954-1974 by Cobb (1976) indicates that the continuously increasing demand for lobster is reflected in its increasing value but not in the quantities landed in the U.S. A similar situation exists on the east coast of Canada (DeWolf 1974; Campbell and Duggan 1980). Numerous investigators have reported apparently declining lobster populations (DeWolf 1974; Thomas 1973) and it is obvious from their data that effective management regulations are required to alleviate the critical situation in this fishery. It is clear that in recent years fishing effort has increased dramatically, whereas lobster landings have slowly declined.

The apparent decline in stocks, coupled with increasing consumer demand, has generated a great deal of interest in commercial lobster rearing. A considerable amount of federal money has recently been made available for research into the various biochemical, technological and economic aspects of lobster aquaculture in Canada, through the Department of Fisheries and Oceans. Similar research is being funded in the United States, through the Sea Grant Program. Until this

research leads to commercially viable lobster operations, the consumer remains dependent upon the natural lobster stocks. Commercially viable lobster culturing opera- tions will most probably emerge in the near future provided that solutions are found for a number of important problems. The areas requiring the most intensive research efforts include lobster nutrition, energy acquisition and utilization, and water treatment technology (Johnston and Botsford 1980).

Most of the commercial catch of Homarus is sold live, requiring storage for periods of up to six months in various types of holding facilities. Traditionally, these holding units utilize ambient temperature sea water, are relatively large, and are restricted to selected coastal intertidal areas. With the expansion of the live lobster market to include numerous non-lobster producing coastal areas, as well as inland sites, has come a more sophisticated approach to live lobster storage in general, and specifically in water quality manage- ment. In an attempt to reduce mortality and maintain a quality product, water treatment technology utilized in other fields has been borrowed and modified, when necessary, to suit the requirements of lobster holding systems. The most complex holding facilities, with respect to water quality control, are those that are situated inland, as these operations require, out of necessity, recirculation of the salt water medium. Partial or complete recycle may also be useful in coastal operations for temperature control and energy conservation.

With recirculation though comes deteriorating water quality over time due to eventual build-up of metabolities (Hughes et al. 1972; Shlesser and Tchobanoglous 1974; Gravitz et al. 1975) and increased pathogen concentration (Fisher et al. 1978).

A number of units and operating procedures have been developed to cope with different aspects of these problems (Getchell 1953; Wilder 1953; Wilder and McLeese 1957; Thomas 1962, MeLeese and Wilder 1964; Ayres and Wood 1977; and Cormick and Stewart 1977). Although the objectives in lobster aquaculture are somewhat divergent from those set out in lobster holding, many of the problems are common to both operations. As a result, the increased interest in lobster culture that has taken place during the last few years has significantly aided in the development of systems designed strictly for the maintenance of market size lobsters (Sastry 1975; Hand 1977).

From a distributor's or retailer's point of view, there are a number of economic advantages to successful live storage. These include (1) increased flexibility of marketing and dispatch which can be carried out at convenient times or on demand, (2) reduced mortality by maintaining animals in water, and (3) insurance that the produce arrives at its destination in good condition. Long-term storage brings further benefits, particularly to those producers who are geographically isolated from major markets. Large consignments can be built up, customers can be supplied with equally graded shipments, advantage can be taken of bulk packing and transportation and,

most important of all, lobsters may be purchased when cheaper and more abundant, and stored and sold when demand and prices are at a peak.

It is a lobster holding system characterized by many of the features listed above that is the subject of this thesis. The facility* exists strictly as a holding operation for wholesale and retail trade, and is relatively simple when compared to prototype lobster culturing facilities. It is this apparent simplicity of design and technology that makes this operation worthy of study. A holding system that utilizes tested and established technology, and is relatively simple to fabricate and manage may have great potential in a variety of other situations and locations. Therefore the overlying objectives of the research reported here were initially, to establish the demands on the existing system mainly from a water quality viewpoint, and secondly to evaluate system response to these demands so that recommendations to upgrade the system could be formulated and presented to the managers of the facility.

It was felt that the knowledge acquired from the study of this particular live lobster holding operation could be applied to the design and maintenance of future live seafood storage units and even intensive and extensive aquaculture projects.

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2.0 SPECIFIC RESEARCH OBJECTIVES

In response to the overlying objectives outlined in the preceeding section, four specific research objectives were formulated and are as follows:

1. To establish, from a literature review, the most important water quality parameters to be considered in the design of a short-term lobster holding facility.
2. To determine changes in the levels of established water quality parameters immediately after introduction of lobsters into an existing short-term holding system.
3. To determine changes in the levels of established water quality parameters during a period of short-term lobster holding.
4. To propose basic design criteria for the maintenance of acceptable water quality in a short-term lobster holding facility based on the results obtained during the fulfillment of Objectives 2 and 3.

3.0 LITERATURE REVIEW

3.1 Introduction

Due in part to recent efforts at commercial lobster rearing and natural stock management and enhancement, a substantial amount of literature regarding environmental requirements of Homarus (americanus and gammarus) is available. Although a great deal of additional investigation is needed before intensive lobster culture becomes a reality, a general picture describing the basic environmental needs of lobsters has developed, and it is this kind of information that is proving to be invaluable to designers and operators of lobster holding facilities. The primary objective of this literature review is to establish which water quality parameters are of greatest importance in lobster holding systems, and over what range these established parameters must be maintained. Information concerned with parameter monitoring methods, and sampling techniques and intervals was also obtained from the literature and will be outlined in more detail in a later section.

Phylogenetically, the lobster is a crustacean belonging to the sub-class Malacostraca, which contains most of the commercially important species. There are two species of lobster: Homarus gammarus, the European lobster, occuring in the eastern Atlantic waters from the Arctic Circle to Morocco and into the Mediterranean, with its centre of distribution the British Isles; and Homarus americanus found on the Atlantic coast of

Northern United States and Canada (Headstrom 1979).

There exists a variety of good general publications on Homarus that provide useful background information on lobster biology, ecology and behavior, as well as numerous references concerned with specific problems in lobster research (Herrick 1909; Doliber 1973; Taylor 1975; Cobb 1976; Richards and Wickins 1979). These publications act as an excellent resource for a more in-depth look at the water quality problems associated with live lobster storage. Unsatisfactory conditions in a lobster holding system arise as a result of either animal related alterations to the environment or alterations caused by external influences. In a land-based holding operation, employing moderately sophisticated technology, external influence, such as ambient air temperature, can be kept to a minimum, providing a suitable water supply is available. Therefore, it is the biochemical and behavioral characteristics of the animal that complicate live lobster storage procedures. Research efforts have reacted in two ways to this situation. Initially investigators established which biochemical and behavioral characteristics were causing problems in lobster holding, and secondly what level of perturbation was acceptable in each case. One very important consideration is that environmental quality is very much dependent upon animal density, the more lobsters there are per unit area or volume the more concentrated or intense the associated problems are likely to be (Sastry and Zeitlin-Hale 1975; Shlesser 1974).

The aggressive nature and cannibalistic tendencies of both juvenile and adult lobsters exist as the most significant behavioral problems that occur in a holding or culturing system. A popular approach in the design of lobster culturing systems is to provide individual growth chambers for each animal so that behavioral affects are minimized (Chanley and Terry 1974). The economics of lobster holding will not allow this approach, so alternative methods must be employed to ensure that these factors are controlled.

3.2 Microbiology/Pathology

Most literature related to lobster culture and storage states that bacterial populations must be closely controlled to reduce or eliminate disease. When lobsters are held at high densities, resulting in stressful conditions, diseases that are virtually unknown in natural environments may appear. A recent review of lobster microbial diseases (Fisher et al. 1978) states that there are basically six diseases that could create problems in holding systems. Included in the review are shell disease, Gaffkemia, microbial epibiont disease, Lagenidium disease, Haliphthoros disease, and Fusarium disease. Gaffkemia and shell disease appear to be the most common diseases affecting lobsters held in a high density situation.

Gaffkemia is a systemic disease and is caused by a Gram-positive, tetrad forming bacterium known as Aerococcus viridans variety homari (Steward and Zwicker 1974). A. viridans is a

normal component of the flora of the exoskeleton and possesses no mechanism for invasion of the host. However it can be introduced into the hemocel by cracks or punctures caused by traditional 'pegging' of the crusher claw or by drying of the exoskeleton in shipment (Steenbergen and Schapiro 1974). Stewart and Rabin (1970) report that Homarus shows an almost complete lack of defense against gaffkemia.

Shell disease on lobsters was first reported by Hess (1937) and was believed to be caused by chitinolytic bacteria. Rosen (1970) has shown that both bacteria and fungi are responsible for shell disease on a variety of crustaceans. The gross signs of the disease are similar in all species; the exoskeleton is pitted and marred with necrotic lesions and, although the disease is not immediately fatal, death may occur. The chitinolytic microorganisms do not penetrate into the soft tissues underlying the chitinous exoskeleton (Rosen 1967; Rosen 1970), but may provide a portal of entry to epidermal tissues for secondary invaders. Sawyer and Taylor (1949) reported that shell disease may also cause discoloration, irritation, and erosion of lobster gills resulting in impaired gas exchange. The disease is contagious especially when the animals are held in mass confinement, but lobsters may overcome minor shell disease by successfully molting (McLeese 1965).

It is clear then that control of microorganisms is essential in a lobster holding operation to reduce mortality

and preserve lobster health and physical appearance. This control can be achieved through a number of techniques including the exposure of holding water to ultraviolet light at the appropriate wavelength and intensity (Ayres 1978) or through ozone sterilization (Wheaton 1977). Therefore the evaluation of any lobster holding operation must include some sort of analysis of the system's ability to control potentially lethal microorganisms.

Along with the aforementioned biological and behavioral demands of stored lobsters the literature lists a number of physical and chemical parameters or characteristics that must be considered in a evaluation of this sort. The most appropriate parameters are temperature, pH, salinity, dissolved oxygen, ammonia, nitrite and nitrate (Hand 1977; Ayres and Wood 1977). Suspended and dissolved solids, and organic carbon are also considered important parameters in various holding and culture systems. Therefore these water quality characteristics will be examined with respect to lobster holding.

3.3 Temperature

As with most other poikilotherms, temperatures that lobsters can tolerate depend to a considerable extent on the temperature to which they were acclimated. In general, market size lobsters can withstand a wide range of water temperatures, providng that fluctuations are gradual. They can survive in water cooled to the freezing point or heated to 32°C (Cobb

1976). Lethal water temperature levels are not determined by the size of the lobster, and are not affected by a two-month period of starvation, an important factor in live lobster storage (McLeese 1956). When other water quality parameters such as dissolved oxygen or salinity are less than optimal, tolerance to temperature is reduced. During storage the water temperature should be maintained between 4.5° and 10°C (Ayres and Wood 1977; Cornick and Stewart 1977). Since the lobster is a cold-blooded animal it responds to reduced environmental temperature by lowering its metabolism and thus its activity, both responses are desirable in a holding tank (McLeese and Wilder 1958). A further advantage in maintaining low water temperatures in a lobster holding system is that the previously mentioned systemic disease gaffkemia is temperature dependent, the lower the temperature the slower the disease progresses (Stewart et al. 1969).

3.4 pH

The pH, or hydrogen ion concentration of a solution, is defined by the equation:

$$pH = - \log_{10} [H^+] \quad (1)$$

where $[H^+]$ = the hydrogen ion concentration (Wheaton 1977). Since many dissociation constants for the chemical reactions occuring in aqueous solutions are pH dependent, the chemical environment for aquatic organisms is strongly influenced by this parameter. The equilibrium between ammonia (NH_3) and

ammonium (NH_4^+) in water is a notable example of this pH effect. There are a number of biologically mediated reactions affecting pH in natural water systems, two are significant in sea water holding systems. The first process is plant and animal respiration which tends to decrease pH by the production of free carbon dioxide (CO_2). The basic reaction is as follows:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \qquad (2)$$

The carbon dioxide then combines with water to produce carbonic acid (H_2CO_3) which results in a pH reduction. The second process is nitrification, which again tends to reduce pH this time through the direct production of hydrogen ions. Equation 3 shows the overall reaction.

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+ \qquad (3)$$

According to Spotte (1970) the acceptable pH range for marine culture and holding water is 7.5-8.3. Although there seems to be general acceptance of this range in the literature, the effect of low pH on animals is poorly understood and methods employed to deal with incorrect pH levels seem to vary. Hirayama (1970) reports that when calcareous gravels are relied on exclusively to buffer water in a closed sea water system, the water eventually equilibrates at about pH 7.5 with an alkalinity of 1.0 meq$\cdot l^{-1}$. Both values are slightly lower than the reported optimum. Partial water changes at a rate of 10% every two weeks, and regular addition of sodium carbonate or sodium bicarbonate are usually necessary to keep the pH and

alkalinity values within the acceptable range (Spotte 1970).

3.5 Salinity

Salinity can be defined as the total amount of solid material, in grams, contained in one kilogram of seawater when all the carbonate has been converted to oxide, the bromine and iodine replaced by cholorine, and all organic matter completely oxidized (Tsurikova and Tsurikov 1971). The salinity of seawater generally varies from 33 to 37 parts per thousand (ppt). However, in tidal estuaries, salinities are generally lower and subject to considerable variation. The most critical aspect of salinity in a live lobster holding system is its influence on osmoregulation. Lobsters exhibit limited osmoregulatory ability when the osmotic concentration of the surrounding water is either above or below that of their blood. Excess salt is excreted by the gut, while excess water is excreted by the antennal gland (Dall 1970).

Lobsters are typically coastal animals found in waters having a salinity of 33 ppt or more. Although they can be acclimated to tolerate low salinities they are usually not found naturally in brackish water. It is possible to store lobsters in water having a salinity down to 20 ppt and less when water temperatures are below 10°C, but the minimum value usually considered acceptable in commercial storage units is 27 ppt (Wood and Ayres 1977). Salinity of about 8 ppt is the lower limit of tolerance for juvenile and adult Homarus

(Phillips et al. 1980).

3.6 Dissolved Oxygen

The extraction of oxygen from water and the addition of this gas to water are operations of critical importance to aquatic animals. In a cold-blooded animal such as the lobster, oxygen demand rises with increasing environmental temperature, however, the solubility of oxygen in sea water is reduced as the temperature rises. The synergistic effect produced by the combination of these two principles is that the amount of sea-water lobsters require to satisfy their oxygen requirements during storage increases disproportionately as the temperature rises (Ayres and Wood 1977). Increased salinity also has the affect of decreasing the oxygen carrying capacity of water.

In studies of oxygen consumption and tolerance it has been shown that when other parameters are optimal, lobsters can survive oxygen levels as low as about 1 mg oxygen per liter (McLeese 1956). The amount of oxygen consumed per gram of body weight decreases with increasing body weight, and increases with increasing water temperature (McLeese 1964). Under normal conditions, coastal waters contain from 7-13 ppm of dissolved oxygen depending on a number of factors including temperature, salinity and biochemical oxygen demand. In lobster storage systems oxygen requirements depend on storage density, animal activity and stress factors. The generally accepted dissolved oxygen level is 7 or 9 ppm but the closer to saturation levels

the better.

The tolerance of lobsters to oxygen, salinity, and temperature is shown in the three-dimensional graph in Figure 1 (any point in the graph represents a specific combination of oxygen temperature and salinity).

3.7 Solids and Organic Carbon

The general term "solids" usually is taken to mean the total solids or residue content of the holding water. In most instances, however, the specific form in which the solids are present in the water must be determined. Therefore, methods have been developed to differentiate between settleable solids, suspended solids, dissolved solids and volatile solids (American Public Health Association et al. 1976). In a lobster holding system most of the large particulate matter or settleable fraction should be removed by mechanical filtration, and assuming that the available holding water supply is of good quality, total dissolved solids should not be a serious problem. The major concern lies in the small suspended and volatile organic fractions. These fractions may have the following effects in a lobster holding system:

1. irritation and/or damage to lobster gills (becoming more severe in the presence of toxic substances),
2. exertion of biochemical oxygen demand and creation of noxious conditions,
3. transport of adsorbed pollutants,

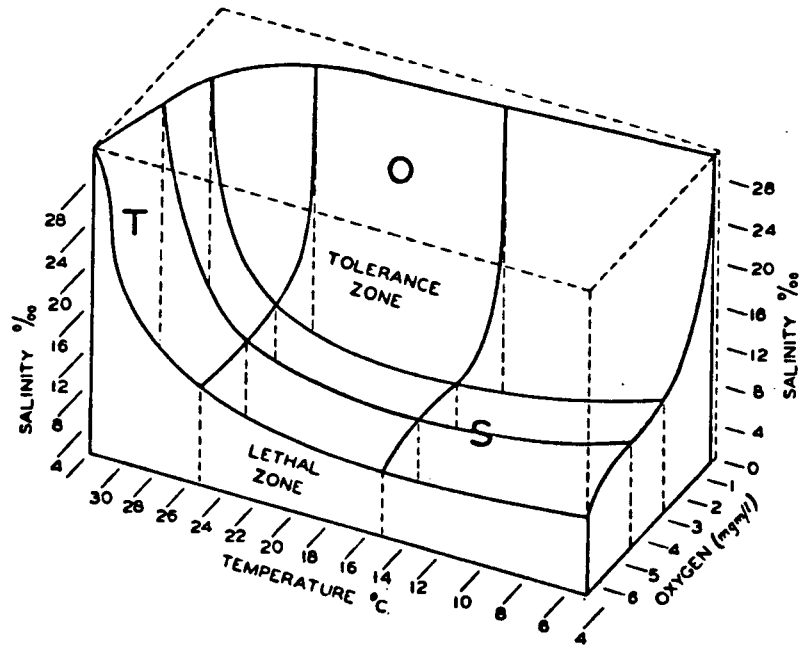

Figure 1. Three dimensional diagram of the boundary of lethal conditions for lobsters under various combinations of temperature, salinity and oxygen.

T - region in which temperature alone acts as a lethal factor.

S - region in which salinity alone acts as a lethal factor.

O - region in which oxygen alone acts as a lethal factor.

[redrawn from McLeese(1956)]

4. increased system maintenance.

The first two effects are the most critical.

The measurement of biochemical oxygen demand (BOD), which defines the biodegradable organic content of a wastewater, and chemical oxygen demand, which measures the total chemically oxidizable organic content, both degradable and refractory, is difficult when using low concentration wastewater such as water from a lobster holding sytem. BOD determinations are particularly difficult to obtain when using a saltwater medium (Iverson 1973). Suspended solids measurements are complicated by fluctuating salinity levels in the holding water.

Since metabolism is low and feeding is not taking place, a very small amount of solid matter would be expected to be generated in a lobster holding unit. As a result, very little emphasis has been placed on measuring this parameter in established systems. Data on lobster tolerance levels to suspended solids in recirculating holding operations are unavailable at present. The National Academy of Sciences and The National Academy of Engineering (1974) suggest that aquatic communities sould be provided with a high level of protection at suspended solids concentrations of greater than 25 $mg \cdot l^{-1}$. In a summary of water quality criteria for salmonid hatcheries the Canadian Department of Fisheries and Oceans (1979) states that the maximum concentration of suspended solids in a salmonid holding pond should not exceed 25 $mg \cdot l^{-1}$. This figure is likely more than adequate for lobster storage as the lobster is

generally more tolerant of poor water quality than most salmonid species.

Since BOD, COD and suspended solids determinations are complicated by various conditions inherent in a lobster holding system, total organic carbon (TOC) levels can be considered as a substitute for the determination of suspended matter and biochemical oxygen demand. Relationships between TOC and COD in various types of wastewaters have been developed (Eckenfelder 1970) and these relationships are discussed in more detail in a later section.

3.8 Nitrogenous Compounds

The final area of evaluation in this study is that of nitrogenous compounds in lobster holding water. The three major end-products of nitrogen metabolism in animals are ammonia, usually as the ammonium ion (NH_4^+), urea, and uric acid (Campbell 1973). The major nitrogenous end-product of marine invertebrates, including Homarus, is ammonia, making these animals ammoniotelic. The term ammonia usually refers to the sum of ammonium ion and free ammonia (NH_3), determined analytically as total NH_4^+-N. As well as direct excretion of ammonia, the lobster does produce minor amounts of urea, uric acid, and amino acids which all eventually undergo mineralization or deamination to form ammonia (Spotte 1979). Nitrogenous wastes are excreted by the gills, the gut, and, to a lesser extent, by the antennal glands in the anterior ventral

part of the body through a pore on the lower side of the basal segment of the first antennae.

When urine alone was examined from the spiny lobster (Jasus edwardsii), urea, ammonia and amino compounds together made up only 21% of the total urine nitrogen, the remainder being unidentified (Binns and Peterson 1969). The rate of urine output has been estimated at approximately 0.5% of the body weight per day for Homarus (Burger 1957).

In a culture or natural situation, where the lobsters are consuming food, ammonia production is closely related to the feeding rate, dietary protein-nitrogen level, protein utilization or conversion, and nitrogen excreted as ammonia or compounds readily converted to ammonia. A theoretical total ammonia-ammonium production rate can be determined from the quantity of food supplied and the protein content of the feed:

$$N = (P/6.25)\ Q_f \qquad (4)$$

where N is the rate of intake of nitrogen, in $g \cdot day^{-1}$, P is the proportion of protein in the ration, and Q_f is the quantity of feed supplied in $g \cdot day^{-1}$ (Allen and Johnston 1976). Production of ammonia gas, the more toxic metabolite, is calculated with the theoretically available nitrogen estimate from Equation 4. The proportion of total ammonium dissociated as gas can be derived from the Henderson-Hasselbach equation (Allen and Johnston 1976):

$$pH - pKa + \frac{X}{C-X} = pKa + \log \frac{(base)}{(acid)}; \qquad (5)$$

where C is the molar concentration of acid or base and X is the amount of strong base that must be added to make the solution electrically neutral. No empirically determined nitrogen conversion factor is presented by Allen and Johnston, therefore, it is impossible to calculate actual ammonia-ammonium production. Colt and Armstrong (1981) state that the protein conversion factor for aquatic animals ranges from 0.65 to 0.80. The estimation of ammonia production by fish has received far more attention due to the importance of this parameter in hatchery and growout facilities. As a result, the variables involved in fish culture have been defined in a much clearer fashion (Pettigrew et al. 1978).

When lobsters are being held and not provided with food, it becomes very difficult to estimate ammonia production. Compared to a lobster growout operation, where feeding is taking place and metabolism is high, the concentration of ammonia in a system designed strictly for lobster storage should be quite low, assuming both systems utilize similar water recycle patterns. Even assuming a low rate of ammonia production in a lobster holding unit, toxic levels of this compound can develop when the holding water is continuously recycled without treatment or dilution.

The hydrolysis of ammonia in natural waters, as shown by the reaction below, has a pK value of about 9.0, so that the percentage of ammonium is always greater than that of free ammonia (Spotte 1970).

$$NH_4^+ + H_2O \rightleftharpoons NH_3 + H_3O^+ \qquad (6)$$

Factors affecting ammonia hydrolysis are of great importance in aquatic culture and holding systems, since it has been shown that in most instances NH_3 is significantly more toxic than NH_4^+. Equation 6 is controlled predominately by pH and to a lesser extent by temperature and salinity. A pH increase of one unit causes the percentage of free ammonia to increase about tenfold (Trussel 1972; Bower and Bidwell 1978). The temperature effect is the result of increased hydrolysis of ammonium ions at higher temperature levels; the salinity effect is the result of the decreasing activity of free ammonia in solutions of increasing ionic strength (Hampson 1976).

Through the biological oxidation process known as nitrification, ammonia is converted into two other toxic nitrogen based compounds, nitrite and nitrate. Nitrification is carried out mainly by two autotrophic bacteria, Nitrosomonas and Nitrobacter. The stoichiometric reaction for oxidation of ammonium to nitrite by Nitrosomonas is:

$$NH_4^+ + 1.5\ O_2 \longrightarrow 2H^+ + H_2O + NO^-_2 \qquad (7)$$

The reaction for oxidation of nitrite to nitrate by Nitrobacter is:

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^- \qquad (8)$$

Nitrite is the ionized form of nitrous acid, a weak acid. This reaction can be written:

$$NO_2^- + H^+ \longrightarrow HNO_2 \qquad (9)$$

At holding water pH and temperatures the percentage HNO_2-N

ranges from .0005 to .05 percent (Russo et al. 1981). Low pH and temperatures favor the nitrous acid form. Nitrates are in general readily soluble in water, showing slight tendancies to form coordination compounds, and for most purposes can be considered to be dissociated completely (Latimer and Hildebrand 1951).

The mechanism of ammonia toxicity in lobsters is as yet not fully understood but as in fish it may be by 'mass law' prevention or reversal of normal nitrogen metabolism. NH_3 is regarded as the form of ammonia that is toxic to freshwater fishes because it is lipid soluble, whereas the permeability of plasma membranes to NH_4^+, hydrated ammonia ions, is relatively low (Milne et al. 1958).

Colt and Armstrong (1981) categorize the toxic effects of ammonia to aquatic organisms under eight general headings - effects on a cellular level, effects on nitrogen excretion, effects on osmoregulation, effects on oxygen transport, effects on tissue, effects on disease, lethal effects and effects on growth.

On the cellular level, the release of NH_3 into the blood from ambient water or metabolic production is converted to NH_4^+ with the release of an OH^-. The subsequent elevation of blood and perhaps intercellular pH can have a pronounced effect on enzyme-catalyzed reactions and membrane stability (Campbell 1973). Campbell also states that high levels of ammonia may cause a reversal of the glutamate

dehydrogenase reaction, withdrawing alpha-ketoglutarate from the tricarboxylic acid cycle as well as decreasing the amount of NADH available for oxidation.

Three principal routes of metabolic ammonia excretion are available to aquatic animals: 1) diffusion of NH_3 from the blood to the water through the gills, 2) exchange transport of NH_4^+ with Na^+, and 3) conversion to non-toxic compound like urea. As previously stated, the most important route utilized by lobsters is diffusion across the gills. Hampson (1976) suggests that high ammonia levels in the blood inhibit ammonia excretion. It has been demonstrated for a number of animals including rainbow trout, (Salmo gairdneri (Olson and Fromm 1971); goldfish, Carassius auratus (Olson and Fromm 1971); crab, Callinectes sapidus (Mangum et al. 1976); and the freshwater shrimp, Macrobrachium rosenbergii (Armstrong 1978), that the addition of ammonia to the external medium will reduce ammonia excretion. This effect is particuarly important in an aquaculture system because the initial reaction of aquatic animals to this inhibition of ammonia excretion may be the cessation of feeding which in turn may reduce growth rates.

It is also quite likely that sublethal concentrations of ammonia may produce severe hyperplasia of the gill epithelium and an increased permability of the animal to water. This effect on osmoregulation was observed in fish by Lloyd and Orr (1969).

Ammonia can have a serious effect on the ability of

aquatic species to transport oxygen to the tissues. These effects include damage to the gills, reduction of the blood's capacity to carry oxygen due to a lowered pH, an increased oxygen demand, and histological damage to the blood cells and the cells producing tissues (Smart 1978). Sublethal and lethal ammonia levels can cause histological changes in the kidneys, liver, spleen, thyroid tissue, and blood parameters of many fish species (Smith and Piper 1975).

Effects on disease are difficult to assess but it is reasonable to expect that an aquatic animal will be more susceptible to disease if suffering the additional stress associated with high ambient ammonia concentrations.

The 96-h LC50 value of un-ionized ammonia ranges from 0.4 to 3.1 $mg \cdot l^{-1}$ for fish (Colt and Tchobanoglous 1976; Ball 1967), 0.40 to 2.31 $mg \cdot l^{-1}$ for crustaceans (Armstrong et al. 1978; Delistraty et al. 1977; Wickins 1976), and 3.3 to 6.0 $mg \cdot l^{-1}$ for marine molluscs (Epifanio and Srna 1975).

Effects of ammonia on growth of aquatic animals is difficult to assess quantitatively and is of minor concern in a lobster holding facility.

Nitrite toxicity in fish is due in part to the oxidation of hemoglobin to ferrihemoglobin or methemoglobin (MHb) by nitrite (Krous et al. 1982). Methemoglobin does not have the capacity to carry oxygen; if suffient methemoglobin is formed hypoxia and cyanosis may result (Kiese 1974). The dominant respiratory transport pigment in malacostracan crustaceans,

such as Homarus, is hemocyanin (Picket et al. 1966). It is likely that the same oxidation reaction can occur with the copper of crustacean hemocyanin but such conversion has not as yet been reported for this animal class. Although only scanty information exists relating to the toxicity of nitrite and nitrate to lobsters, Hand (1977) reports that Homarus exhibits a much greater tolerance to both inorganic nitrogen species than most other aquatic organisms, most notably freshwater fish. This situation is not surprising as it has been observed that nitrite is significantly less toxic to fish in a seawater environment (Crawford and Allen 1977). Various chemical constituents and processes associated with seawater may be involved in this apparent increased tolerance. Perrone and Meade (1977) suggested that the protective effect of environmental ions may be the result of a competitive inhibition of nitrite uptake through the gills and other integumentory tissues, and/or an increase in plasma and body fluid ion levels. A number of ions (Ca^{++}, Cl^{-}, Na^{+}, and $SO_4^{=}$) have been tested for their effectiveness in reducing nitrite-induced mortality and the formation of MHb, with most findings supporting the suggestion of a competitive inhibition of nitrate uptake at the gills (Crawford and Allen 1977; Wedermeyer and Yasutake 1978; Tomasso et al. 1979). It is likely that a number of other complex factors such as behaviour and biochemical processes are involved in the comparatively high tolerance to nitrite and nitrate exhibited by lobsters but no

literature is available at present relating to these factors.

Although high levels of water quality must be maintained, due to the potential build-up of toxic metabolic wastes and because water discharged from any commercial operation must meet standards set by environmental laws, lobsters can survive in water with comparatively high levels of nitrogenous compounds (Cobb 1976). In fact, they can tolerate concentrations of ammonia, nitrate and nitrite, considerably higher than those acceptable to many other animals in rearing or holding enviroments. Gravitz et al. (1975) reports that for 24 hours, most juvenile lobsters of one to three grams will survive concentrations of total ammonia-N up to 25 $mg \cdot l^{-1}$, if other conditions are optimal. However, longer exposure to ammonia at lower concentrations can result in death. Values of 96h LC_{50} have been estimated at 1.2 $\pm$ 0.1 mg NH_3-N $\cdot l^{-1}$ for 1g lobsters and 1.4 $\pm$ 0.1 mg NH_3-N $\cdot l^{-1}$ for 3g lobsters but no reliable information on concentrations causing chronic toxicity is known to be available (Allen and Johnston 1976). Similar assays with nitrite and nitrate showed these species of nitrogen to be nontoxic at concentrations of 100 and 500 $mg \cdot l^{-1}$ respectively (Hand 1977). In each case though animals that survived the toxicity tests and were allowed to recover in running ambient seawater were found to be far more sensitive upon re-exposure to these compounds.

Despite the relatively inoccuous nature of NO_2^- and NO_3^-, monitoring of these compounds is important in the

evaluation of lobster holding systems, since they act as indicator species of various chemical processes (such as nitrification) that take place in a recirculating seawater system.

Table 1 summarizes the results of this literature review by listing the appropriate water quality parameters to be monitored during evaluations of lobster holding systems and indicates an acceptable level for each parameter. Also listed are potentially lethal values and levels to be expected under natural conditions.

Table 1. Important water quality parameters to be monitored in a live lobster storage system. Values are listed for holding, natural, and potentially lethal conditions.*

Parameter	Holding Conditions	Natural Conditions	Potentially Lethal
Temperature (°C)	6-7	0-25	-2 and 32
pH	8.0-8.3	7.5-8.4	5 and 9
Salinity (ppt)	27	29-34	8 and 45
Dissolved Oxygen ($mg \cdot l^{-1}$)	7-8 or just below saturation	4-10	2 and supersaturation
Suspended Solids ($mg \cdot l^{-1}$)	25	-	-

Nitrogenous Compounds ($mg \cdot l^{-1}$)

Parameter		Holding Conditions	Natural Conditions	Potentially Lethal
NH_3-N**	1st larval stage		to 0.3	1.3
	4th larval stage			3.8
	1st juvenile	1.3		9.4
	12th juvenile			94.0
NO_2-N	juvenile	100, not lethal at 96 hr	0.014	possibly 100
NO_3-N	juvenile	500, not lethal at 96 hr	0.07-21.0	possibly 500

*Figures quoted are referenced in the text.
**Based on LC_{50}, 24-48 hr

4.0 SYSTEM DESCRIPTION

The general system layout and water flow pattern at Pacific Rim Shellfish is outlined in Figure 2. The basic components of the system include 13 fiberglass holding tanks (Figure 3), a water storage reservoir, a centrifugal intake pump and a centrifugal circulation pump, three silica-sand filters (Figure 4), three ultraviolet (UV) sterilizer units (Figure 5), and a refrigeration unit (Figure 6). Both the silica-sand filters and the ultraviolet sterilizers are arranged in a parallel fashion so that each unit receives one-third of the water flow. System components and their specifications are listed in Table 2.

Water flow through the various components originates at the reservoir, which is fed on a demand basis by the intake pump. From the reservoir, water flows through the circulation pump and into the silica-sand filters where much of the particulate matter is removed. After filtration the water is exposed to high intensity ultraviolet light from the UV sterilizers to destroy any microorganisms present. Water is then pumped into the fiberglass holding tanks where it eventually flows by gravity back into the reservoir. The refrigeration unit exists as a self-contained circulation loop. Water is extracted from the reservoir, chilled and returned to the reservoir in a cyclic fashion. A variety of valves and bypass points exist in the water treatment system to

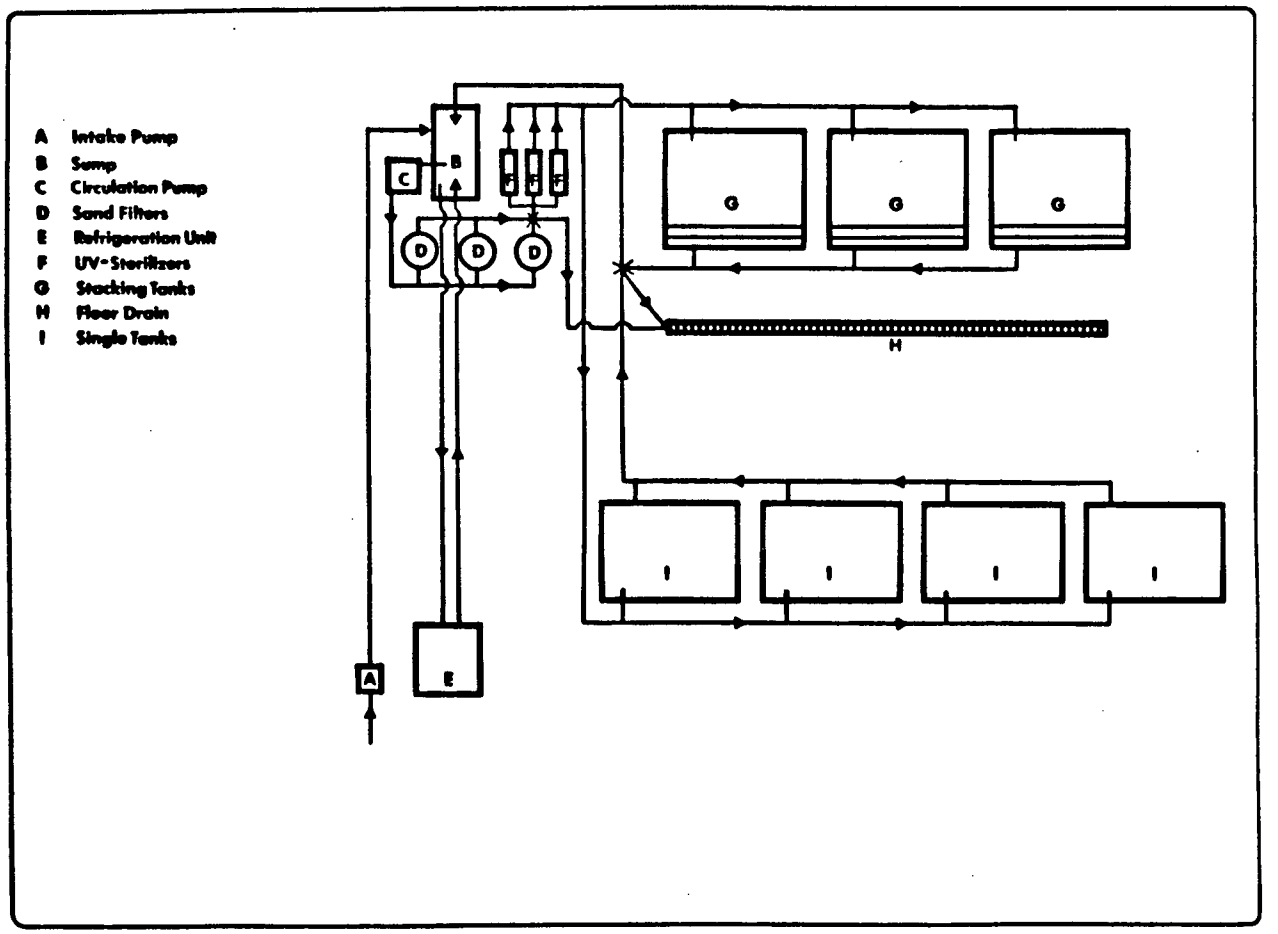

Figure 2. System components and water flow pattern of lobster holding unit.

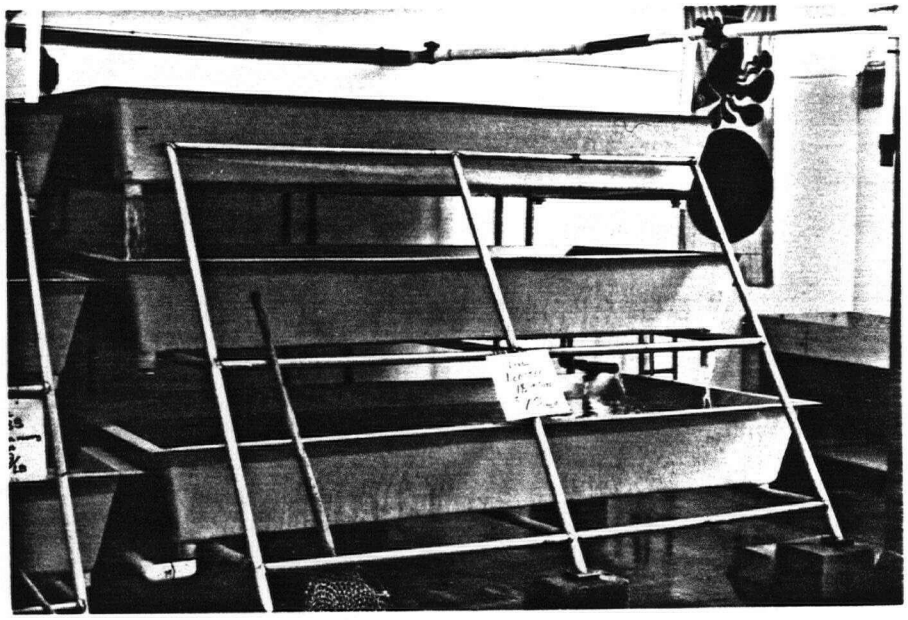

Figure 3. One of the three sets of stacked fiberglass holding tanks used for lobster storage.

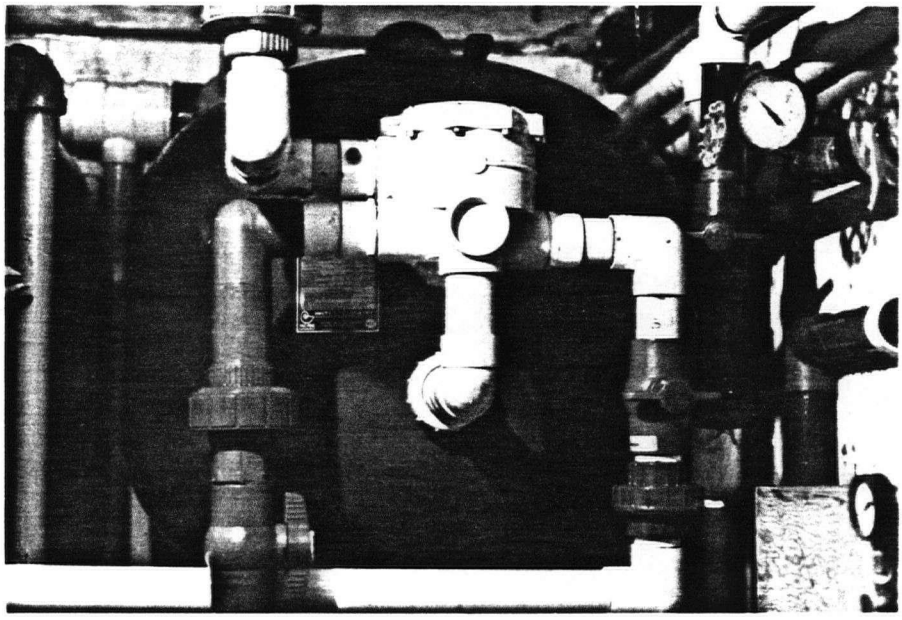

Figure 4. One of three silica sand filters used for treatment of the lobster holding water.

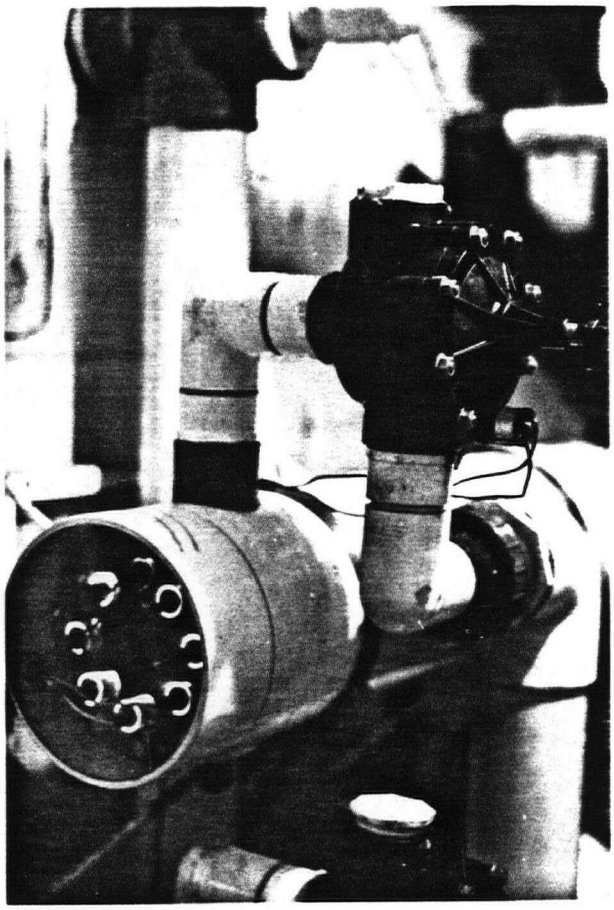

Figure 5. One of the three UV sterilizer units showing circular arrangement of UV lamps.

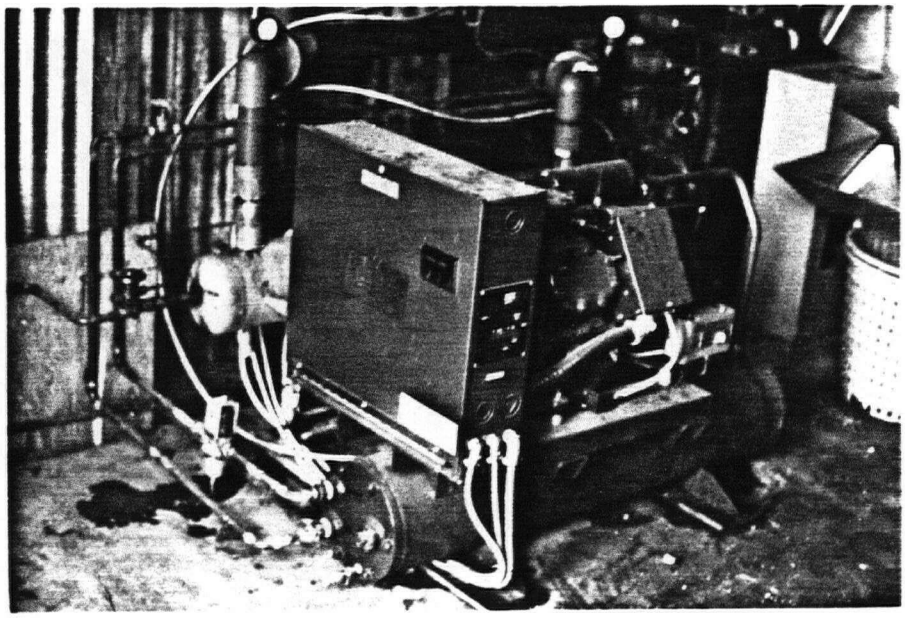

Figure 6. Refrigeraton unit used to maintain lobster holding water temperature.

Table 2: Lobster holding system equipment and specifications.

Component	Specifications
intake pump	0.47 $l \cdot s^{-1}$
centrifugal circulation pump	5.6 kW motor rated at 9.5 $l \cdot s^{-1}$ - actual approximate output 6.5 $l \cdot s^{-1}$
silica sand filters	operating pressure, 345 kPa individual filter area, 0.64 m^2
Ultraviolet sterilizers	60,000 $\mu Ws \cdot cm^{-1}$ at 2537 A
refrigeration unit	560 W motor, Freon-12, rated at 30160 W
fiberglass holding tanks	inside dimensions: 1.37x3.05x0.38 m average water depth: 0.24 m average tank volume: 1003 l
reservoir	inside dimensions: 0.97x3.35x2.64m average volume: 4500 l
average system volume	17,539 l

facilitate filter backwash and component maintenance.

The system functions mainly on a complete recycle basis (one cycle approximately every 45 minutes) with the addition of 'fresh' seawater coming on a demand basis and during filter backwash operations. The filters are backwashed usually once daily resulting in the addition of approximately 10% of the system volume in fresh seawater. Therefore a complete water change occurs every 10 days. Since the 'fresh' seawater obtained from the marine inlet adjacent to the holding operation is of poor quality (i.e. low salinity and usually too warm) energy and materials must be expended to treat intake water. Therefore, in an attempt to conserve energy, water is recycled in the system for as long a period as possible. The water quality problems associated with the intake water exhibit seasonal variability, being of minor concern during the winter months but becoming progressively more acute during spring and summer.

Lobster introduction into the holding tanks at Pacific Rim Shellfish is a routine procedure. Upon arrival of a shipment from the airport (approximately 1000 kg of lobster) the insulated containers holding the animals are sorted according to weight class and moved to a site adjacent to tanks allocated for the particular class. Four weight classes are presently being stocked: 1/ "chix" - one pound; 2/ "quarters" - one and a quarter pounds; 3/ "halves" - one and a half pounds; and 4/ "selects" - two pounds and over. The lobsters are then

individually placed into the holding tanks as quickly as possible. The entire procedure requires approximately 20 minutes depending on the size of the shipment and manpower available.

5.0 MATERIALS AND METHODS

5.1 Introduction

Prior to a detailed description of materials and methods, it is important to point out how seasonal variation in make-up water (False Creek) quality, and operational procedures at the holding facility influenced the empirical portion of this project. In general the situation resulted in uncontrollable background conditions as well as difficulties in isolating individual system components for independent study. A brief outline of some of the operational procedures employed at the facility will aid in illustrating the research problems.

Two geographically distant markets for live lobster have been established by the company, one locally and the other in Japan. As a result lobster storage duration varies with final market destination, the animals destined for Japan may only require holding for one or two days, whereas lobsters held for local distribution may remain in the system for a period of up to several weeks depending on demand. This dynamic supply and demand situation had a definite influence on experimental design. Necessary day-to-day system maintenance such as filter backwashing also affected the timing and design of experiments. In addition water treatment equipment upgrading and replacement made controlled experimentation very difficult. A prime example of this type of situation resulted from the upgrading of the refrigeration system after one season of operation when it

became obvious that the original unit was inadequate during the warmer months.

Fluctuating intake water quality, resulting from seasonal changes in the natural sea water supply, also had a significant impact on the investigations carried out during this study. The most consequential and chronic problem was that of fluctuating salinity levels. Temperature control of the intake water proved to be very difficult during the first year of operation at the holding facility, contributing directly to high lobster mortality at times and complicating experimental design and procedures. The upgrading of the refrigeration system helped to alleviate the intake water temperature problem, but an efficient mechanized method of dealing with the continuously changing salinity levels in the system has not been developed.

These problems associated with water quality investigations at the project site served in part as a basis for the formulation of the materials and methods summarized below. The experiments carried out on the system included an initial test to establish sampling requirements during time-series monitoring, followed by the time-series monitoring, and finally a look at the functional capabilities of two system components, the silica sand filters and the ultraviolet sterilizer unit. The efficiency and functional capability of the refrigeration unit was not examined during this investigation but is included in a detailed independent study of heat

balances on site at the lobster holding facility (Monk 1980). A test was also run to establish a relationship between total organic carbon content and chemical oxygen demand of the lobster holding water.

5.2 Sampling Requirements

Thirteen holding tanks were in use at the study site during the research outlined in this thesis. In order to establish the number of water samples required from the system during the time-series monitoring experiments it was essential to determine how the chemical parameters, ammonia, nitrate, and nitrite varied from tank to tank and within individual tanks. This was accomplished by comparative chemical analysis of a number of water samples taken at approximately the same time from five common locations in each tank. Samples were analysed for ammonia-N (total ammonia) only. Ten samples were taken, two from each of five locations in 12 of the 13 tanks for a total of 120 samples. The 12 tanks included the 9 stacked tanks and 3 of the 4 unstacked tanks. One of the unstacked tanks was randomly omitted to create a balanced experimental design. The five sample locations included the four corner areas and the centre, with samples at all locations coming from mid-water. All samples were collected when the system was operating at full holding capacity. The results were statistically analysed and a decision on sample requirements was made. The objective in the statistical analysis was to find the most

suitable tank(s) and location with the tanks to sample. This was accomplished by carrying-out a three-level nested analysis of variance as outlined in Sokal and Rohlf (1969). The three factors that were tested included: the stacked versus the unstacked tank format; tank position - top, middle, bottom or alone; and the location within each tank.

All nitrogen analyses required for this experiment and the other experiments outlined in this section were performed in duplicate on a Technicon Auto Analyser II(R). The methods used are outlined in Technicon Auto Analyzer II. Industrial Method No. 33-69W (1969) and Technicon Auto Analyzer II. Industrial Method No. 98-70W (1971). Detection limits for ammonia-N, nitrite and nitrate determinations on the auto analyser are $0.2\ mg \cdot l^{-1}$, $0.04\ \mu g \cdot l^{-1}$ and $0.04\ mg \cdot l^{-1}$ respectively. Respective coefficients of variation are 0.25% for ammonia-N, 0.95% for nitrite and 1.8% for nitrate. The following convention will be used in reporting the results obtained from nitrogen analyses:

NH_3-N = un-ionized ammonia nitrogen

NH_4^+-N = ionized ammonia (ammonium) nitrogen

ammonia-N = un-ionized + ionized ammonia nitrogen

NO_2^--N = nitrite as nitrogen

NO_3^--N = nitrate as nitrogen

In most instances throughout this document ammonia values are reported as ammonia-N due to minimal pH influence on the formation of NH_3-N. For example when pH, temperature and

salinity values of 7.7, 17°C and 15°/oo respectively are used in the calculation of percent NH_3-N ionized to NH_4^+-N (Bower and Bidwell 1978), a maximum value of 1.40% results, with an overall acid hydrolysis constant of ammonium ions in the seawater (pKa^s) of 9.55.

5.3 Total Organic Carbon vs Chemical Oxygen Demand

A literature review revealed that virtually no information exists regarding total organic carbon (TOC) levels in lobster holding water. Some comparative data have been reported on solids characteristics and chemical oxygen demand (COD) of lobster waste (Iverson 1973) but not on the holding water. Therefore, a series of tests were run to establish background levels of TOC and COD in the lobster holding water prior to use of the TOC analysis in the time-series experiments. Results obtained were used to examine the relationship between these two parameters for compartive purposes.

Water samples used for the test were collected from the holding system during a variety of biomass loading conditions in an attempt to secure a range of TOC and COD levels. Three samples were collected at each loading level and analysed in duplicate for TOC using a Beckman Model 915 total organic carbon analyzer, and for COD using the method described by Burns and Marshall (1965) which involves the use of additional mercuric sulfate for the suppression of chloride oxidation. The total organic carbon analyzer was operated using the

procedures outlined in American Public Health Association et al. (1976). Prior to injection into the analyzer with a Hamilton automatic hypodermic syringe, the samples were thoroughly homogenized. All samples were analysed within one hour of collection.

5.4 Time-Series Experiments

The purpose of this set of experiments was to monitor, over time, system response and environmental change resulting from dramatic increases in lobster biomass. These rapid increases in lobster biomass occur during the introduction of large shipments (usually about 1000 kg) from east coast suppliers. The incoming animals are transferred rapidly (within 15 minutes of arrival at the holding facility) into the tanks, typically increasing animal density from 5 or 10 percent to 95 or 100 percent of system capacity.

A total of ten time-series experiments were carried out under a variety of initial background water quality conditions (e.g. temperatures ranging from 7 to 19^{o}C). Except for the first experiment, which ran for 24 hours, duration was set at 12 hours and involved a total of 20 sampling times. Eight samples were collected during the first two hours at 15 minute intervals, followed by a set every 30 minutes for the next two hours, and finally a set every hour for the remaining eight hours. An initial set of samples was obtained prior to each run to establish background values. Sample requirements were

established experimentally as previously outlined with each sample set consisting of four 0.5 l samples obtained from the center of tanks 2, 5, 8 and 12 (Figure 2). Conditions of 100 percent recycle were in effect throughout the system during all the experiments.

The water quality parameters that were monitored during this principle set of experiments were pH, salinity, dissolved oxygen, temperature, ammonia-N, nitrate-N, nitrite-N, and total organic carbon. Instruments employed to determine parameter values included a Beckman Chem-Mate portable pH meter, a YSI Model 33 salinity meter, a YSI Model 57 dissoled oxygen meter, a standard alcohol thermometer, a Technicon Auto Analyser II$^{(R)}$ for nitrogen analysis, and a Beckman Model 915 total organic carbon analyser for total organic carbon determinations. Prior to chemical analysis, which took place within 18 hours of collection, all samples were stored in darkness at 4°C.

5.5 Silica Sand Filter Experiments

Mechanical filtering efficency of silica sand filters has been well documented by manufacturers and independent investigators. It was therefore considered redundant to include experiments designed to test this mechanical function during the present study. Instead the filters were evaluated with respect to their performance as biofilters. Along with its primary function of mechanical filtration the filter media acts as a suitable substrate for the growth of nitrifying

bacteria which oxidize ammonia to nitrite and nitrate. In order to evaluate the possible significance of this nitrification process, ammonia, nitrite and nitrate levels in the lobster holding water immediately before and after passage through the silica sand filters were monitored.

On-site tests to determine the appropriate filter medium grain size were conducted prior to the nitrification experiments. A decision to use a #16 silica sand was reached based on a number of parameters including particulate removal efficiency, interstitial blockage, and backwash frequency. Once the medium was installed and the water treatment system was operating at maximum capacity a period of approximately 75 days was allowed for bacterial seeding and population growth prior to experimentation. Included in this conditioning period were five consecutive days in which the ultraviolet sterilizer unit was not operating. Spotte (1970) defined a 'conditioned system' as one in which the nitrifying baceria are in dynamic equilibrium with the routine formation of their energy source.

Duplicate experiments were conducted at three temperatures, 7°, 12° and 17°C, and were scheduled for a period shortly after times of peak ammonia loading in the holding system. The peak period usually occurred within four hours after the introduction of a large lobster shipment, and was of relatively short duration. This period was choosen so that a relatively consistent inflow level of nitrogen compounds could be ensured. Since the three sand filters were arranged in a parallel configura-

tion, each was studied individually during the test periods. It was calculated from the specifications outlined in Table 2 that system turnover time (i.e. the amount of time required for a volume of water equivalent to the approximate volume of the system to pass through the treatment system) was approximately one hour. Therefore, to avoid as much as possible testing previously treated water, experiment duration was set at one hour. Every five minutes during the one hour long experiments duplicate water samples were drawn from the holding water before and after passage through the silica sand filters. Sampling points were conveniently established in the main water lines less than one meter from the filter outflow and inflows. Samples were analysed for NO_3^--N, NO_2^--N, and ammonia-N. Dissolved oxygen and pH were monitored during each experiment in the main holding tanks.

Prior to both experiments at each of the three temperatures a series of 10 samples was collected in rapid succession from one of the input sampling ports and analysed for ammonia-N and NO_3^-N. Standard deviations and coefficients of variation were calculated for each set of results. The objective of this testing was to ensure that changes observed in ammonia-N and NO_3^-N concentrations were not totally a result of individual sample variation.

5.6 Ultraviolet Sterilizer Experiments

This set of experiments was designed to establish the

effectiveness of the ultraviolet sterilizer units at controlling bacterial levels in the lobster holding water. A time-series approach was adopted with test durations varying from four to seven days depending on water temperature. Four experiments were performed, two at 7°C, and one each at 12° and 17°C., An attempt was made to schedule all experiments during periods of minimal biomass fluctuation in the holding tanks so that variations in bacterial production would result only from changes in temperature and the influence of the ultraviolet sterilizers.

To establish the performance characteristics of the ultraviolet unit it was imperative to monitor, over time, bacterial levels in the recycling holding water with and without the influence of the sterilizers. Due to pratical constraints on the use of the entire holding system as an experimental unit a subsystem was constructed to operate without ultraviolet sterilization but at the same time provide all the other primary water treatment functions. A 341 l glass aquarium tank was filled with lobster holding water and placed in one of the main holding tanks, effectively isolating a portion of the holding water while insuring a consistent ambient temperature. The aquarium tank was fitted with a standard air pump and coarse filtration unit to insure adequate dissolved oxygen and particulate removal respectively. After the system had stabilized (i.e. water quality consistent with main system) the aquarium tank was stocked with lobsters at a

density equivalent to that of the main holding system and sampling was initiated.

Duplicate water samples were collected from both the aquarium and the holding tank immediately after lobster introduction and then on a daily basis until bacterial levels reached a level considered potentially harmful to the test animals (approximately 10^4 colonies$\cdot$ml^{-1}). Bacterial levels were determined by standard aerobic plate counts according to methods outlined in International Association of Microbiological Societies (1978) and American Public Health Association (1976).

A number of other experiments and tests of direct practical significance to the successful managing of the live lobster storage facility were conducted throughout the study period. The results obtained from this peripheral work are not reported here but are outlined in part in a separate operations manual submitted to Pacific Rim Shellfish Ltd.

6.0 EXPERIMENTAL RESULTS

6.1 Sampling Requirements

Sample quantity and sampling location for the time-series monitoring experiments was based on a statistical analysis of comparative water samples collected throughout the holding system as outlined in the preceeding section. Generally the variation among samples was very low. The range of ammonia-N values for the 120 samples analysed was from 7.2 and 9.0 $mg \cdot l^{-1}$. Despite this very low range it was felt that an analysis of variance was a worthwhile exercise as it might reveal subtleties about the data that could be useful in determining sample requirements. To determine whether there was significant variance within the holding system the combined results of ammonia-N determinations from the sampling runs were subjected to a three-level nested analysis of variance with unequal sample sizes. Calculations revealed that choice of location within each tank was significant at the 0.01 level (Table 3). Further analysis of the data employing the multiple comparison Student-Newman-Keuls test to compare sample acquisition from the five sample locations was carried out. Results showed that the central sampling position in each tank was the most representative of the five locations tested.

A series of ten water samples collected simultaneously at location 5 (middle of each tank) had a mean ammonia-N value of 8.14 $mg \cdot l^{-1}$ with a standard deviation of 0.07 $mg \cdot l^{-1}$ and a

Table 3. ANOVA table showing results of three-level nested analysis of variance on ammonia-N samples to determine sample requirements.

Source of variation	df	SS	MS	Fs
position	3	0.66	0.22	0.73 ns
Tank (within position)	8	2.37	0.30	1.07 ns
Location (within tank)	48	13.52	0.28	28.0**
error	60	0.58	0.01	
Total	119	17.13		

F.05 [3.8] = 4.07 F.05 [8,48] = 2.14 F.01 [48,60] = 1.89

coefficient of variation (V) of 0.86%, indicating that duplicate sampling during the time series experiment should not be necessary.

In summary, statistical analysis of the measured ammonia-N levels throughout the holding tanks indicated that to obtain a representative picture of water quality conditions in the system, water samples should be collected from the central location in any tank sampled. Since no significant difference between the unstacked and stacked format or position of tank was found, an arbitrary assignment of sample tanks was made. Efficient and adequate coverage of the system should include samples from the three middle tanks in the stacked format and one of the unstacked tanks.

6.2 Total Organic Carbon versus Chemical Oxygen Demand

The relationship between total organic carbon (TOC) and chemical oxygen demand (COD) was based on analysis of water samples collected during four different lobster loading rates in the holding system (Figure 7). TOC levels ranged from a mean low of 51 ppm to a high of 192 ppm, with a COD maximum and minimum of 283 and 42 ppm respectively. With the exception of the lowest concentration, where mean TOC and COD levels were virtually identical, COD levels were consistently greater than TOC values by approximately 30 percent. Triplicate COD determinations showed significantly higher variation than TOC triplicates, as indicated by the range bars associated with

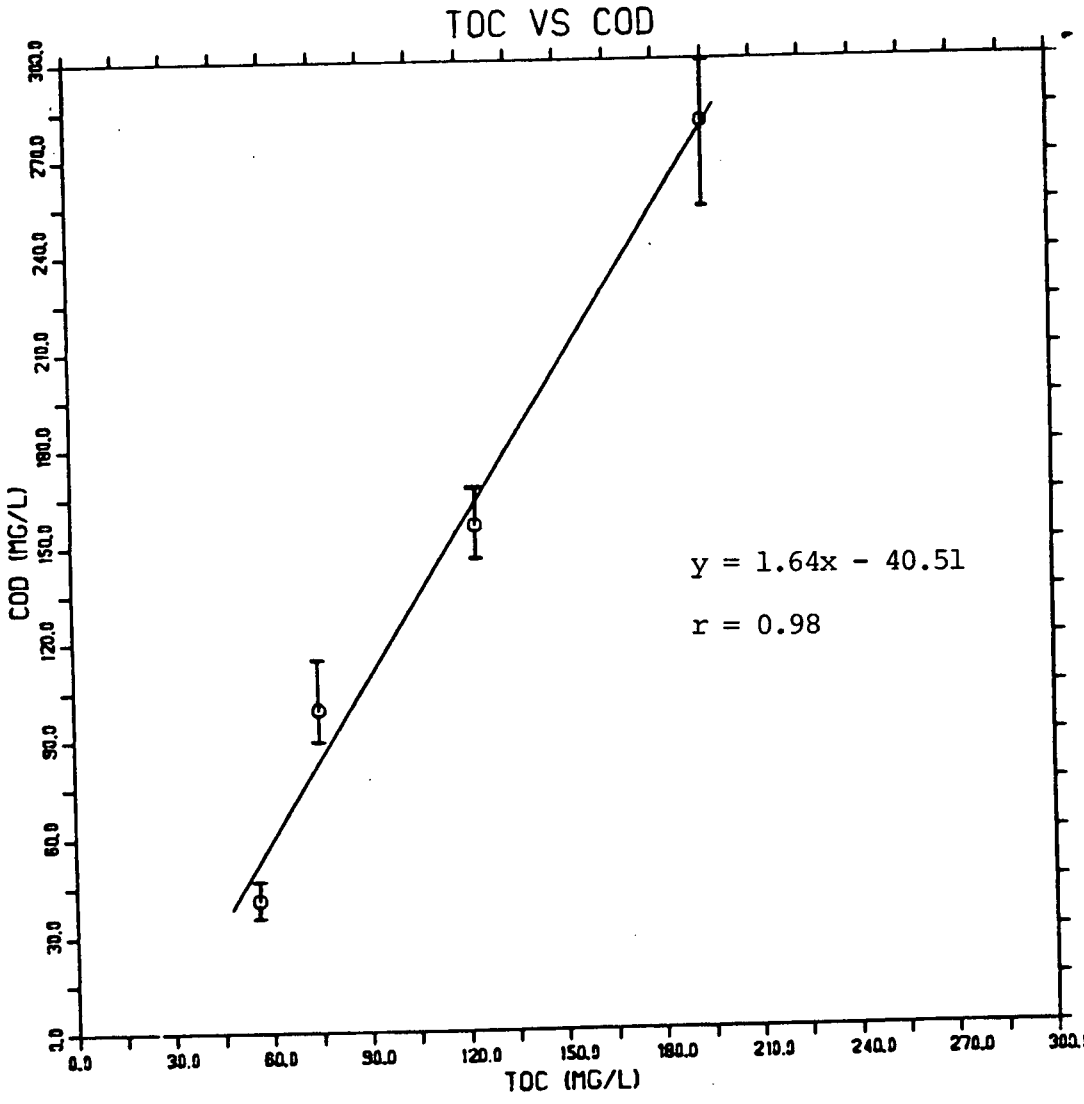

Figure 7. Relationship between TOC and COD of lobster holding water. Range bars are provided for COD concentrations.

each point. Maximum variation was recorded for the three highest COD values, where as the range for the lowest value was small. Since variation for TOC was minimal, range bars are not shown. The COD/TOC ratio, determined from points on the regression line, is 1.67.

6.3 Time-Series Experiments

A summary of conditions for the ten time-series experiments (Table 4) shows that water temperatures varied from a low of 7.0°C during the January, 1980 experiment to a high of 19°C recorded in July of the previous year. Experimental biomass load also varied having a minimum of 675 kg during the January, 1980 experiment, a maximum of 1200 kg in February, 1980 and a mean weight of 962.5 kg $\pm$ 174.4. A closer look at the temperature data shows that experiments were run at basically three water temperatures, a low of between 7.0-8.0°C, a high of 18.0-19.0°C and a group at mid-range temperatures. Salinities remained constant during each experiment ranging from a high of 27 parts per thousand (0/00) during experiment 6 to a low of 17 0/00 during experiment 1.

To avoid repetition and simplify presentation, data from three experiments (1, 3 and 10) representative of the three temperature groups is presented below, the remaining data is listed elsewhere (Appendices 1,2,3,4,5,6). Selection of the three representative data sets was also based on the size of the experimental biomass load. The estimated load was virtually

Table 4. Conditions for time-series experiments.

Experiment #	Date	Time(hr)	Temperature (°C)		Salinity (o/oo)	Biomass Load (kg)	
			Water	Air		Residual	Experimental
1	May 29/79	13:40	13.0	14.0	17	75	1050
2	June 27/79	14:45	18.5	22.0	21	85	820
3	July 21/79	13:45	19.0	26.0	23	105	1100
4	Aug 24/79	16:45	18.7	24.0	21	70	770
5	Sept 14/79	15:50	16.0	21.0	19	55	840
6	Nov 9/79	16:05	12.0	13.0	27	150	1080
7	Jan 14/80	14:35	7.0	9.0	24	50	675
8	Feb 26/80	15:20	8.0	13.0	26	200	1200
9	May 23/80	13:30	7.5	17.0	25	80	980
10	Aug 19/80	16:15	8.0	23.0	28	110	1110

identical for the three experiments choosen, with a mean of 1087 kg and a standard deviation of 32 kg. In some instances additional data sets will be displayed to highlight a specific feature of the results.

Due to technical problems with the inorganic combustion column in the carbon analyser during most of the time-series experiments, only total carbon (TC) values were obtained. Therefore 50 samples of lobster holding water were analysed for TC and total inorganic carbon when the analyser was fully operational and a correction factor developed which allowed for the estimation of TOC from the time-series TC values. TC values ranged from a low of 19.1 to a high of 305.8 $mg \cdot l^{-1}$. The range of inorganic carbon values was 0.1 to 7.8 $mg \cdot l^{-1}$. The mean TOC content of these samples was calculated to be 97.02% with a standard deviation of 1.39 and a coefficient of variation (V) of 1.43%. These results were considered significant based on the low V value and therefore the TOC conversion factor applied to all the TC results from the time-series experiments was set at 0.97.

Representative results from the analysis of TOC during the time-series experiments are presented in Figure 8. Several characteristics are common to the data at all three temperatures. An initial dramatic increase in TOC concentration within the first hour after the lobster introduction was observed in all experiments, followed by a gradual decline in concentration. In most instances TOC values reached a steady

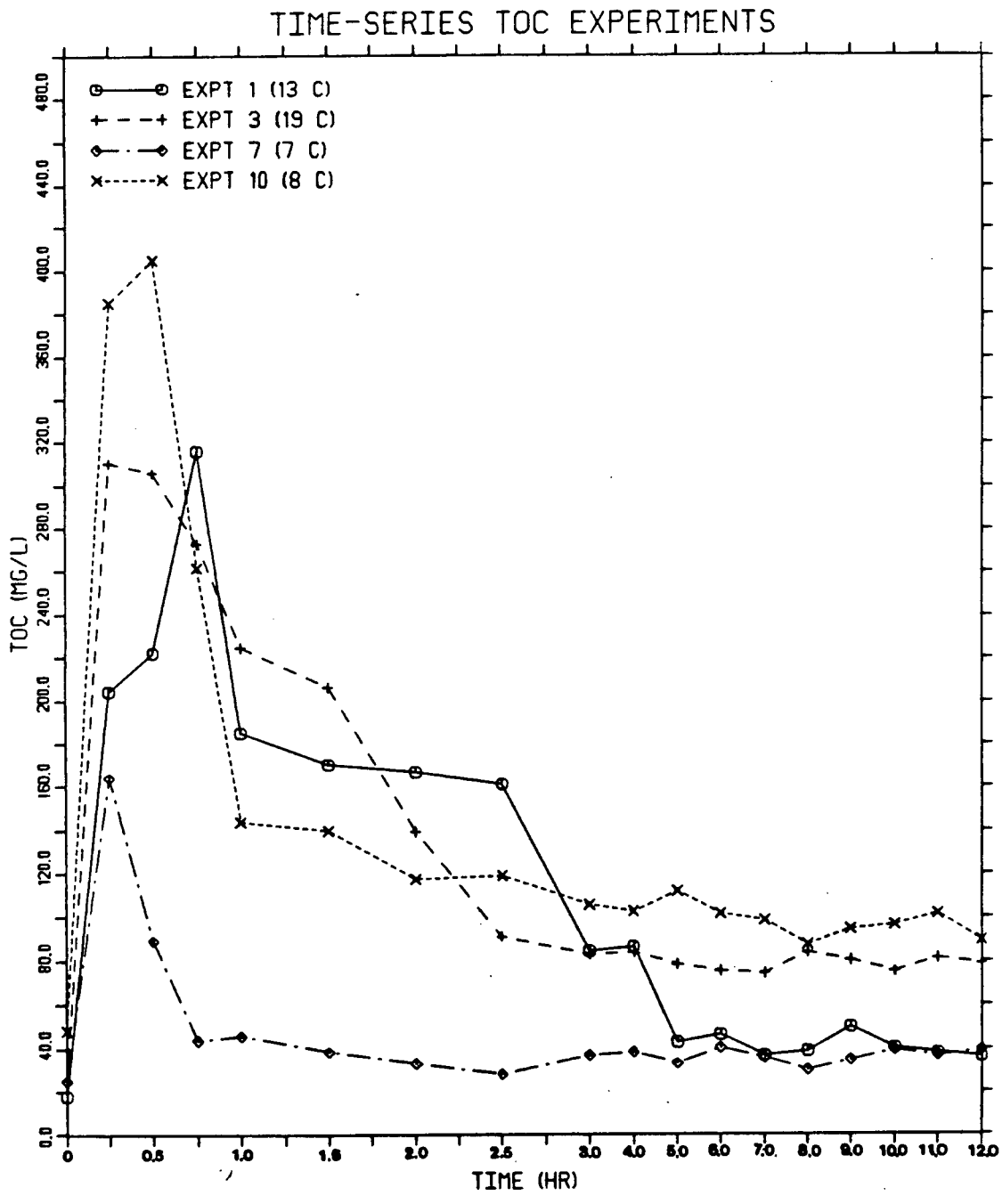

Figure 8. Changes in TOC concentrations recorded during representative time-series experiments. A discontinuous time-scale was used to emphasize changes in TOC concentrations during the first three hours of each experiment.

state at approximately 3 to 4 hours and final TOC levels were all higher than initial (Time 0) values. In experiment number 10 at 8°C the initial TOC concentration was 48.4 $mg \cdot l^{-1}$ with a maximum value during the experiment of 405.1 $mg \cdot l^{-1}$ at 0.50 hours, and a 12 hour level of 89.4 $mg \cdot l^{-1}$. At 13°C in experiment 1 the initial concentration was 18.1 $mg \cdot l^{-1}$, the peak was 316.1 $mg \cdot l^{-1}$ at 0.75 hours and the final, 12 hour, value was 36.1 $mg \cdot l^{-1}$. Experiment 3 was run at 19°C and had an initial concentration of 25.4 $mg \cdot l^{-1}$, a peak of 305.7 at 0.50 hours, and a final value of 78.9 $mg \cdot l^{-1}$.

For comparative purposes data from experiment 7 which was run under much lower biomass loading (approximately half of the previously discussed experiments) conditions is also presented. The initial TOC concentration was recorded at 25.1 $mg \cdot l^{-1}$, the peak value was 164.0 $mg \cdot l^{-1}$ at 0.25 hours, with a final 12 hour value of 39.1 $mg \cdot l^{-1}$.

In all four experiments, DO concentrations in the holding water dropped, in some cases dramatically, within the first 15 to 30 minutes of the runs and then slowly increased to a steady state level which was lower than the initial (Time 0) value (Figure 9). At 19°C (Expt. 3), the highest experimental temperature, an initial DO concentration of 8.1 $mg \cdot l^{-1}$ was recorded. Within 30 minutes after the introduction of the lobster shipment, the DO had dropped to a level of 3.4 $mg \cdot l^{-1}$. Within 2.5 h DO concentrations had reached a steady state at approximately 5.5 $mg \cdot l^{-1}$. This value represents a reduction

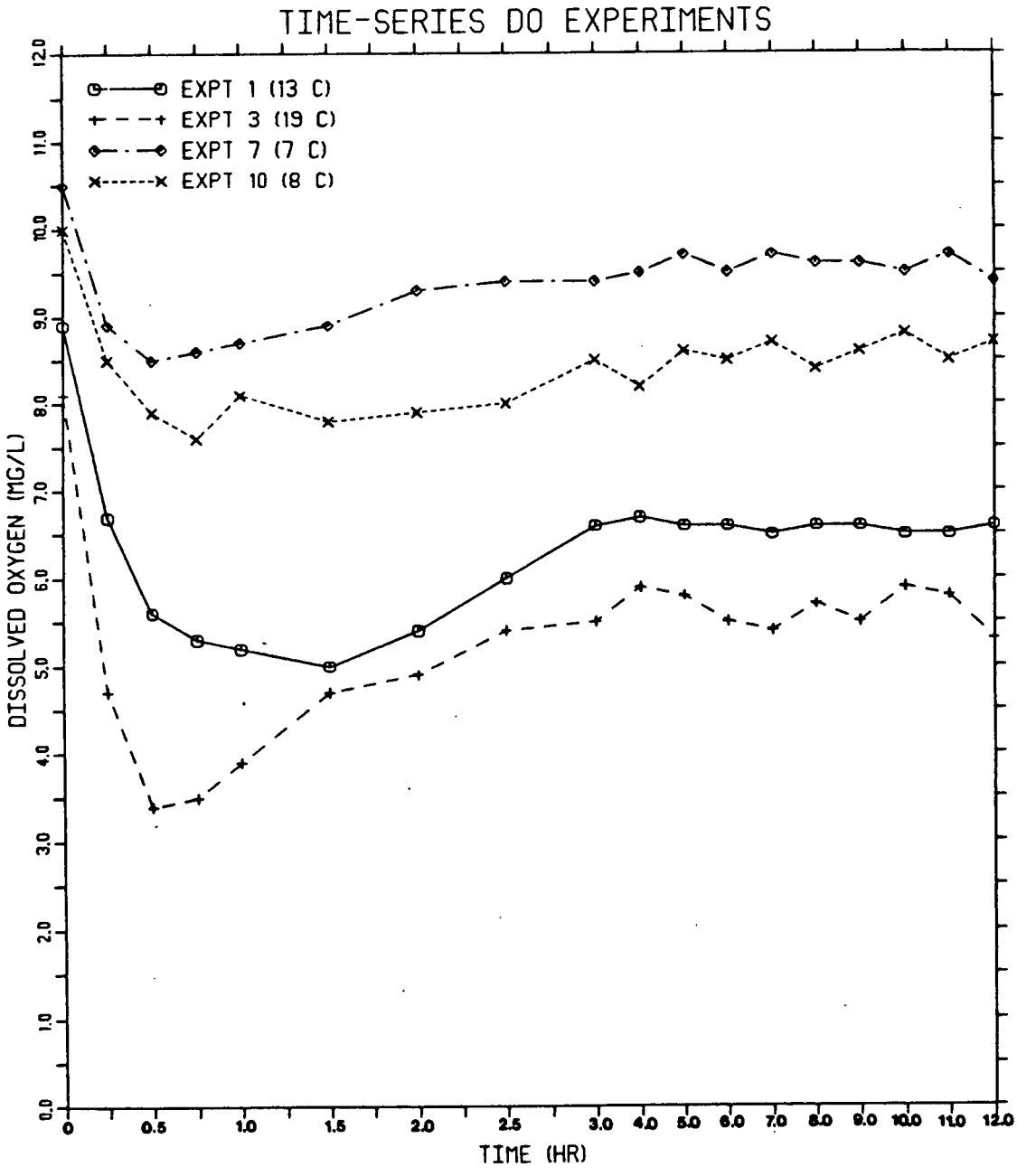

Figure 9. Changes in dissolved oxygen concentrations recorded during representative time-series experiments. A discontinuous time-scale was to emphasize changes in dissolved oxygen during the first three hours of each experiment.

in ambient DO concentration of nearly 3 $mg \cdot l^{-1}$.

At 13°C in experiment 1, the initial DO value was measured at 8.9 $mg \cdot l^{-1}$. A minimum DO concentration of 5.3 $mg \cdot l^{-1}$ occurred within the first 1.5 hours of the experiment. This level was maintained for an additional 0.5 hours at which time the DO concentration began to gradually increase to a final steady state value of 6.6 $mg \cdot l^{-1}$.

Experiment 10 was run at 8°C with an initial DO concentration of 10.0 $mg \cdot l^{-1}$. A decrease in DO was observed during this run reaching a minimum value of 7.6 in 0.75 h. A steady state concentration of approximately 8.5 $mg \cdot l^{-1}$ was reached after 3 h.

The three runs were all carried-out under approximately the same biomass loading conditions. For comparative purposes results from experiment 7, in which a much lower biomass load occurred, are presented. Other than biomass, experimental conditions were similar to those recorded for experiment 10. The initial DO concentration was 10.5 $mg \cdot l^{-1}$ which dropped to a minimum level of 8.5 $mg \cdot l^{-1}$ at the 0.5 h mark in the experiment. From this point DO concentration gradually increased to a steady-state of 9.5 $mg \cdot l^{-1}$.

pH levels measured in the holding water during experiments 1, 3 and 10 are presented in Figure 10. Changes in pH were generally small but detectable with results from experiments at higher temperatures showing changes of greater magnitude. At 8°C in experiment 10 an initial pH of 7.1 was recorded with

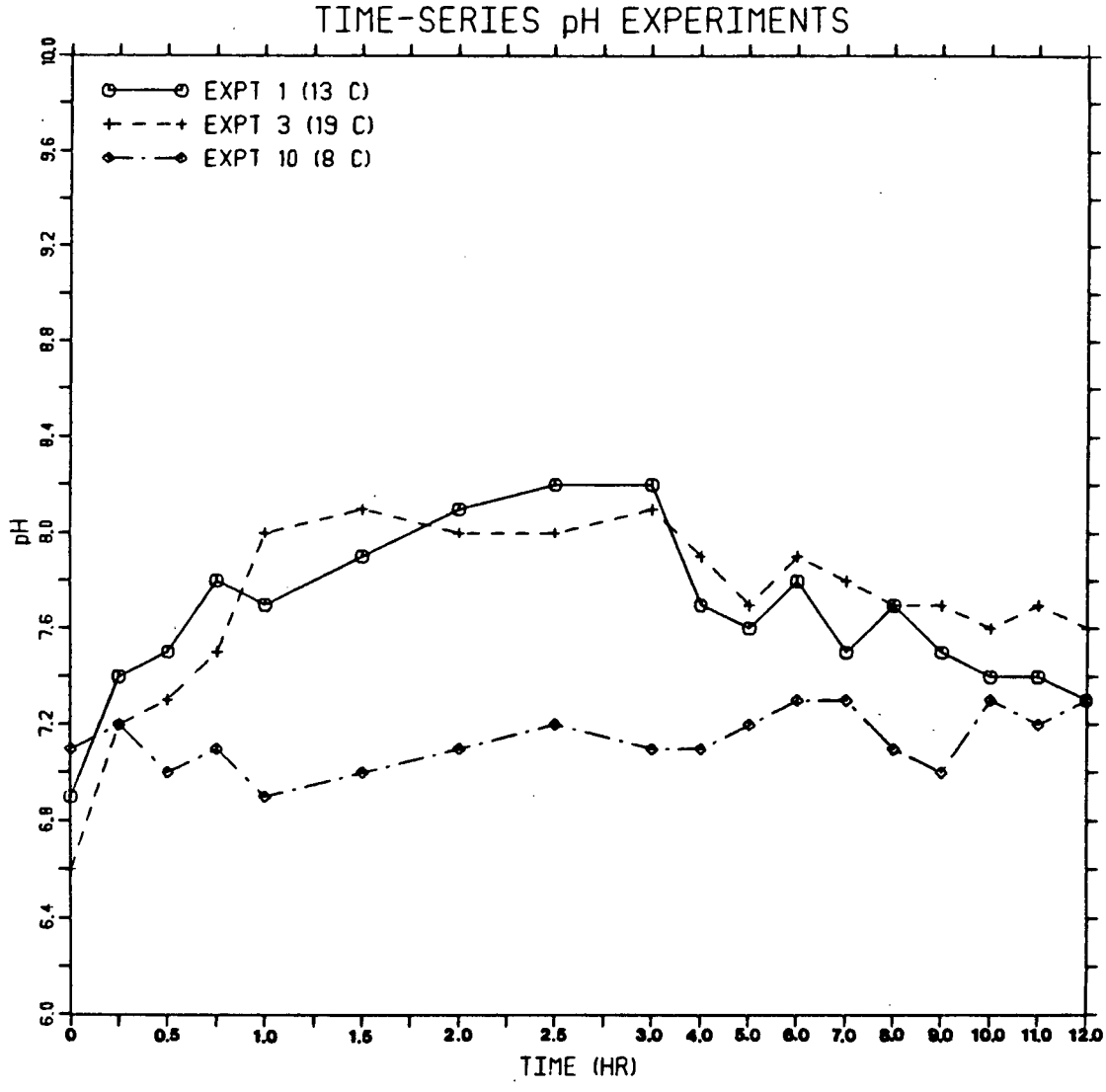

Figure 10. pH changes recorded during representative time-series experiments. A discontinuous time-scale was used to emphasize changes in pH during the first three hours of each experiment.

subsequent measurements showing only minor variation. A maximum and minimum pH of 6.9, at 1 hour, and 7.3, during the final 6 hours of the experiment, respectively, were recorded.

Results from experiment 1 (13°C) show pH beginning to increase immediately after the lobster introduction at Time 0 from a level of 6.9 to a maximum value of 8.2 at 2.5-3.0 h. At this point, pH was observed to decline gradually to a minimum level of 7.3 at the termination of the run.

Initial pH in experiment 3 (19°C) was 6.6. A similar time-series pattern to that observed for experiment 1 was recorded during this run. A substantial increase in pH was observed to take place shortly after the lobsters were introduced into the system. A maximum pH level was reached approximately 1-2 hours after the experiment was initiated, at which point a gradual decline was recorded until the termination of the experiment, when the pH was measured at 7.6. As with the other experiments reported here, the final pH level of the holding water was higher than the level recorded at Time 0 of the experiment.

Ammonia-N, NO_2^- and NO_3^--N concentrations measured during the representative time-series experiments are presented in Figures 11, 12, and 13 respectively. At the lowest temperature of 8°C in experiment 10 the initial ammonia-N concentration was 1.3 $mg \cdot l^{-1}$. A steady increase in ammonia-N was observed for the first 0.75 h. of the experiment, followed by a much more gradual increase to the maximum level of 2.3

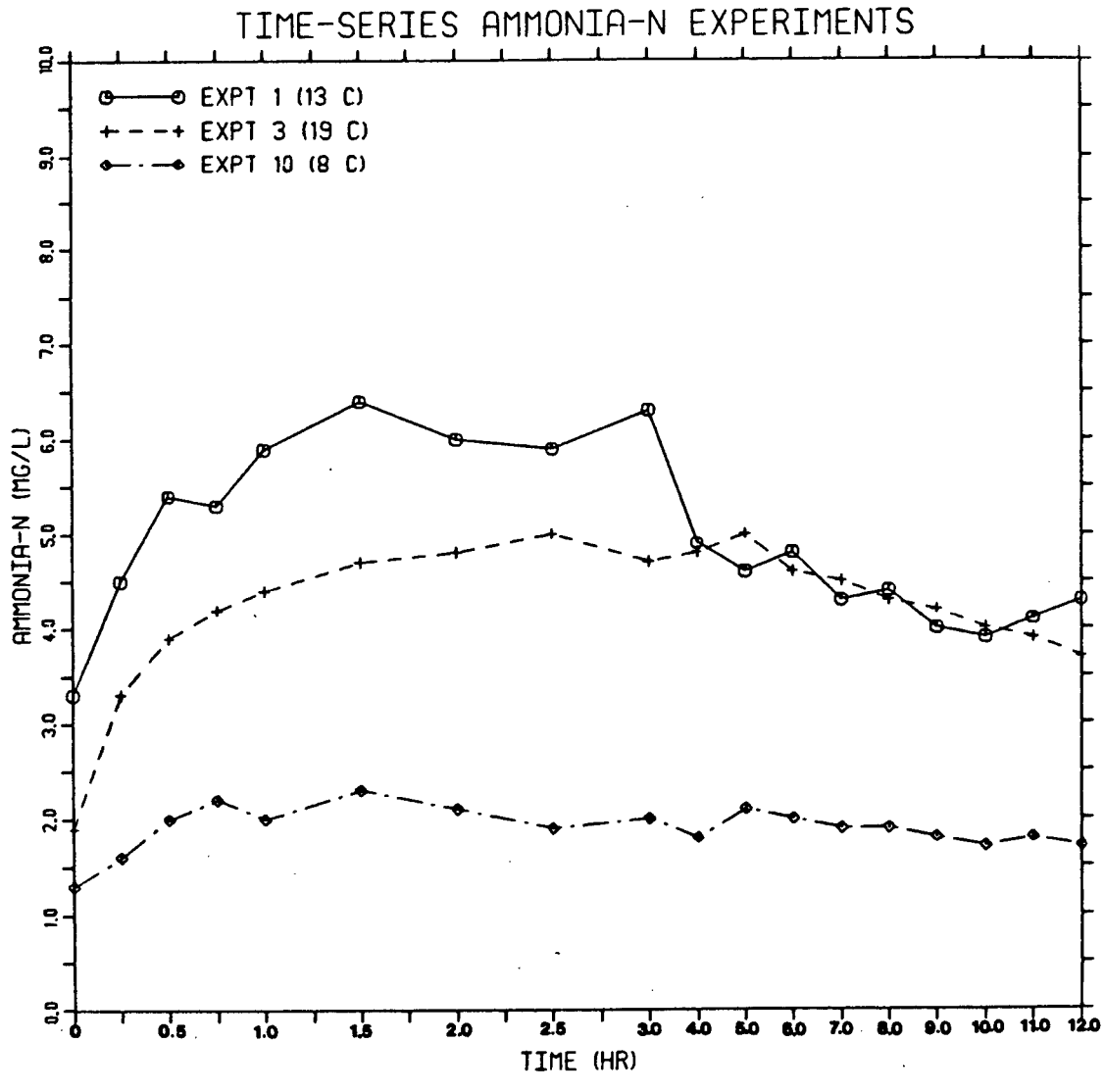

Figure 11. Changes in ammonia-N concentrations recorded during representative time-series experiments. A discontinuous time-scale was used to emphasize changes in ammonia-N concentrations during the first three hours of each experiment.

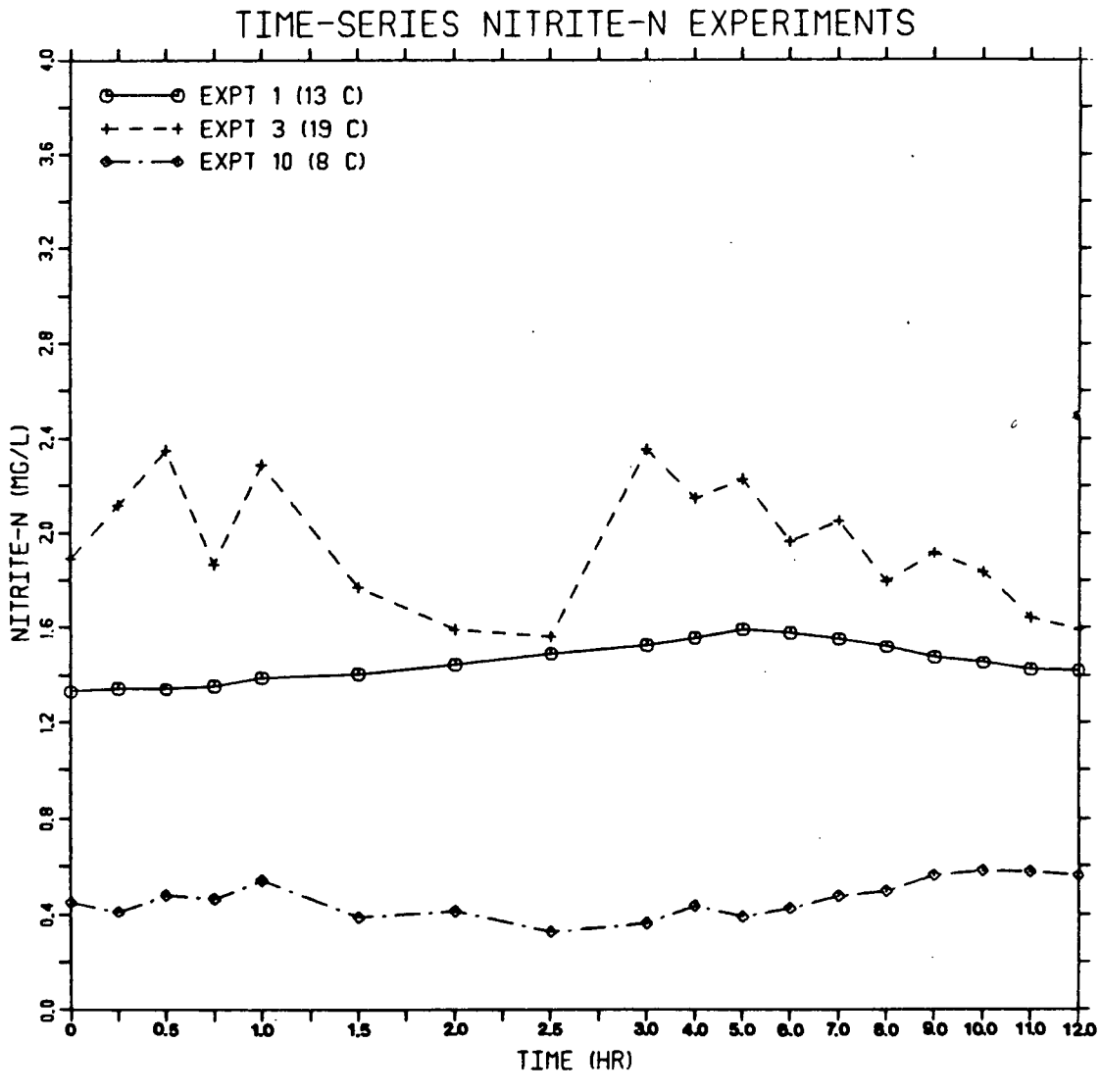

Figure 12. Changes in nitrite-N concentrations recorded during representative time-series experiments. A discontinuous time-scale was to emphasize changes in nitrite-N during the first three hours of each experiment.

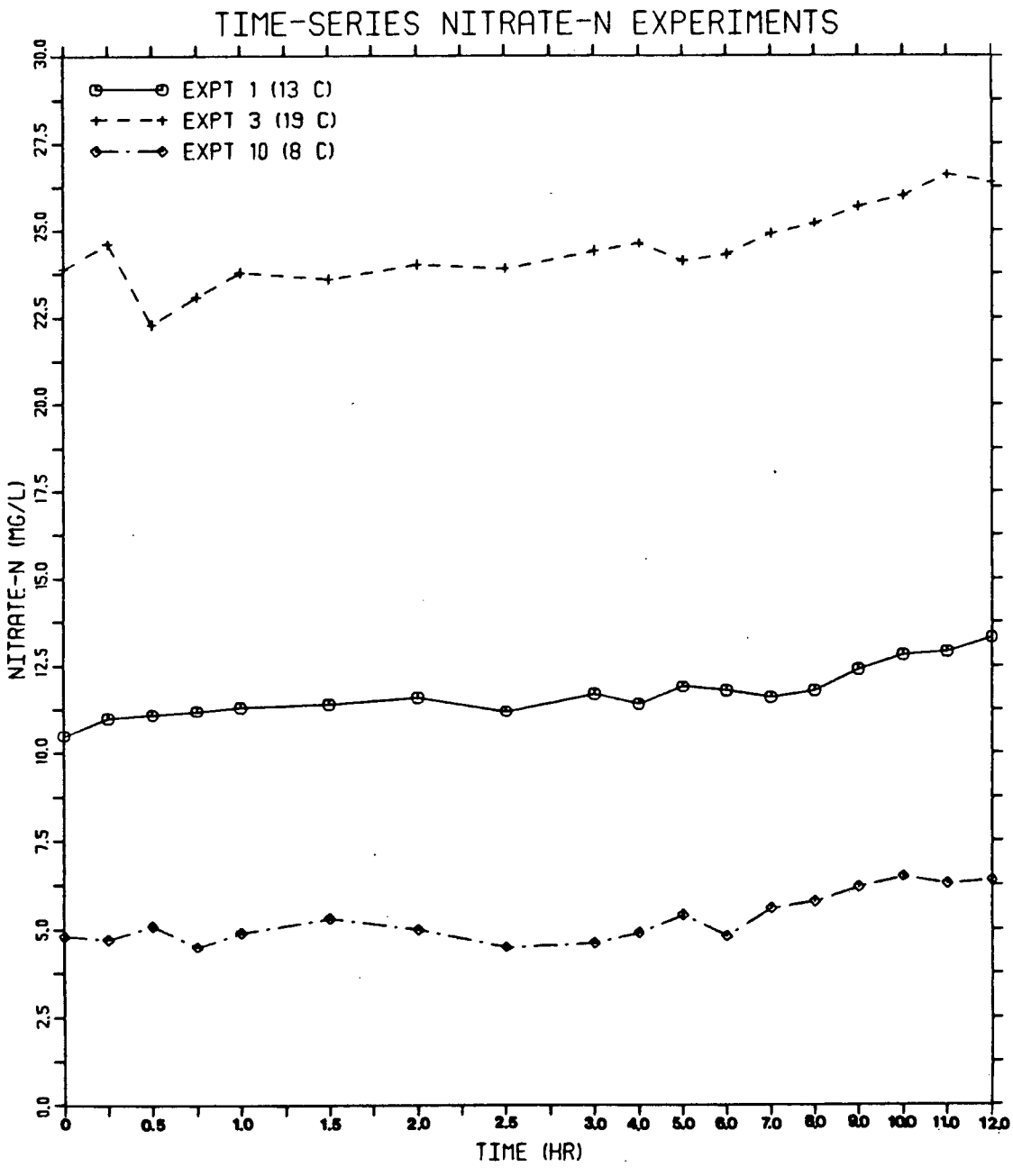

Figure 13. Changes in nitrate-N concentrations recorded during representative time-series experiments. A discontinuous time-scale was used to emphasize changes in nitrate-N during the first three hours of each experiment.

$mg \cdot l^{-1}$, recorded at 1.5 h. From this point ammonia-N concentrations gradually declined to 1.7 $mg \cdot l^{-1}$ at 12 h.

At 13°C in experiment 1 the ammonia-N level was recorded at 3.3 $mg \cdot l^{-1}$ at time 0. Immediately after the introduction of the lobsters ammonia-N began to increase in concentration rapidly to a maximum level of 6.4 $mg \cdot l^{-1}$ at 1.5 h. This concentration was maintained in system for an additional 1.5 h. at which point a decline was observed. The most dramatic decline was recorded over the one hour period from hour 3 to hour 4 when the concentration dropped from 6.3 to 4.9 $mg \cdot l^{-1}$. A general decline in ammonia-N concentration was observed for the remainder of the experiment to a final level of 4.3 $mg \cdot l^{-1}$.

An initial ammonia-N concentration of 1.9 $mg \cdot l^{-1}$ was recorded for experiment 3 at 19°C. Results were similar to those produced during experiment 1 as ammonia-N increased rapidly immediately after the lobser introduction to a maximum level of 5.0 $mg \cdot l^{-1}$ at 2.5 h, at which point a gradual decline was observed to a minimum level of 3.7 $mg \cdot l^{-1}$ at 12 h.

NO_2^--N concentrations measured during the time-series experiments are presented in Figure 12. In experiments 10 and 1 at 8°C and 13°C respectively, NO_2^--N concentrations remained relatively constant throughout the experiments. In other cases fluctuations were less than 0.5 $mg \cdot l^{-1}$. At 19°C fluctuations in NO_2^--N concentration were much more pronounced. A maximum NO_2^--N value of 2.35 $mg \cdot l^{-1}$ was

recorded at 3.0 h, at which point a slow gradual decline was observed which continued until the termination of the run.

NO_3^--N results for the three experiments are all similar in that NO_3^--N concentrations remained basically constant for the first 5 or 6 hours of each experiment at which point levels began to increase gradually. At 8°C NO_3^--N remained at approximately 5 $mg \cdot l^{-1}$ for 6 hours when it began to increase to a maximum level of 6.4 $mg \cdot l^{-1}$ at hour 12. The intial NO_3^--N concentration at 13°C was 10.5 $mg \cdot l^{-1}$. The gradual increase in NO_3^--N throughout the experiment resulted in a final value of 13.3 $mg \cdot l^{-1}$ at hour 12. At 19°C the initial and final NO_3^--N concentrations were 23.9 and 26.6 $mg \cdot l^{-1}$ respectively.

6.4 Silica Sand Filter Experiments

Results reported from this series of experiments are mean values calculated from duplicate samples taken at each sampling period.

To establish levels of sample variation a series of 10 water samples were taken and analysed for ammonia-N and NO_3^--N prior to each experiment as outlined in Section 5.5. The mean, standard deviation and coefficient of variation were calculated for each set of results and are presented in Table 5 for ammonia-N and Table 6 for NO_3^--N. In all cases the coefficient of variation (the standard deviation expressed as a percentage of the mean value) was less

Table 5. Mean ($\bar{Y}$) with 95% confidence intervals, standard deviation (S.D.) and coefficient of variation (V) calculated for ammonia-N levels measured in sample series collected prior to silica sand filter experiments.

Temp.(°C)	Expt. #	$\bar{Y}$ ($mg \cdot l^{-1}$)	S.D.($mg \cdot l^{-1}$)	V (%)
7	1	0.4 ± 0.02	0.01	2.5
	2	1.3 ± 0.22	0.11	8.5
12	1	1.5 ± 0.18	0.09	6.0
	2	7.6 ± 1.35	0.69	9.1
17	1	13.6 ± 2.63	1.34	9.9
	2	6.3 ± 0.71	0.36	5.7

Table 6. Mean ($\bar{Y}$) with 95% confidence intervals, standard deviation (S.D.) and coefficient of variation (V) calculated for NO_3-N levels measured in sample series collected prior to silica sand filter experiments.

Temp.(°C)	Expt. #	$\bar{Y}$ ($mg \cdot l^{-1}$)	S.D.($mg \cdot l^{-1}$)	V (%)
7	1	16.4 ± 2.57	1.31	8.0
	2	24.8 ± 3.41	1.74	7.0
12	1	19.7 ± 1.55	0.79	4.1
	2	23.9 ± 2.00	1.02	4.3
17	1	4.3 ± 0.22	0.11	2.6
	2	29.5 ± 3.06	1.56	5.3

than 10%.

The silica sand filter experiments were designed to detect, and if possible quantify, any nitrification activity taking place in the filter beds during periods of heavy ammonia loading. To achieve this objective ammonia-N, NO_3^--N and NO_2-N levels in the holding water were monitored before and after passage through the sand filters at three temperatures (7, 12 and 17°C). Dissolved oxygen (DO), pH and salinity were monitored in the main holding tanks and a summary of the results is given in Table 7. Salinity levels remained constant during each experiment but fluctuated between duplicate experiments, for example salinities were 25 and 28 ppt for experiments 1 and 2 respectively at 7°C. Maximum and minimum values are presented for dissolved oxygen and pH with both pH and DO maxima occuring usually during the initial phase of each experiment and the minimum at some point close to the end of the run. A DO drop over time was recorded for experiments at each of the three tempertures. For both runs at 7°C the DO drop was minor going from 9.5 to 9.1 $mg \cdot l^{-1}$ during the first experiment and from 9.8 to 8.8 $mg \cdot l^{-1}$ in the second. A somewhat greater drop was recorded for the duplicate runs at 12°C with DO going from 7.3 to 5.9 during the first experiment and from 7.5 to 5.0 $mg \cdot l^{-1}$ in the second. At 17°C maximum DO values were generally low due to the low carrying capacity of the water at that temperature. A minimum DO drop from 4.8 to 4.1 $mg \cdot l^{-1}$ was recorded for the first experiment at 17°C, with a much more substantial loss recorded for the second run as DO went

Table 7. Summary of dissolved oxygen (DO), pH and salinity levels measured during the silica sand filter experiments.

		D.O. ($mg \cdot l^{-1}$)		pH		
Temp.(°C)	Expt.#	max	min	max	min	Salinity (o/oo)
7	1	9.5	9.1	8.1	7.2	25
	2	9.8	8.8	7.7	6.9	28
12	1	7.3	5.9	6.9	6.4	19
	2	7.5	5.0	7.5	6.6	23
17	1	4.8	4.1	7.8	6.7	21
	2	6.1	3.3	7.9	6.8	25

from 6.1 to 3.3 $mg \cdot l^{-1}$. Except from the first experiment at 12°C, pH drops were all in the order of one pH unit over the one hour test period.

Although ammonia-N, NO_3^--N and NO_2^--N were all measured during these experiments only ammonia-N and NO_3^--N values are presented in this section. Although some minor fluctuation in nitrite level was measured during several experiments, concentrations of this parameter tended to remain fairly stable throughout the duration of each run. As a result, these data are excluded from the graphical presentation. Initial data analysis has involved determining whether ammonia-N and NO_3-N concentratons in the holding water have increased or decreased after passage through the three filters at each temperature. During the process of nitrification ammonia-N is oxidized to form nitrate-N thus decreasing the concentration of ammonia and increasing the level of nitrate. This conversion is based on the assumptions that the oxidation from nitrite to nitrate is rapid and that no other process, such as volatilization, is affecting the concentrations of these nitrogenous compounds. Increasing or decreasing concentrations of ammonia-N and NO_3^--N were then plotted over time for the three filters at each of the test temperatures.

Presentation of the data in this fashion presupposes a relatively constant inflow concentration of nitrogenous compounds throughout the duration of each experiment. As

outlined in a previous section, features were incorporated into the experimental design to ensure that fluctuation was reduced to a minimum. To illustrate the effect of these measures inflow concentrations of ammonia-N, NO_3^--N and NO_2^--N were statistically analysed and the results are presented in Tables 8, 9, and 10 respectively. It is evident from these figures that inflow concentrations remained relatively constant during most experiments. The coefficients of variation (V) for ammonia-N ranged from a low of 1.3% in experiment 1, filter 1, at 7°C to a maximum of 10.5% in experiment 1, filter 1, at 17°C. V values for NO_3^--N inflow samples varied from a low of 1.1% in experiment 2, filter 2, at 17°C to a high of 2.6% in experiment 1, filter 2, at 7°C. NO_2^--N V values ranged from a maximum of 7.0% at 17°C experiment 1, filter 3, to a minimum value of 1.9% in experiment 2, filter 2, at the same temperature.

Figures 14, 15 and 16 show the results from the three filters for experiment number 1 at 7°C. Although minor fluctuations in ammonia-N and NO_3^--N levels were detected in the holding water after passage through filter #1 at this temperature, no general pattern is evident from the results. Initially both ammonia-N and NO_3^--N concentrations begin to rise followed by a decline in both parameters and finally a minor increase in NO_3^--N accompanied by a equally small decrease in ammonia-N. Although the magnitude of the changes was slightly larger, results obtained from filters 2 and 3

Table 8. Initial and final concentrations, range, mean ($\bar{Y}$) with 95% confidence intervals, standard deviation (S.D.) and coefficient of variation (V) of ammonia-N in time series samples taken prior to passage through the silica sand filters.

Temp.(°C)	Expt.#	Filter #	Conc. ($mg \cdot l^{-1}$) Initial	Final	Range ($mg \cdot l^{-1}$) Max	Min	$\bar{Y}$		S.D.	V (%)
7	1	1	0.7	0.6	1.3	0.6	0.9	±0.06	0.03	3.3
		2	0.5	0.8	0.9	0.3	0.6	±0.02	0.01	1.7
		3	0.9	0.9	1.1	0.5	0.8	±0.02	0.01	1.3
	2	1	1.7	1.8	2.1	1.4	1.7	±0.41	0.21	12.4
		2	1.8	1.5	2.0	1.2	1.7	±0.53	0.27	15.9
		3	1.2	1.2	1.5	1.0	1.2	±0.38	0.19	15.8
12	1	1	3.4	3.5	3.8	3.0	3.4	±0.41	0.21	6.2
		2	2.9	3.1	3.5	2.8	3.1	±0.43	0.22	7.1
		3	3.0	2.8	3.4	2.7	3.0	±0.37	0.19	6.3
	2	1	7.4	7.3	8.1	7.0	7.6	±0.59	0.30	3.9
		2	7.0	7.3	7.8	7.0	7.4	±0.57	0.29	3.9
		3	7.3	7.7	7.9	7.0	7.6	±0.53	0.27	3.6
17	1	1	2.1	2.5	2.6	1.9	2.2	±0.41	0.21	9.5
		2	2.9	2.7	2.9	2.1	2.6	±0.47	0.24	9.2
		3	1.8	2.3	2.3	1.7	2.0	±0.41	0.21	10.5
	2	1	3.8	3.3	3.8	3.0	3.4	±0.59	0.30	8.8
		2	3.4	3.1	3.9	2.9	3.4	±0.65	0.33	9.7
		3	3.9	3.8	4.1	3.0	3.6	±0.65	0.33	9.2

Table 9. Initial and final concentrations, range, mean ($\bar{Y}$) with 95% confidence intervals, standard deviation (S.D.) and coefficient of variation (V) of nitrate-N in time series samples taken prior to passage through the silica sand filters.

Temp.(°C)	Expt.#	Filter #	Conc. ($mg \cdot l^{-1}$) Initial	Final	Range ($mg \cdot l^{-1}$) Max.	Min.	$\bar{Y}$		S.D.	V (%)
7	1	1	16.6	17.0	17.2	16.1	16.7	±0.71	0.36	2.2
		2	16.1	17.0	17.0	15.8	16.4	±0.82	0.42	2.6
		3	15.8	16.6	16.7	15.8	16.3	±0.55	0.28	1.7
	2	1	25.4	26.4	26.2	24.3	25.3	±1.06	0.54	2.1
		2	26.1	25.9	26.9	25.3	26.1	±0.84	0.43	1.6
		3	24.8	26.0	26.4	24.8	25.7	±1.04	0.53	2.1
12	1	1	19.1	19.7	19.8	18.8	19.3	±0.62	0.32	1.7
		2	18.9	19.5	19.5	18.7	19.1	±0.51	0.26	1.4
		3	19.4	20.2	20.2	18.9	19.7	±0.67	0.34	1.7
	2	1	31.1	31.7	32.1	30.4	31.4	±0.95	0.50	1.6
		2	33.2	32.9	33.4	31.4	32.5	±1.02	0.52	1.6
		3	31.9	32.1	32.4	30.7	31.5	±1.04	0.53	1.7
17	1	1	18.9	19.7	19.7	18.8	19.2	±0.55	0.28	1.5
		2	19.6	19.3	19.9	18.8	19.3	±0.69	0.35	1.8
		3	19.2	20.4	20.4	18.7	19.5	±0.96	0.49	2.5
	2	1	39.4	39.6	41.0	38.6	39.8	±1.14	0.58	1.5
		2	38.6	39.2	39.2	37.7	38.5	±0.86	0.44	1.1
		3	39.9	39.9	40.8	38.8	39.5	±1.16	0.59	1.5

Table 10. Initial and final concentration, range, mean ($\bar{Y}$) with 95% confidence intervals, standard deviation (S.D.) and coefficient of variation (V) of nitrite-N in time-series samples taken prior to passage through the silica sand filters.

Temp.(°C)	Expt.#	Filter #	Conc. ($mg \cdot l^{-1}$) Initial	Final	Range ($mg \cdot l^{-1}$) Max.	Min.	$\bar{Y}$		S.D.	V (%)
7	1	1	54	56	60	54	56	±3.3	1.7	3.0
		2	59	53	61	50	55	±6.5	3.3	6.0
		3	50	56	58	50	55	±4.7	2.4	4.4
	2	1	74	75	80	69	75	±7.3	3.7	4.9
		2	66	68	74	66	69	±4.7	2.4	3.5
		3	64	71	72	64	69	±4.5	2.3	3.3
12	1	1	105	103	112	99	106	±8.2	4.2	4.0
		2	100	109	110	100	106	±5.9	3.0	2.8
		3	111	108	115	108	111	±4.5	2.3	2.1
	2	1	85	94	99	85	92	±7.4	3.8	4.1
		2	91	93	99	86	93	±6.3	3.2	3.4
		3	89	96	98	87	92	±6.9	3.5	3.8
17	1	1	69	74	77	65	61	±6.5	3.3	4.6
		2	65	72	75	64	69	±6.9	3.5	5.1
		3	61	75	75	61	69	±9.4	4.8	7.0
	2	1	164	163	174	160	168	±8.8	4.5	2.7
		2	171	174	177	165	171	±6.3	3.2	1.9
		3	158	169	174	158	168	±9.6	4.9	2.9

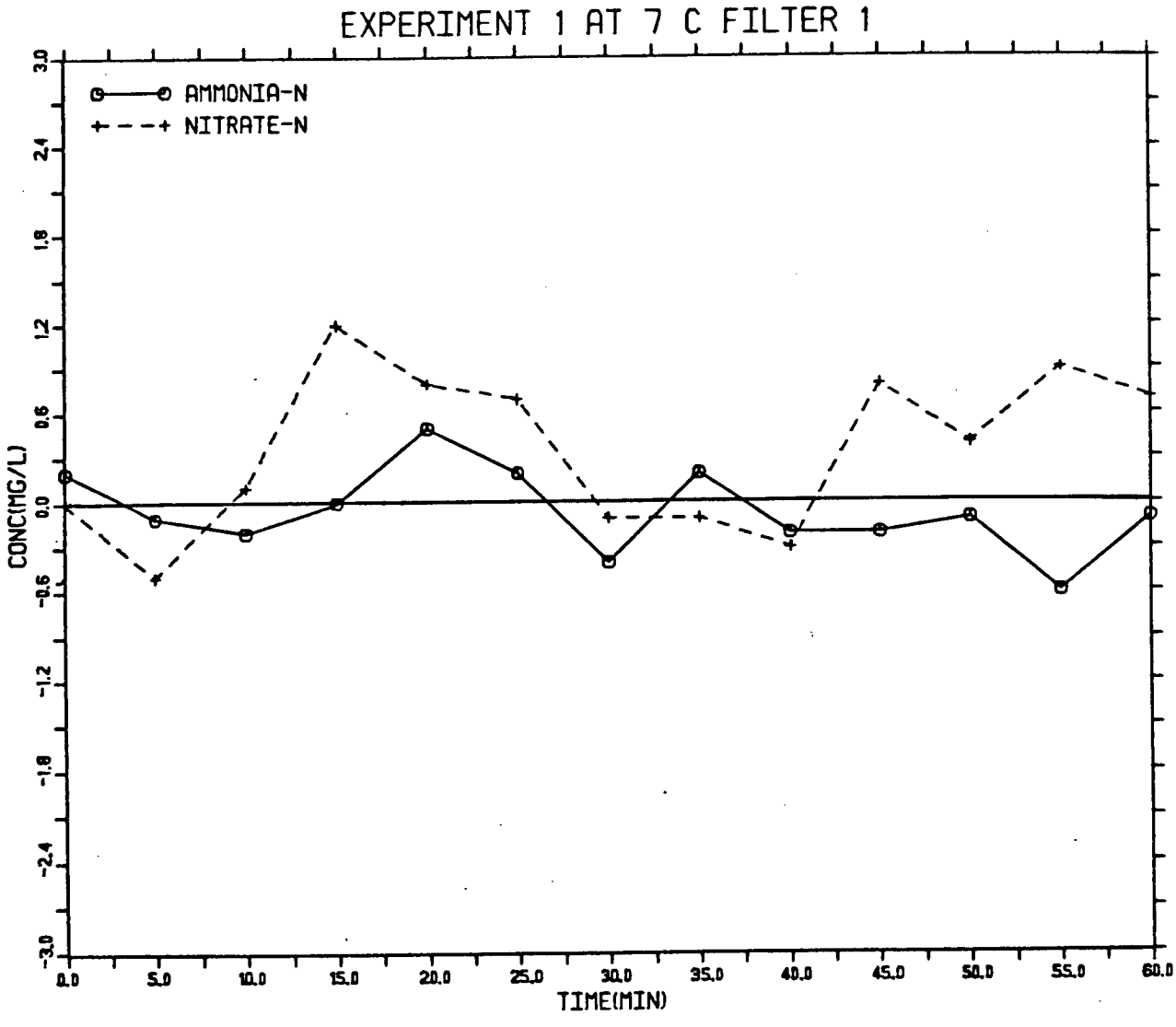

Figure 14. Differences between filter 1 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 7°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

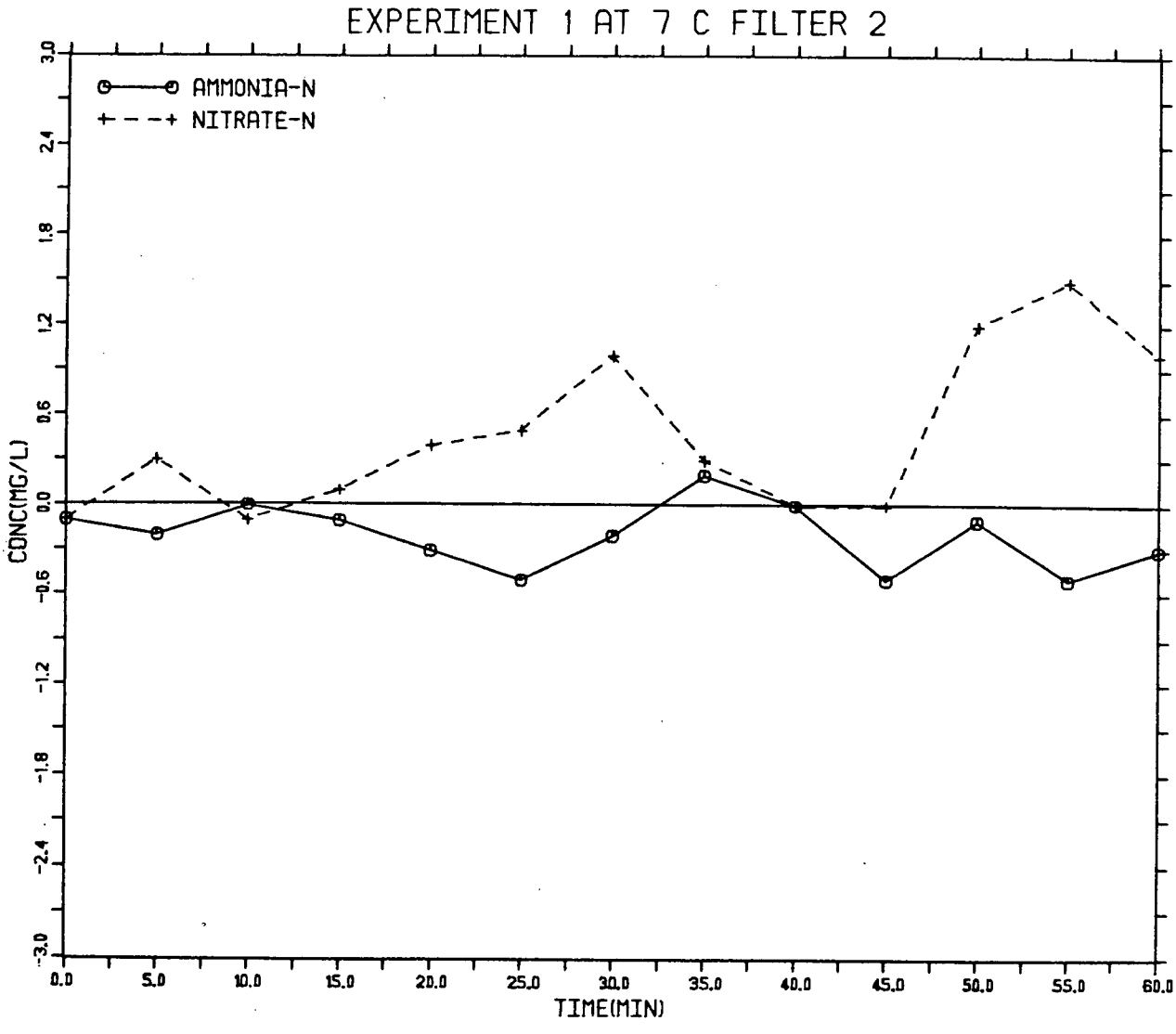

Figure 15. Differences between filter 2 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 7°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

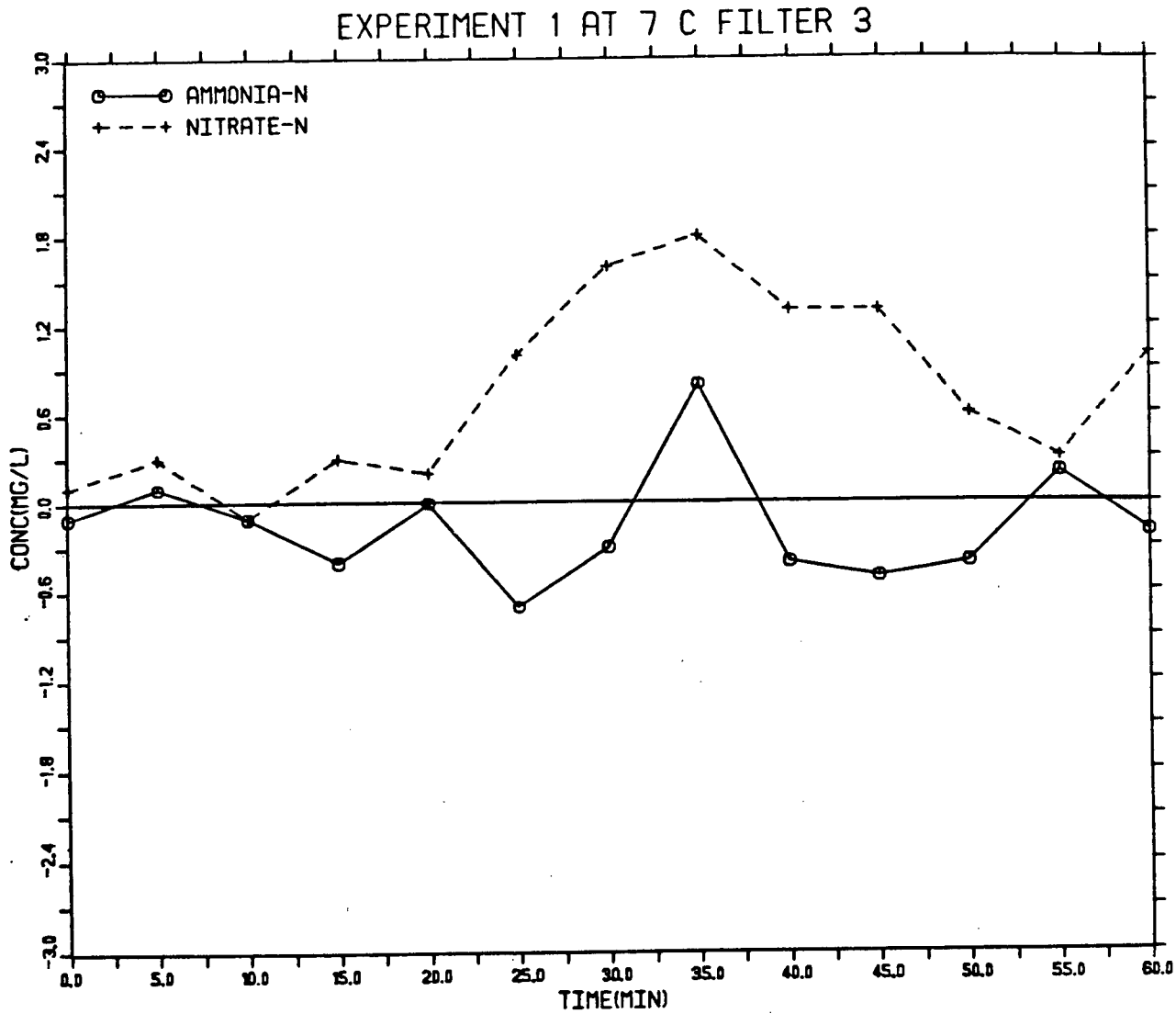

Figure 16. Differences between filter 3 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 7°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

appear similar to those measured for filter 1.

Results from the second experiment at 7°C are presented in Figures 17, 18 and 19. Again, as with the results of the first run at this temperature, there does not appear to be any regular pattern of ammonia-N decrease or NO_3^--N increase over time. This is particularly true for filters 2 and 3 where both ammonia-N and NO_3^--N levels showed almost no change after passage through the filters at the termination of the experiment. It is likely that any changes in the parameter levels measured during the experiment are a result of sample variation.

At 12°C a similar pattern to that at 7°C emerged. Results from the first run at 12°C (Figures 20, 21 and 22) show that ammonia-N and NO_3^--N levels changed very little after passage through filter 1 for the duration of the experiment. Results from filter 2 reveal that ammonia-N and NO_3^--N levels changed very little after passage through the sand for the first 20-25 minutes of the experiment. At this point, NO_3-N outflow concentrations began to increase at approximately the same rate as ammonia-N outflow levels were decreasing. By the end of the experiment ammonia-N discharge concentrations were approximately 1.5 $mg \cdot l^{-1}$ lower than inflow levels. The reverse was true for NO_3^--N. Results from filter 3 showed that ammonia-N and NO_3^--N outflow concentrations remained generally the same as corresponding inflow levels for approximately 35 minutes into the

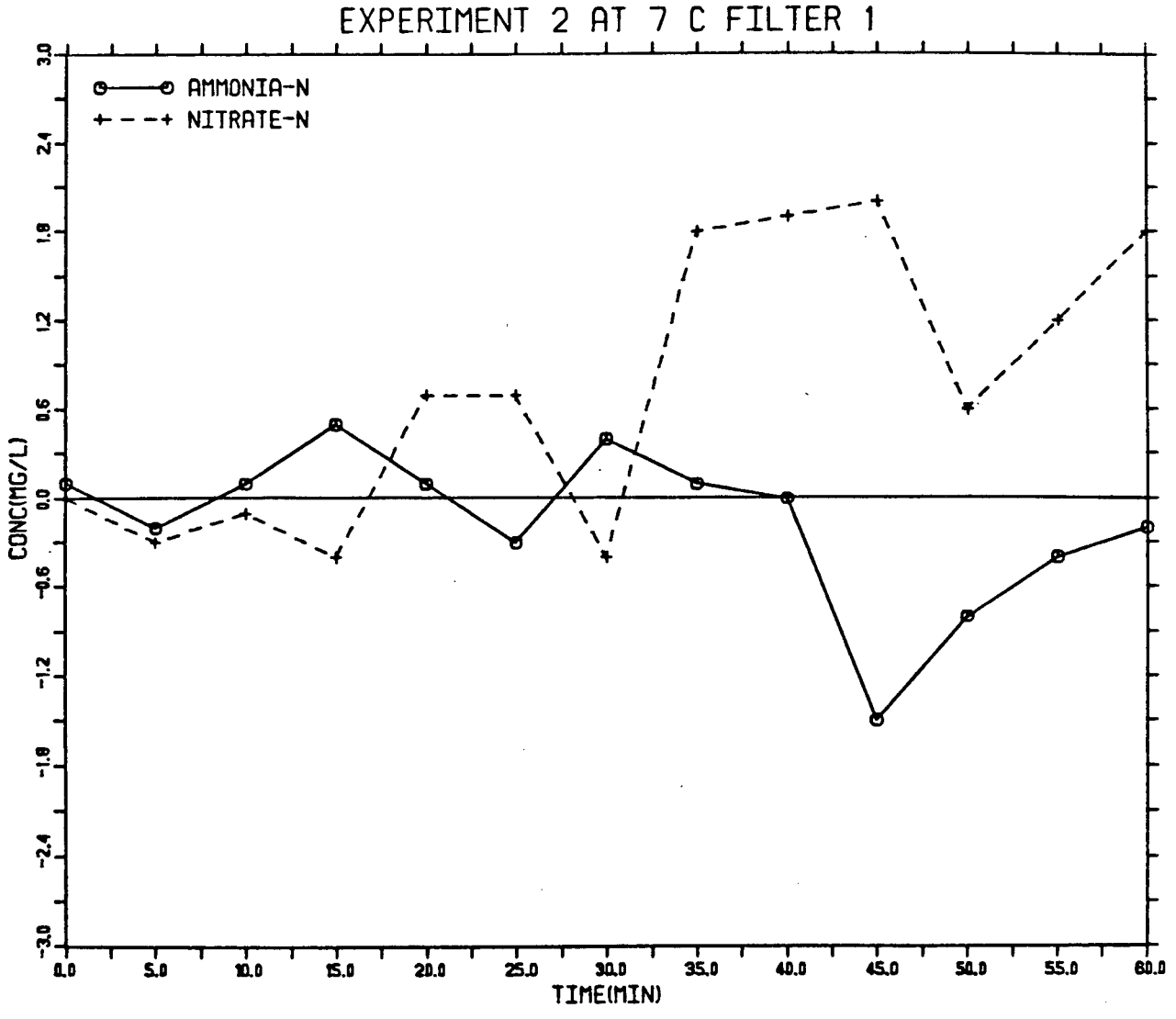

Figure 17. Differences between filter 1 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 7°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

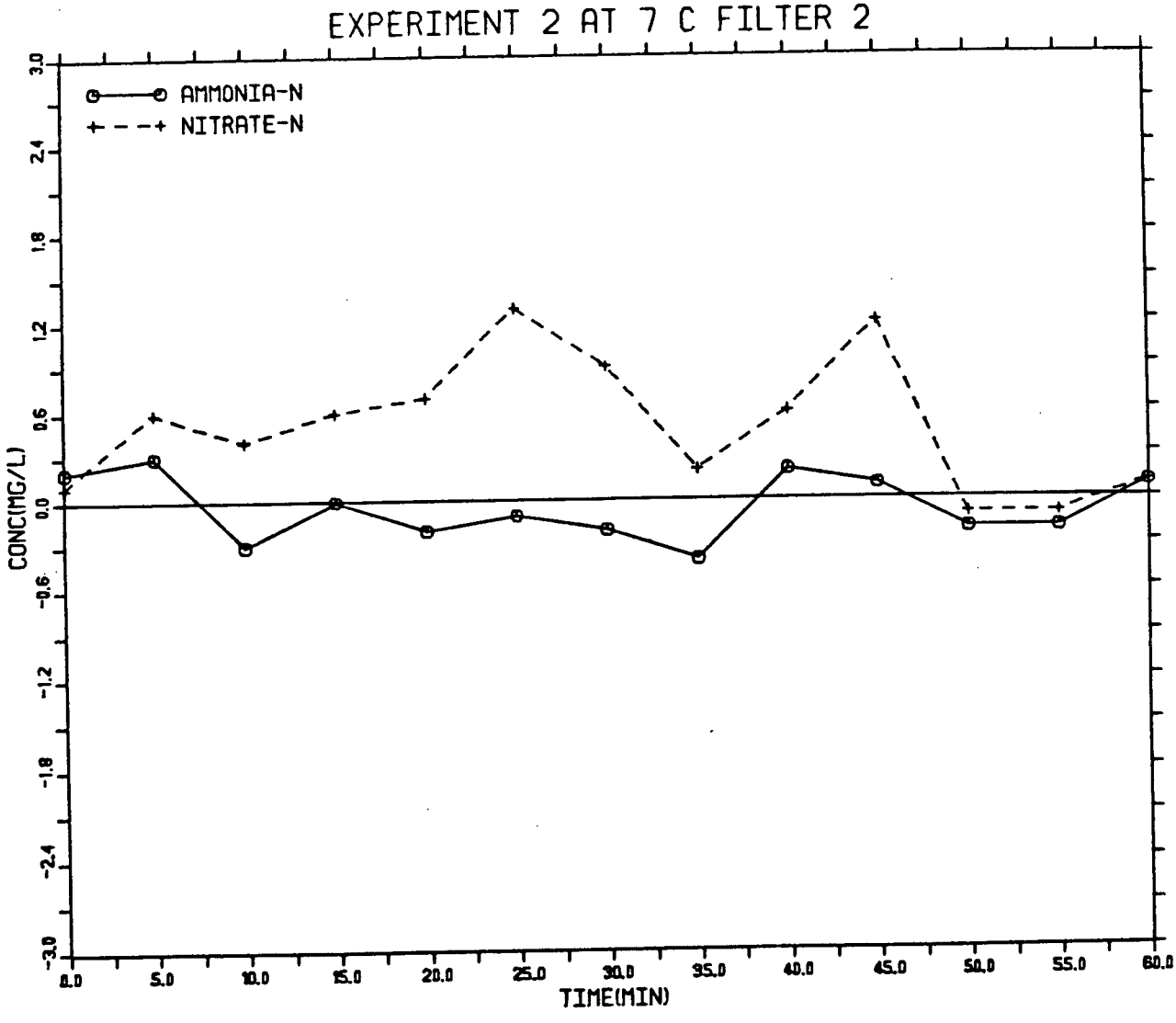

Figure 18. Differences between filter 2 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 7°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

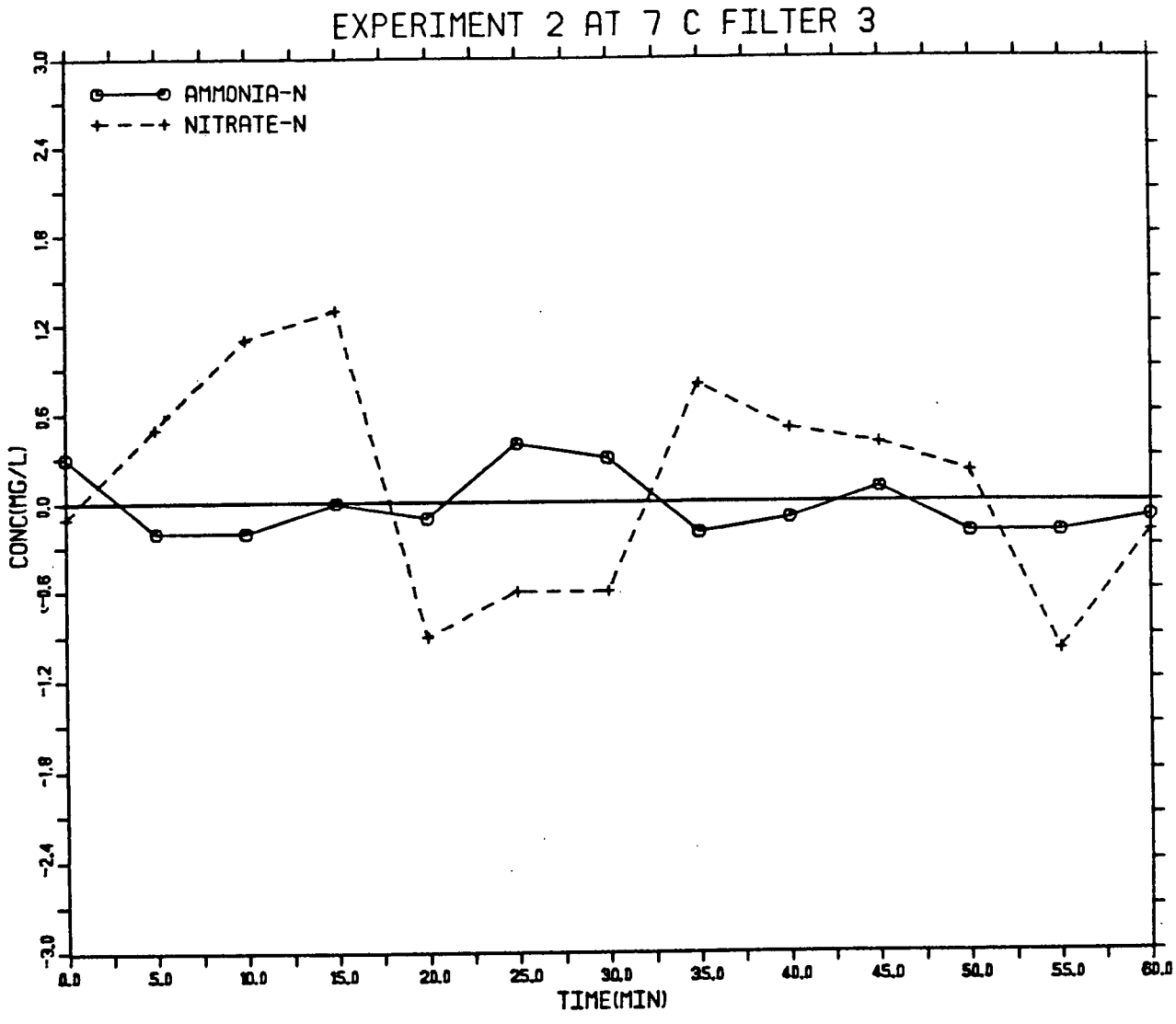

Figure 19. Differences between filter 3 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 7°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

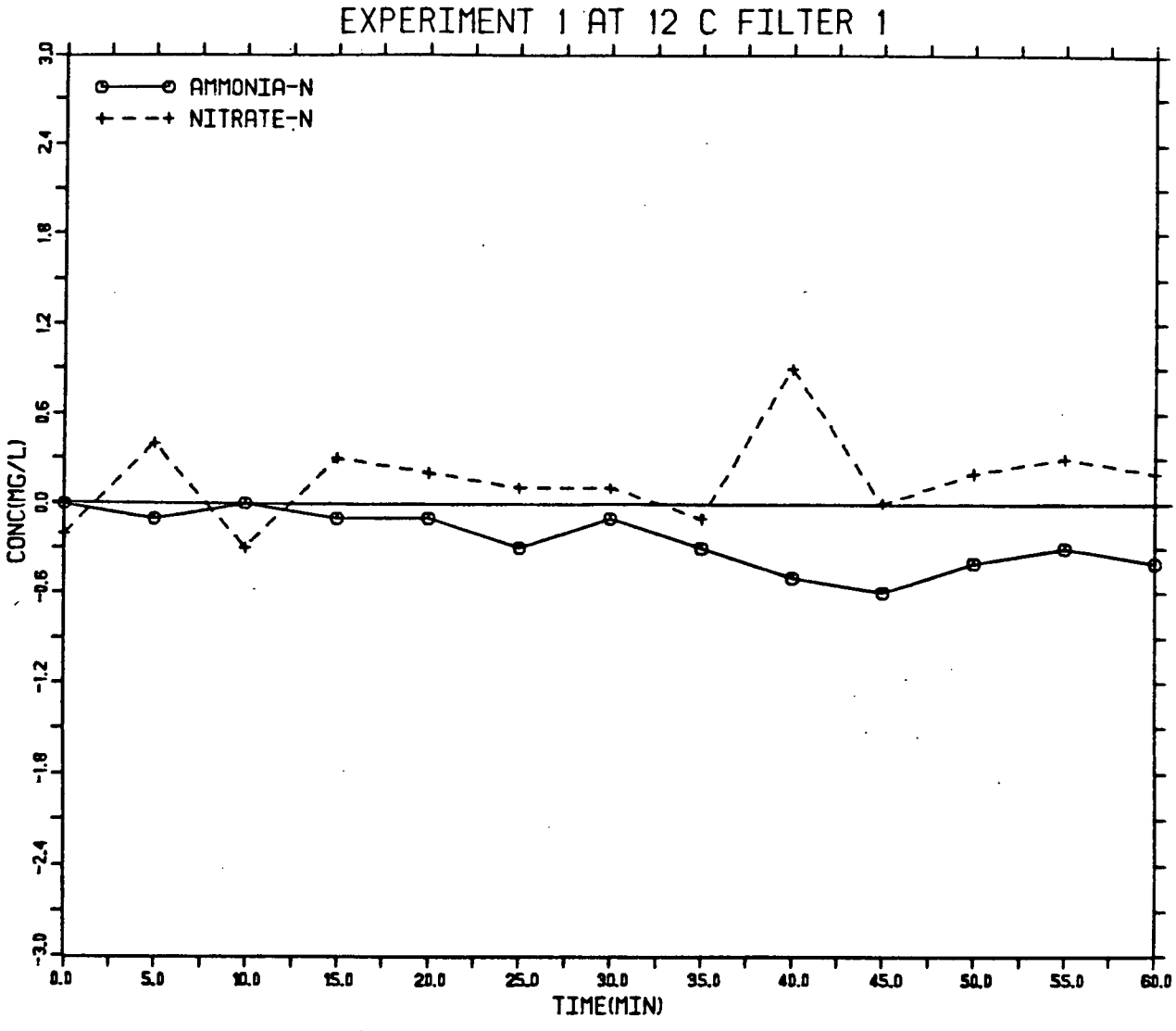

Figure 20. Differences between filter 1 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 12°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

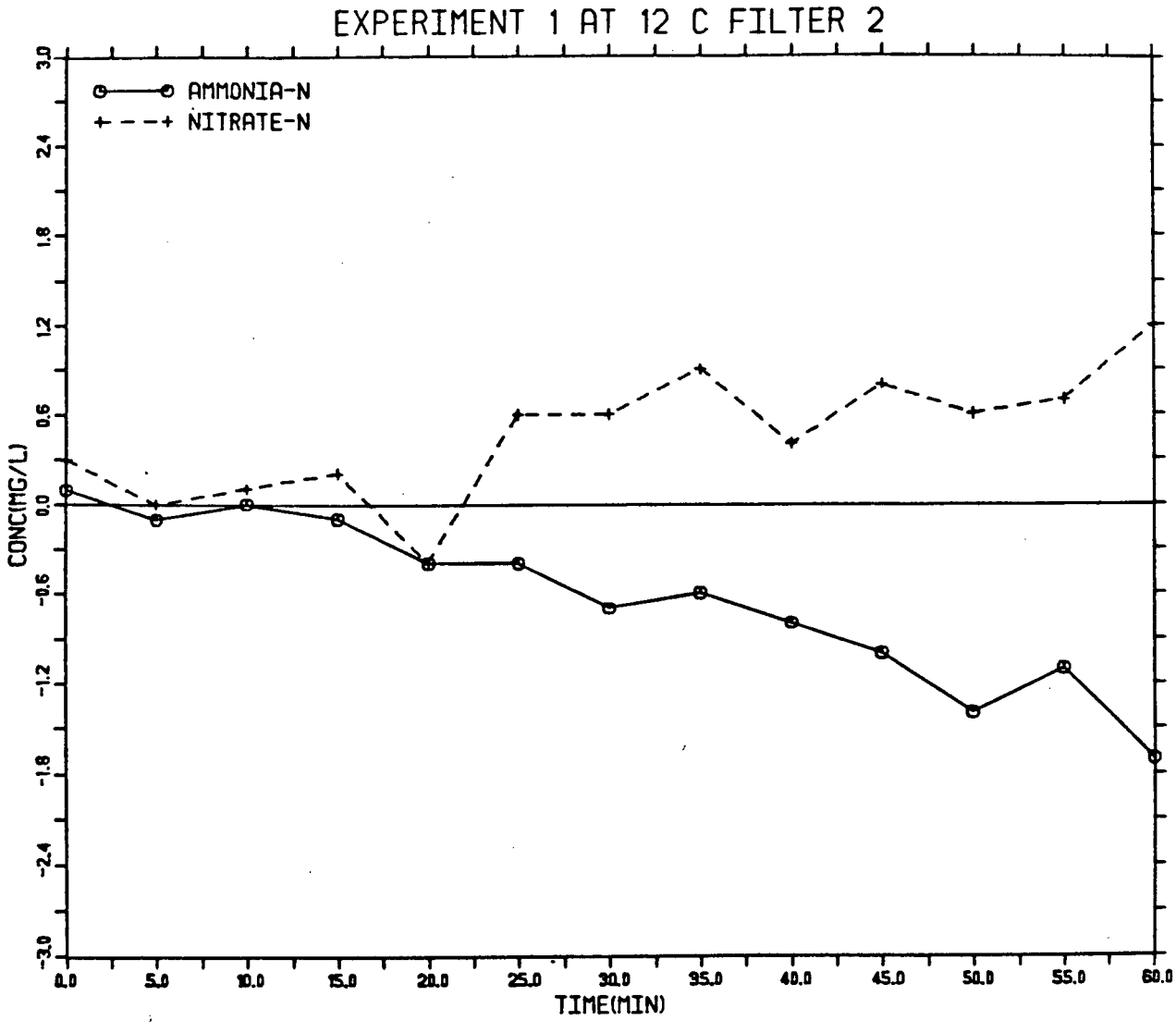

Figure 21. Differences between filter 2 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 12°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

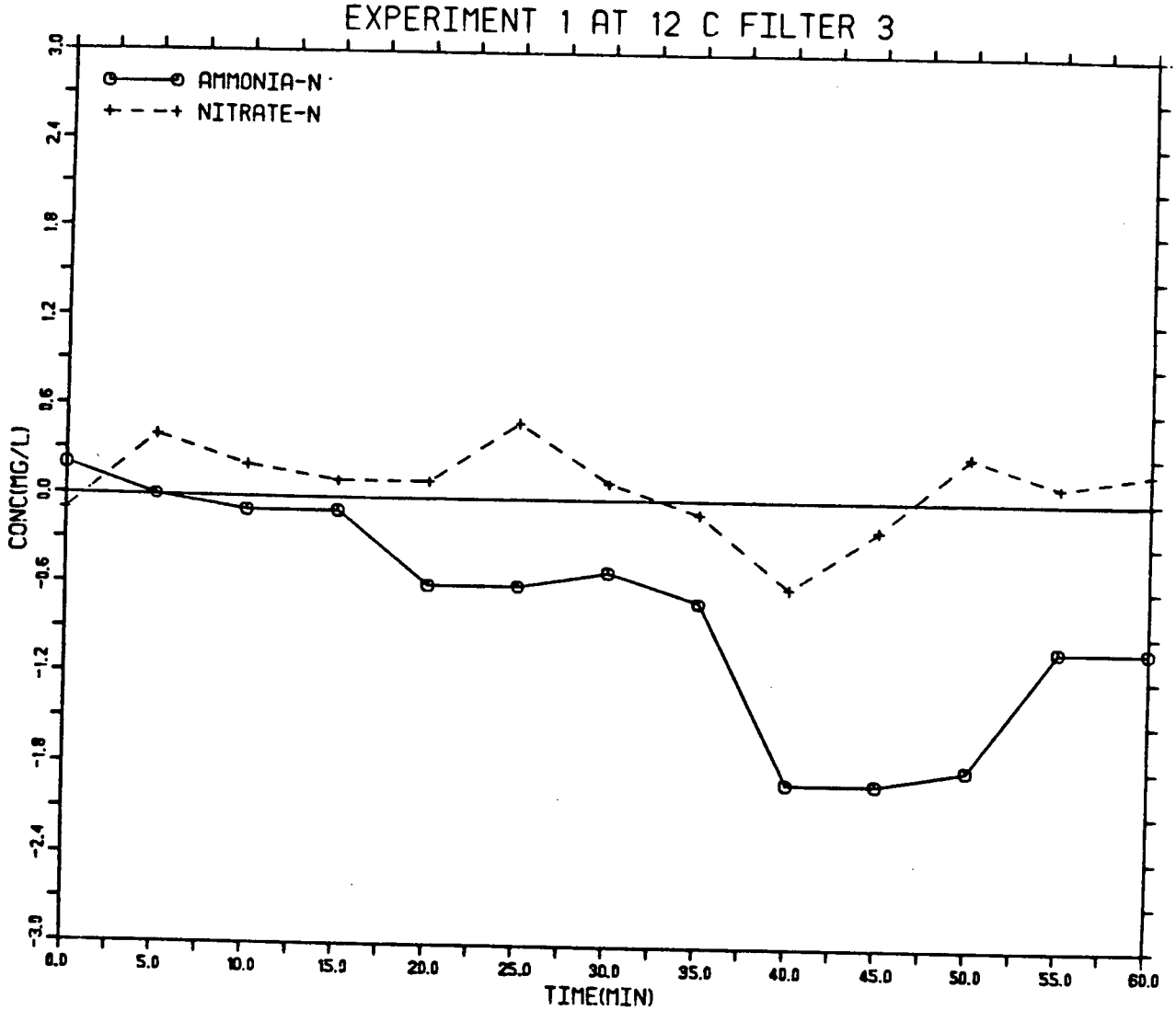

Figure 22. Differences between filter 3 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 12°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

experiment. At that point NO_3^--N remained relatively constant whereas ammonia-N dropped in concentration. At 50 minutes ammonia-N inflow and outflow concentrations began to move significantly closer together which is curious since NO_3^--N outflow levels continued to remain similar to inflow concentration until the termination of the experiment at 60 minutes.

Filters 1, 2, and 3 produced very similar results in the second experiment at 12°C (Figures 23, 24 and 25). Inflow and outflow concentrations of both nitrogenous compounds remained relatively constant for the first 15-20 minutes of the experiment. At that point ammonia-N outflow concentrations began to decrease when compared to inflow values. The final difference between inflow and outflow concentrations was between 1.5 and 2.0 mg l^{-1} at the end of each experiment.

Results from experiment 1 at 17°C are presented in Figures 26, 27 and 28. No significant increase or decrease in either ammonia-N or NO_3^--N was recorded for outflow water when compared to inflow water from filters 2 or 3. In filter 1 NO_3^{-3}-N outflow concentrations rose to about 1 mg l^{-1} above inflow levels at approximately 30-35 minutes and remained close to that level for the duration of the experiment. The reverse is true for ammonia-N inflow and outflow concentrations as the outflow level dropped to approximately 1 mg l^{-1} below the inflow concentration. A much different picture emerged from experiment 2 at 17°C (Figures 29, 30 and 31). Results

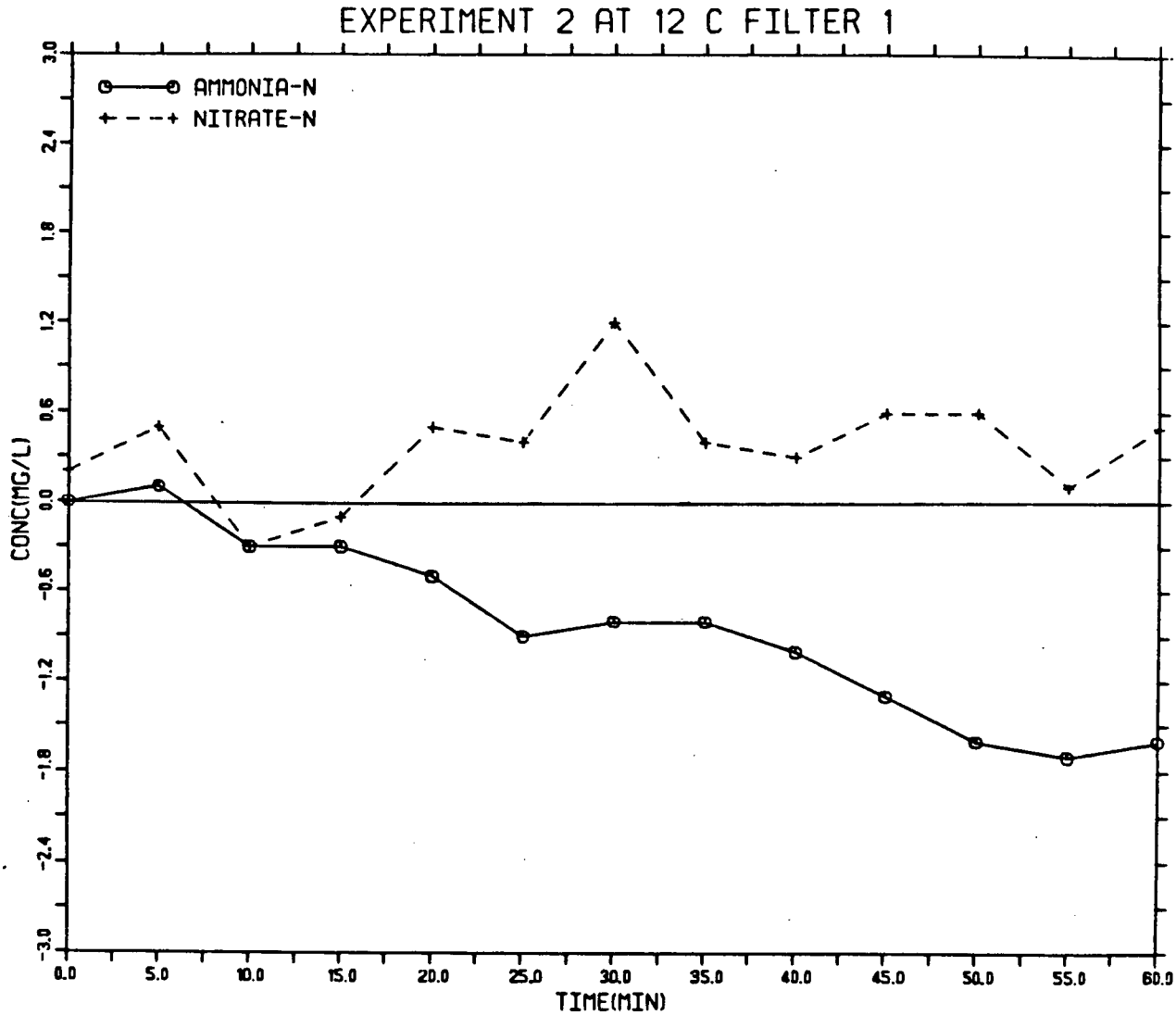

Figure 23. Differences between filter 1 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 12°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

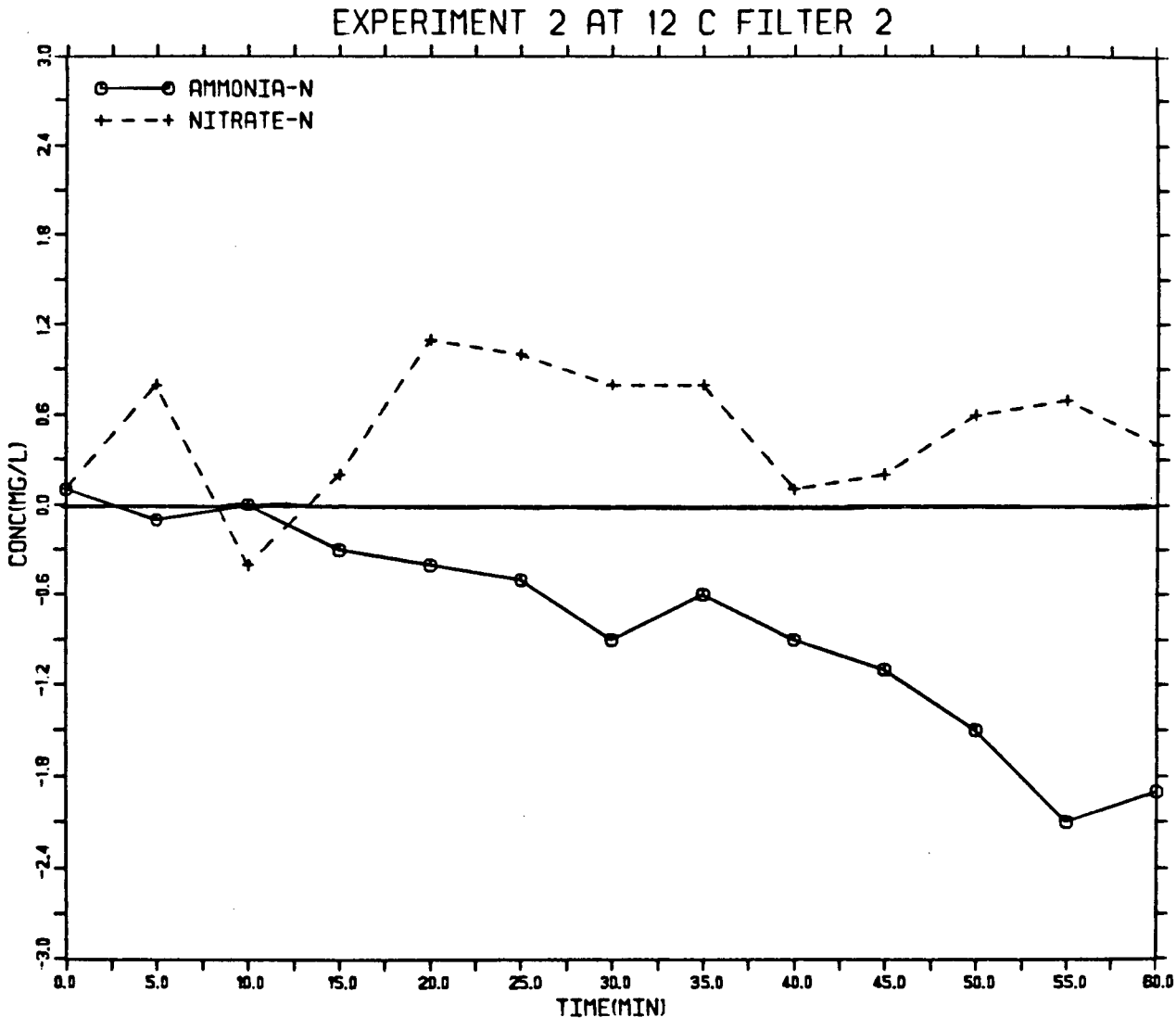

Figure 24. Differences between filter 2 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 12°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

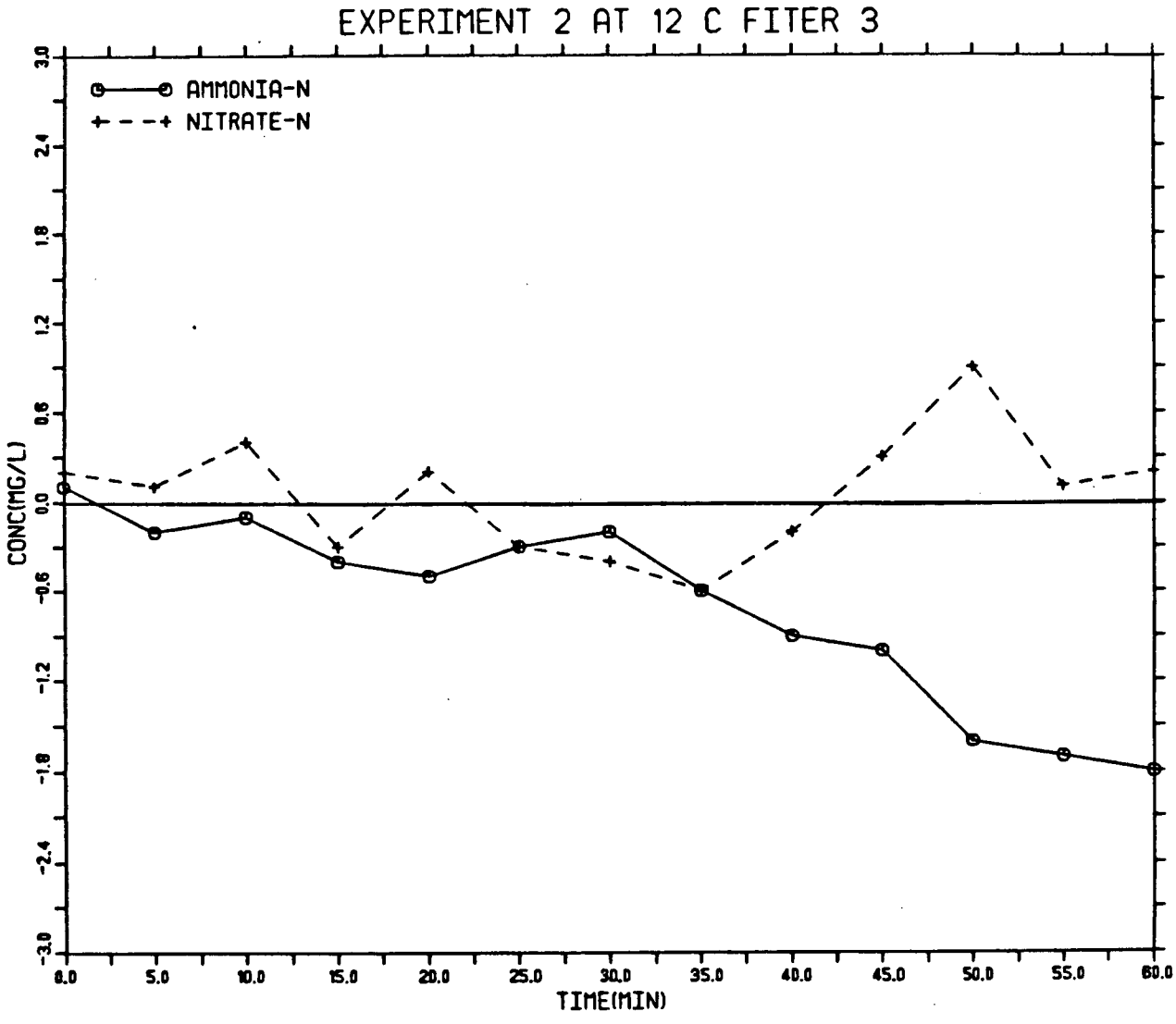

Figure 25. Differences between filter 3 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 12°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

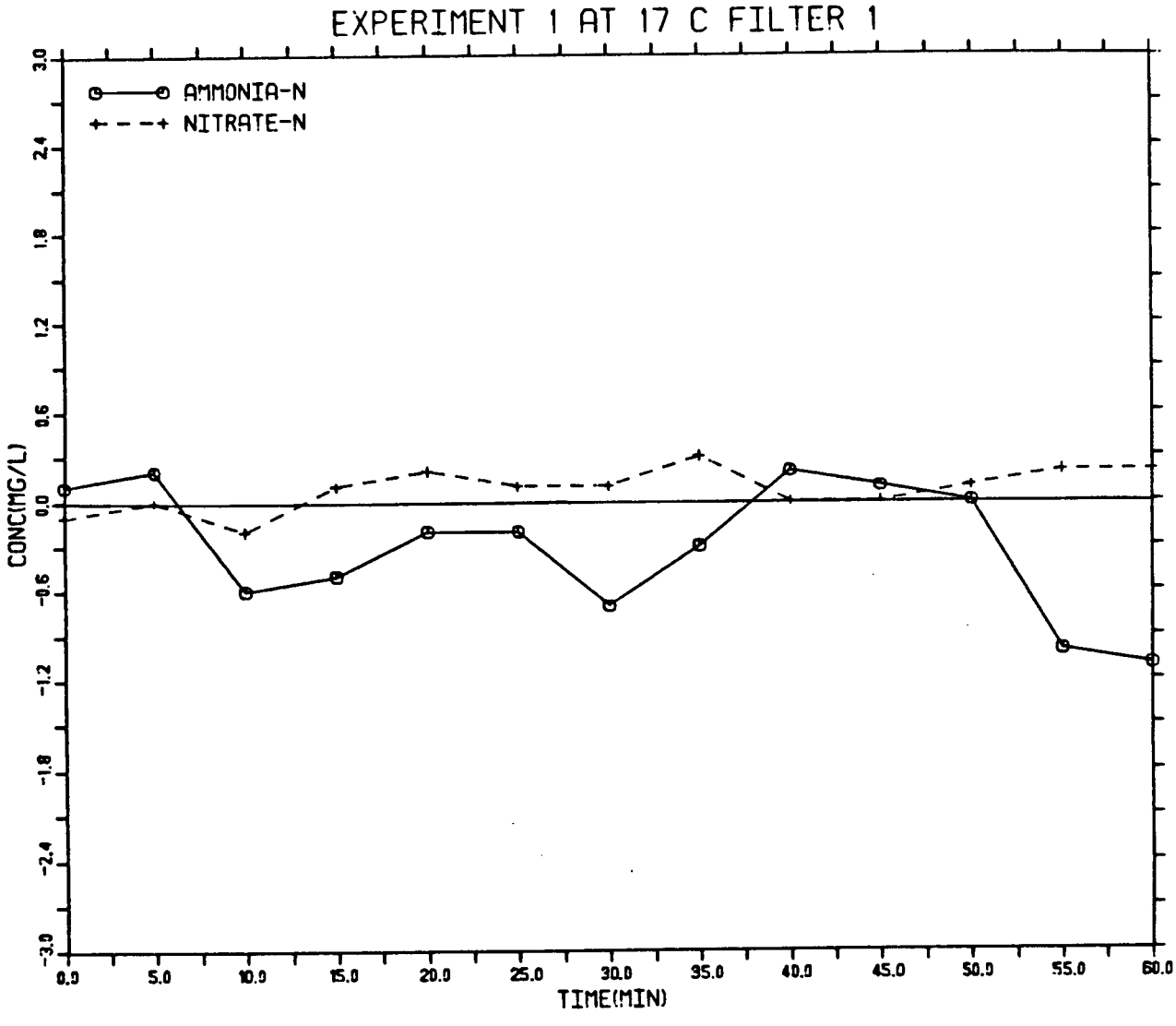

Figure 26. Differences between filter 1 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 17°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

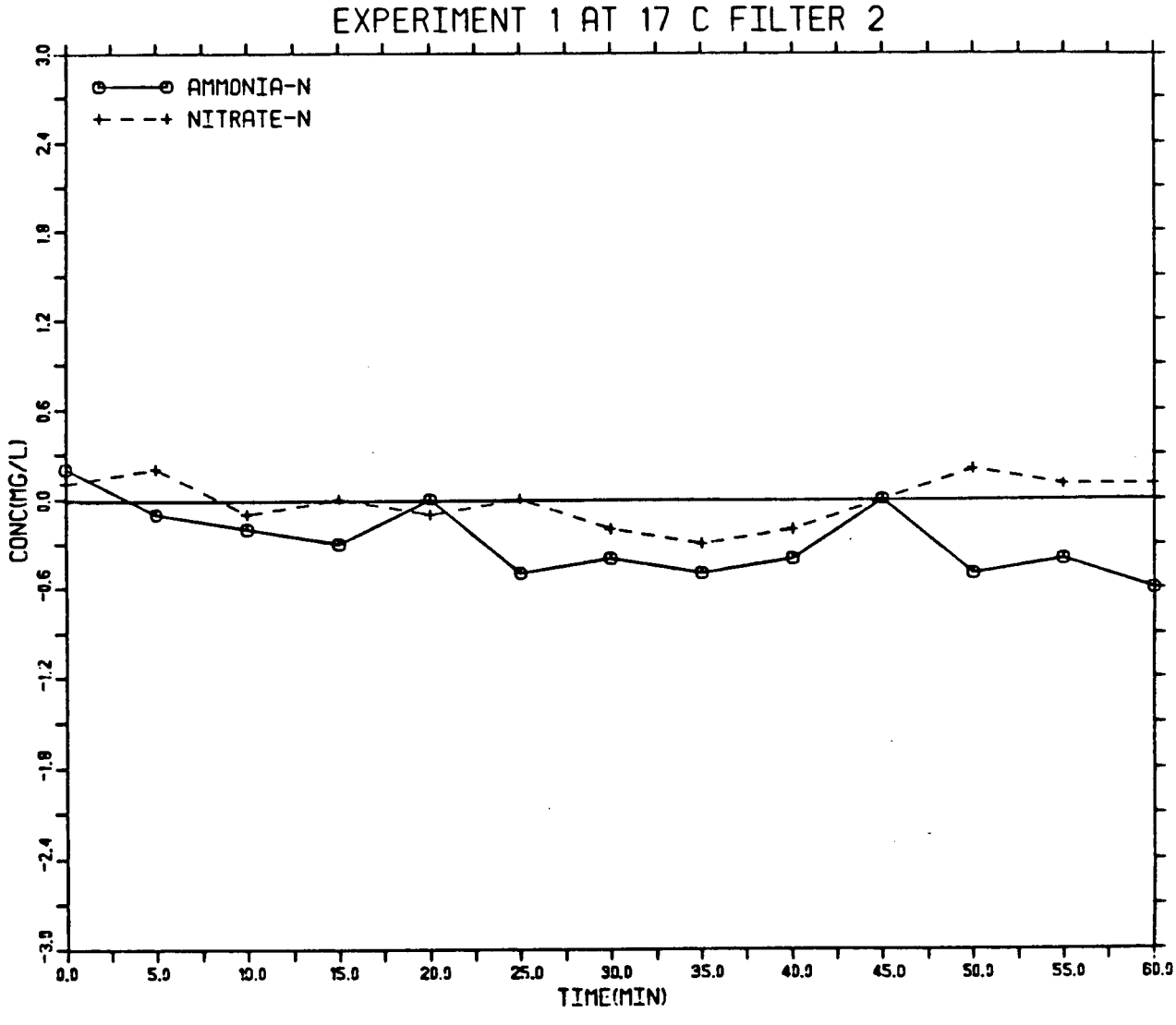

Figure 27. Differences between filter 2 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 17°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

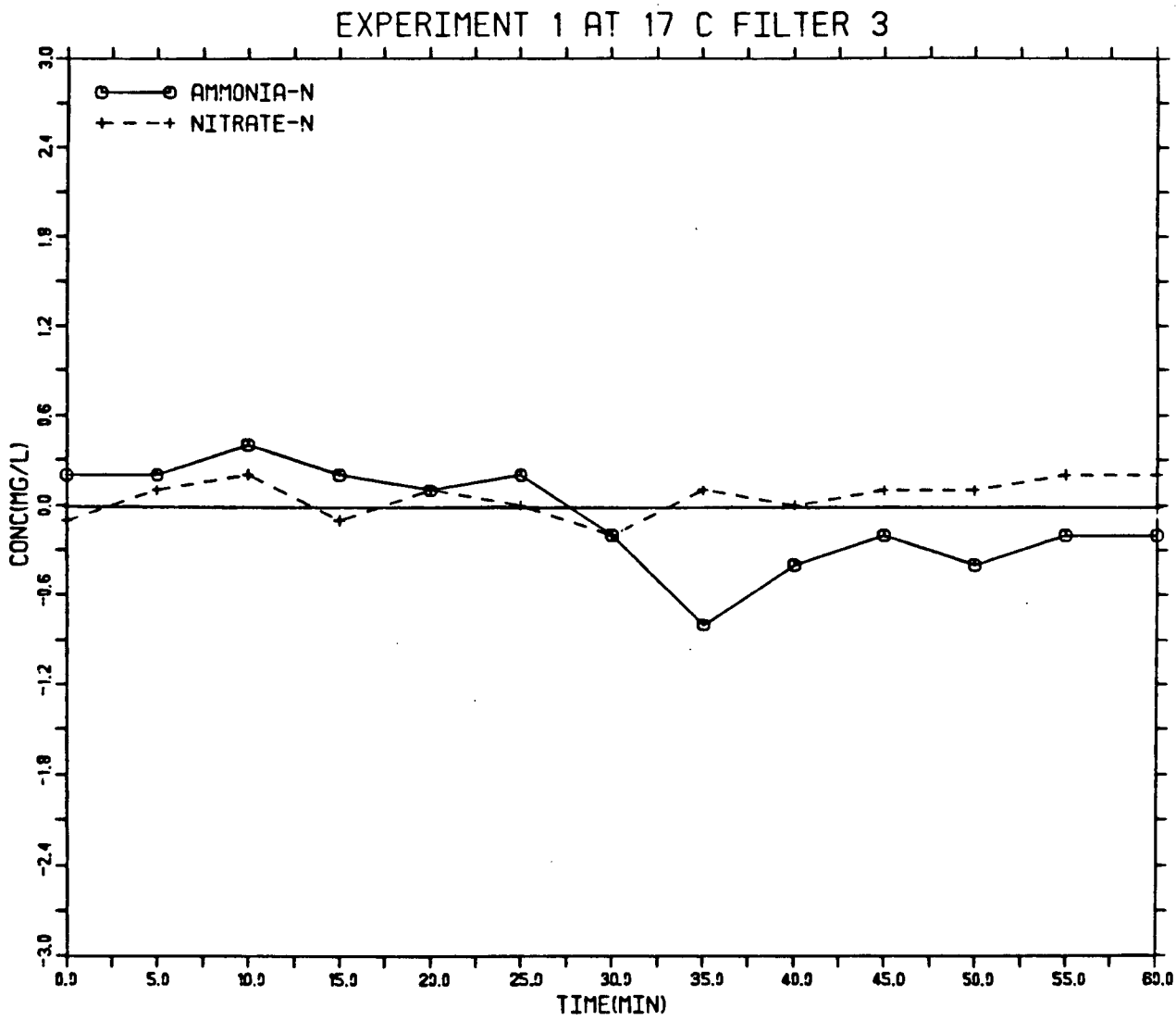

Figure 28. Differences between filter 3 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 1 at 17°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

from filters 1 and 2 show a dramatic increase of NO_3^--N outflow concentration with respect to inflow levels and a substantial decrease in ammonia-N in the outflow water. Both events occurred at approximately 15 minutes after the initiation of the experiment. For both filters the magnitude of change is slightly greater for NO_3^--N than that recorded for ammonia-N. No significant increase or decrease was measured in water passing through filter 3.

6.5 Ultraviolet Sterilizer Experiments

Ultraviolet sterilizer evaluation was carried out at three temperatures, one experiment each at 12° and 17°C, and duplicate runs at 7°C. Experiment lengths varied from a minimum of four days at 17°C to a maximum of seven days for each of the runs at 7°C. Measured plate counts were transformed to Log 10 counts per ml for graphical presentation (Figures 32, 33, 34, 35).

Bacterial concentrations in the main control tank remained at consistently low levels throughout the entire series of experiments. Background concentrations increased slightly with increasing temperature, a result which is not surprising since temperature is an important factor in controlling bacterial population dynamics. A certain amount of daily fluctuation in the control tank was recorded, but variations were considered insignificant when compared to the steadily increasing concentration observed in the aquarium system as experiments

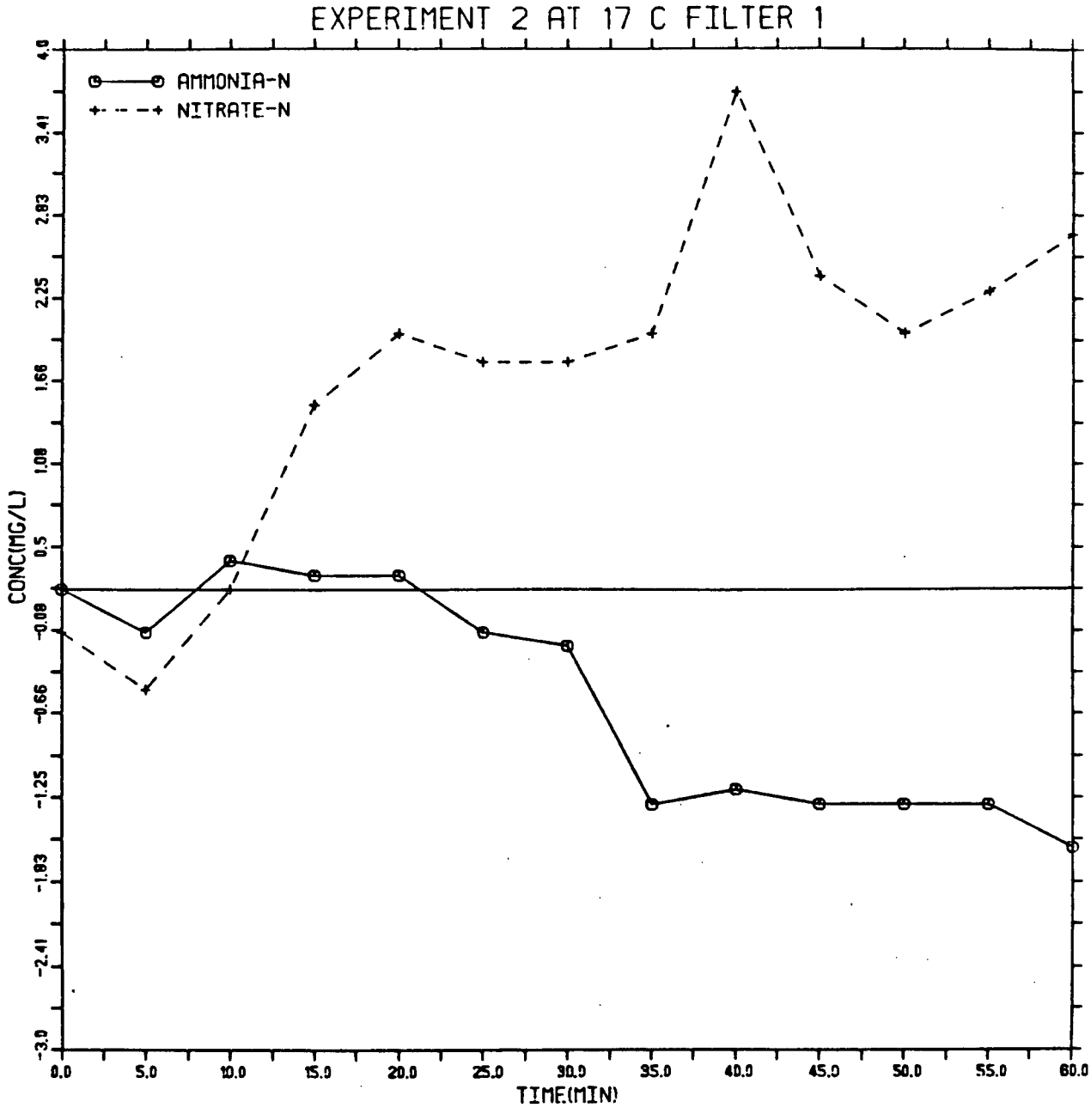

Figure 29. Differences between filter 1 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 17°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

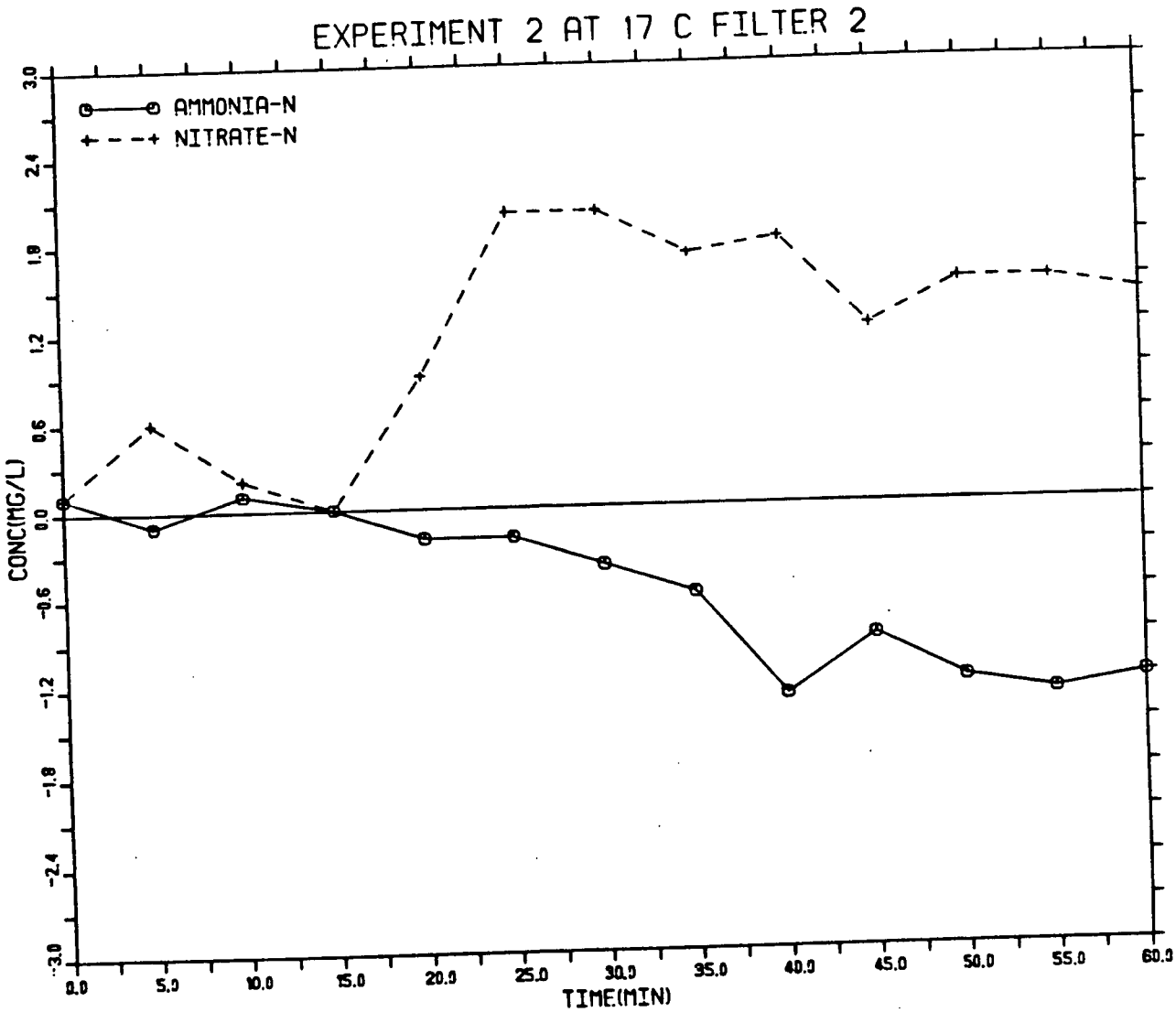

Figure 30. Differences between filter 2 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 17°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

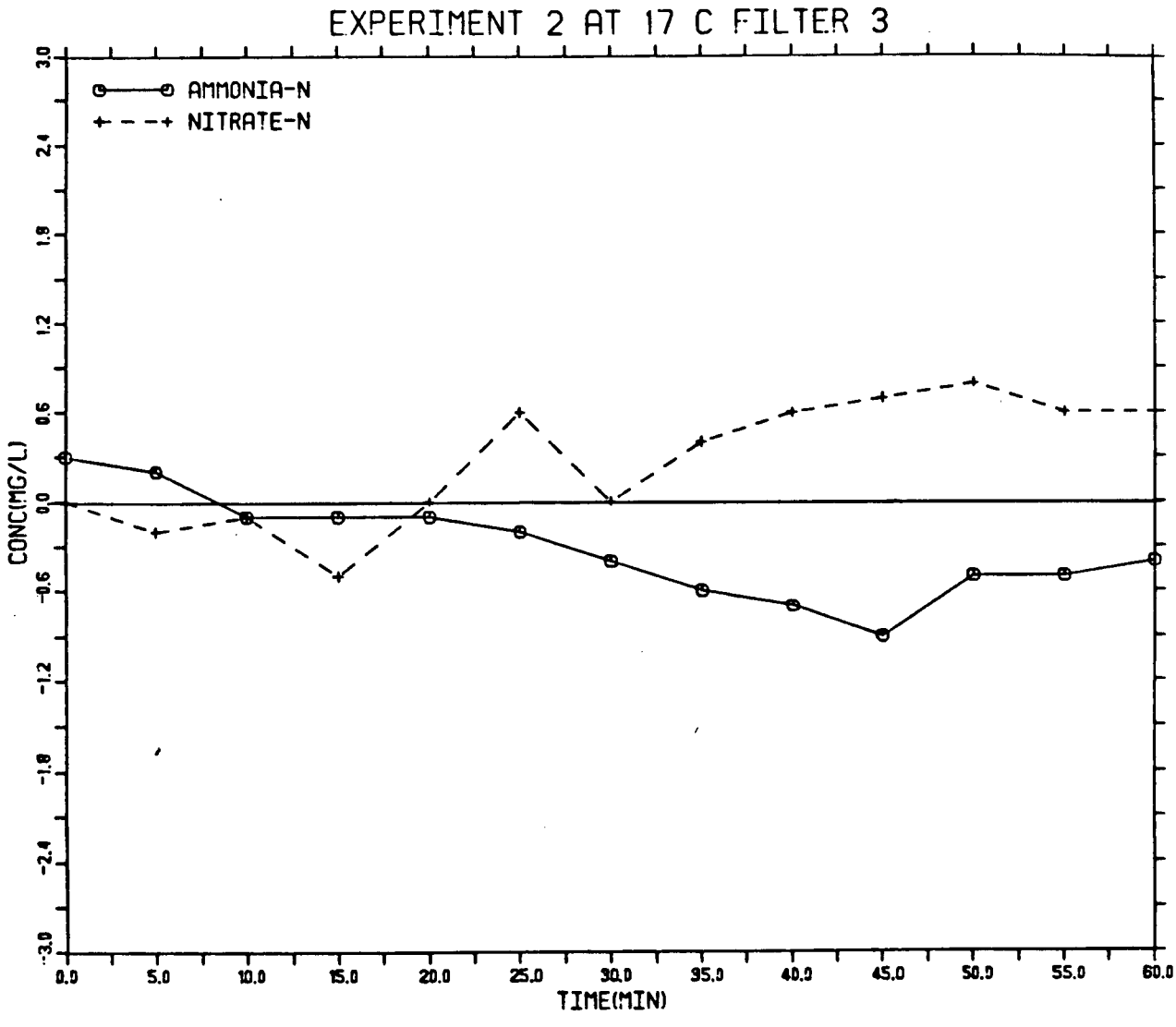

Figure 31. Differences between filter 3 inflow and outflow ammonia-N and nitrate-N concentrations during experiment 2 at 17°C. Increasing and decreasing concentrations are represented by positive and negative values respectively.

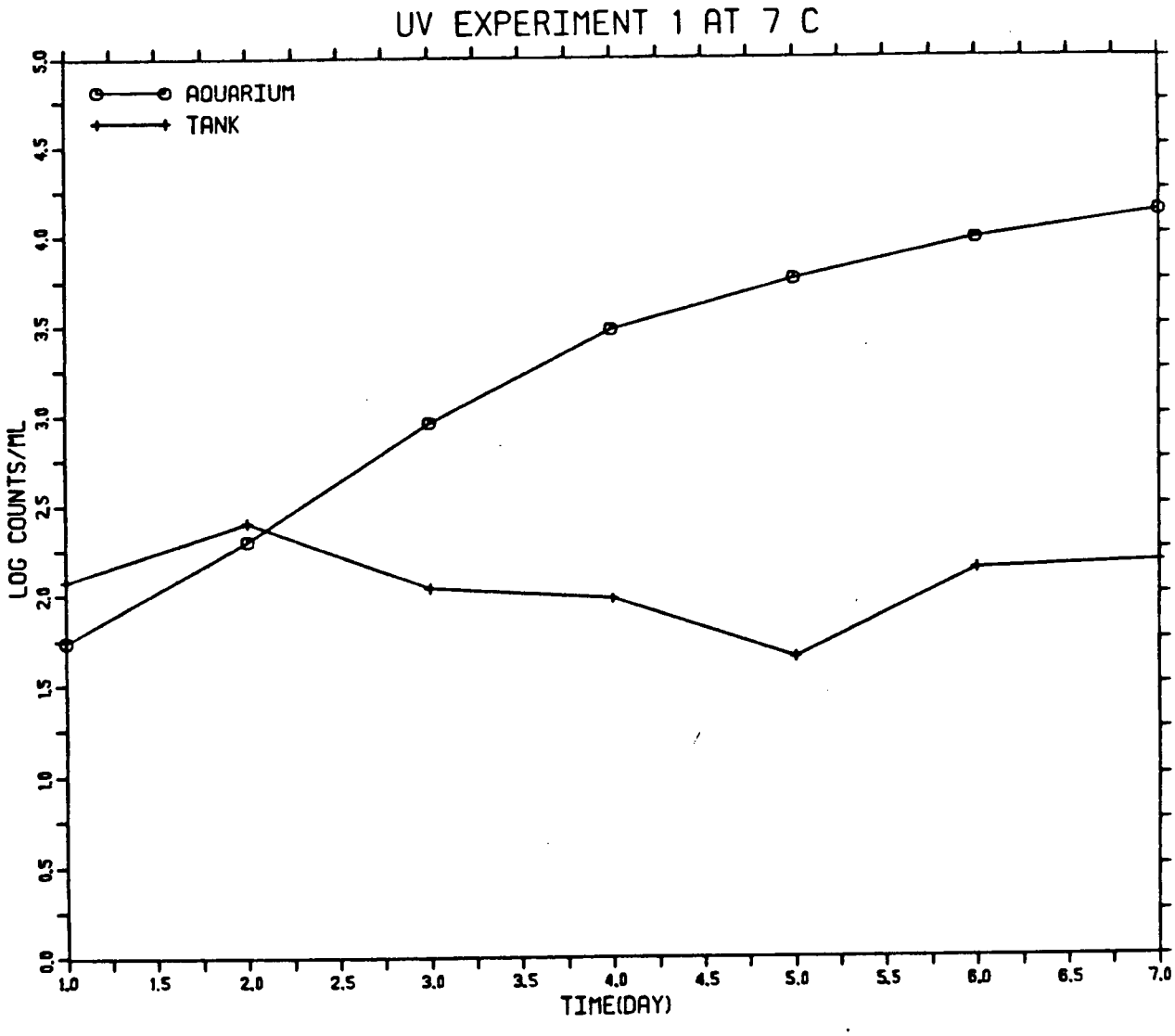

Figure 32. Changes in bacterial concentration recorded over time in the aquarium and tank during UV experiment 1 at 7°C.

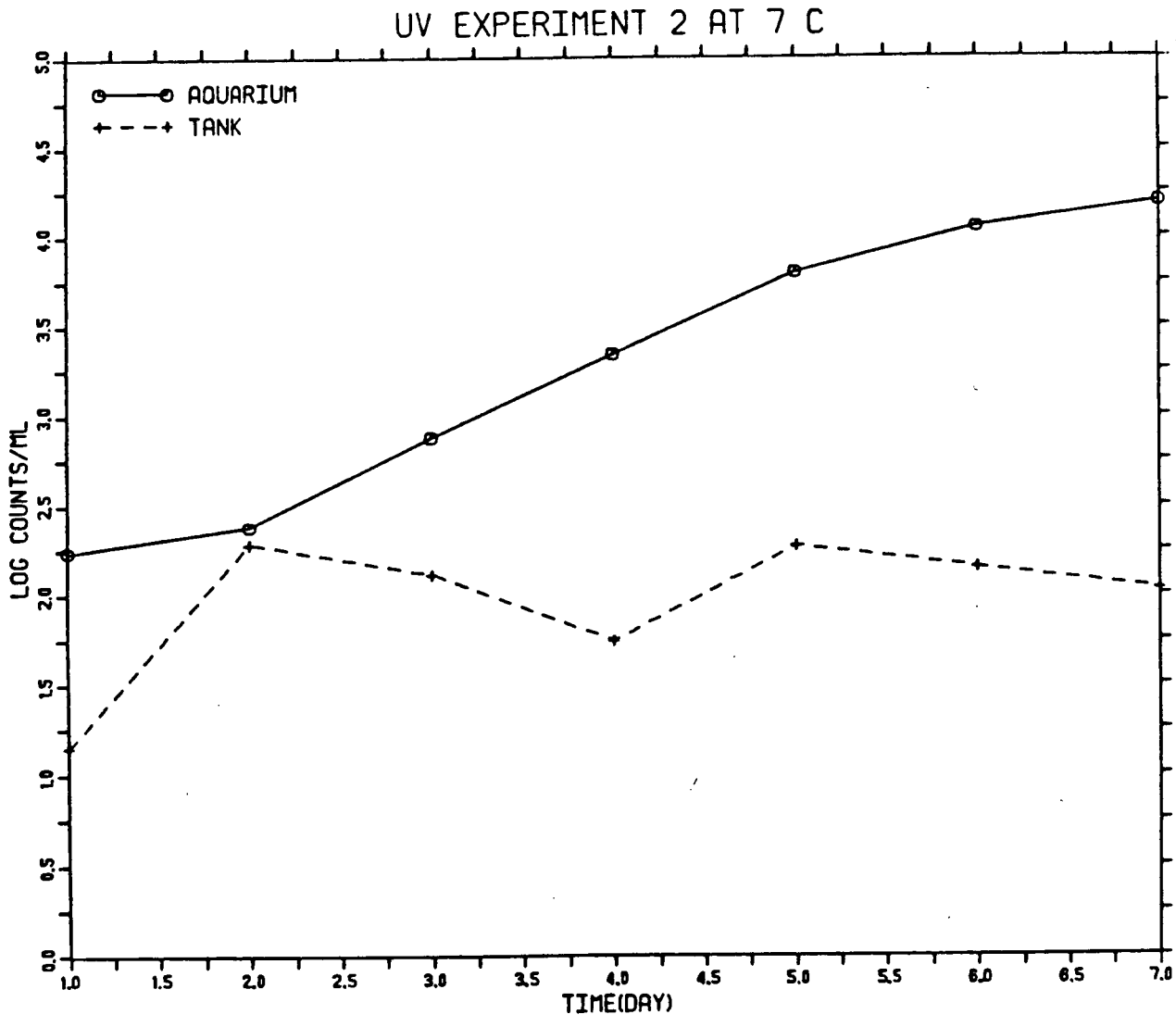

Figure 33. Changes in bacterial concentration recorded over time in the aquarium and tank during UV experiment 2 at 7°C.

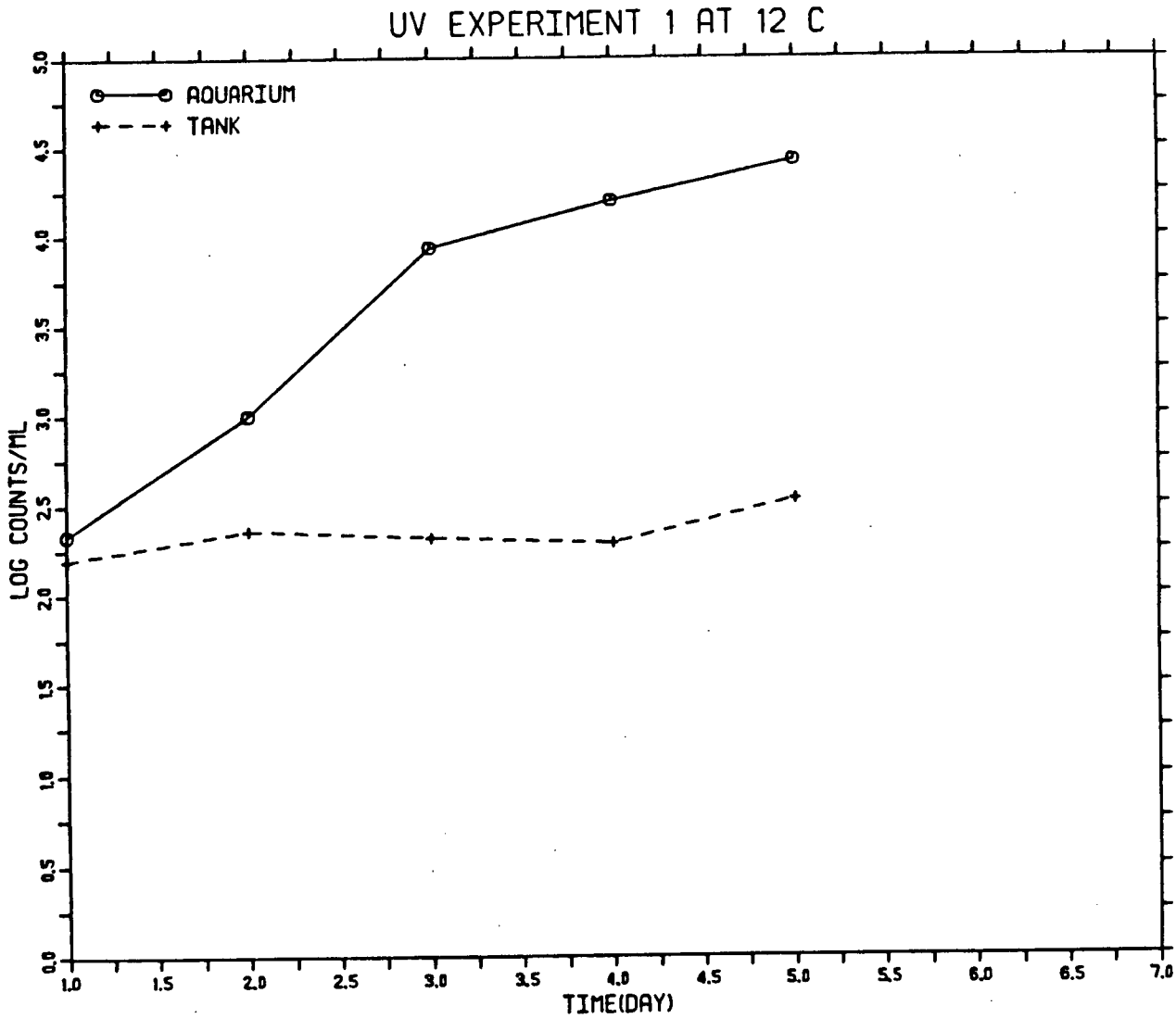

Figure 34. Changes in bacterial concentration recorded over time in the aquarium and tank during the UV experiment at 12°C.

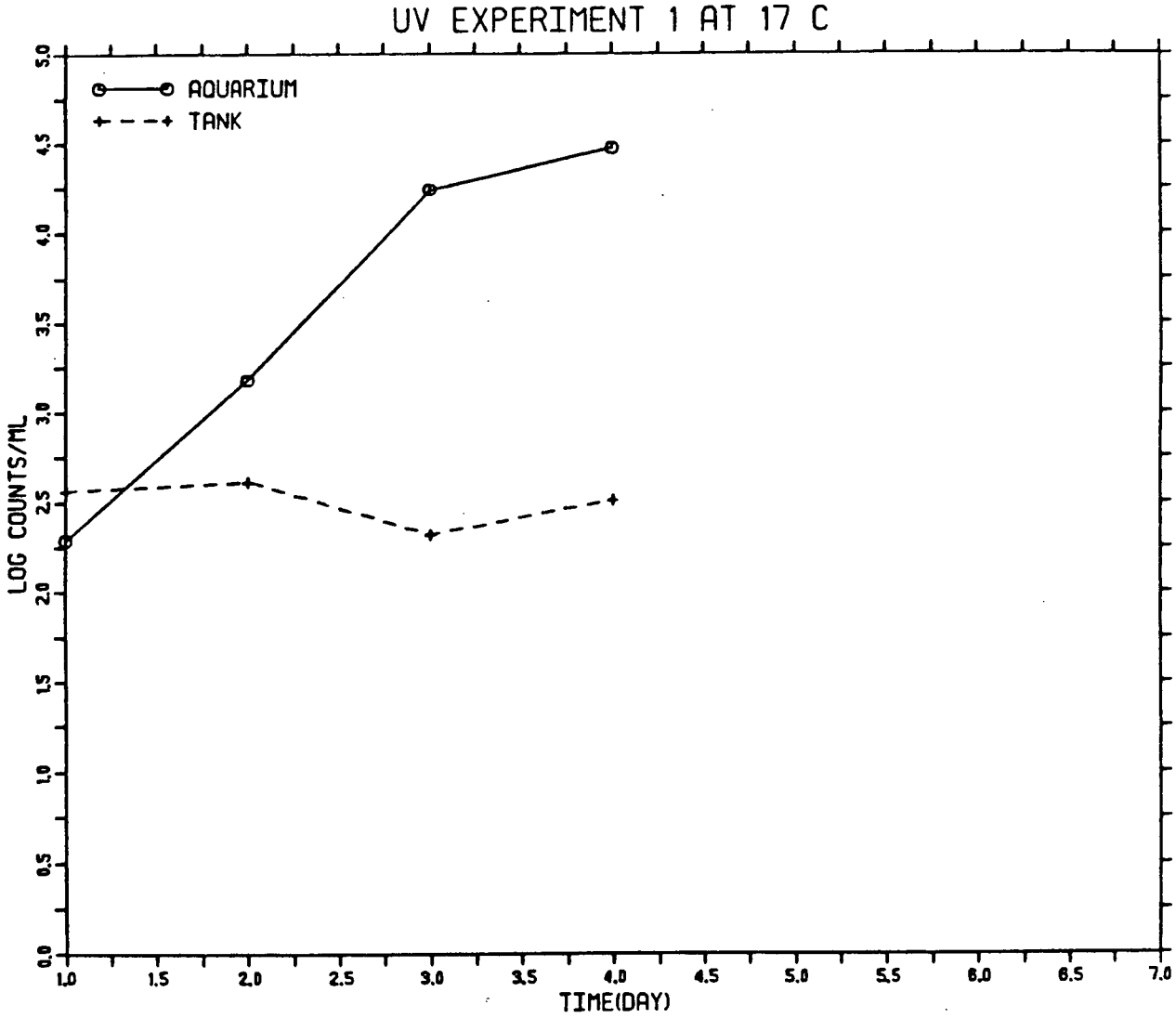

Figure 35. Changes in bacterial concentration recorded over time in the aquarium and tank during the UV experiment at 17°C.

progressed.

All experiments were terminated when bacterial concentrations in the aquarium tank reached approximately 10^4 counts ml^{-1}. In the aquarium, bacterial concentrations appeared to be related to water temperature. AT 7°C the 10^4 counts m^{-1} level was reached in approximately 7 days during both experiments at this temperature (Figure 32 and 33) whereas only 4-5 days were required at 12°C (Figure 34) and approximately 3 days at 17°C (Figure 35) to reach the same concentration.

7.0 DISCUSSION

7.1 Sampling Requirements

Although the overall variation in ammonia-N concentrations was low for the samples analysed in this segment of the study, statistical analysis of the results did reveal some noteworthy features of the data. Initially, it was expected that no significant difference would be found between the stacked and unstacked format since the three stacked tanks were average and compared to the unstacked. In the stacked format the centre tank was not found to be most representative of the three, as it might be expected to have a median level of ammonia-N in a cascading system. Although these results may hold true under most cases, a certain amount of variation should occur depending on a number of factors such as temperature, animal load, pH and aggitation of the holding water. The central position in each tank was found to be the most representative of ammonia-N concentrations throughout the tank at the 0.01 level of significance. An analysis of hydrualic movement in the tanks would be required to fully explain this result.

By reducing the number of samples taken during the experiments in the holding tanks, sample analysis was not delayed and storage time was kept to a minimum. By minimizing these two factors, greater accuracy and precision was achieved in the sample analysis. It is believed that the analysis conducted in this segment of the work provided adequate

justification for the sampling regime adopted.

7.2 Total Organic Carbon vs. Chemical Oxygen Demand

Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are methods that have been traditionally used in culture and holding systems to determine oxygen demand of the holding waters. These methods have associated with them inherent problems and time constraints as outlined in Section 5, therefore, it was decided to evaluate the possibility of using the Total Organic Carbon (TOC) analysis as a replacement for BOD and COD, thus minimizing the time constraint problems. The TOC values inturn could be compared to a series of COD measurements carried-out on the lobster holding water so a ratio could be derived. Since no results from TOC measurements on lobster wastes or holding water have been published this ratio is important if any comparisons are to be made with published COD values.

Although variation was relatively large for the COD determinations, a strong correlation between COD and TOC was demonstrated (Figure 7). An equally strong correlation was established for swine wastes by Bulley and Husdon (1974). This strong correlation is a good indication that TOC can be used as a reliable indicator of oxygen demand for the lobster holding water. The ratio found for COD/TOC of 1.67 falls within the range of values published for other waste types. A value of 2.20 for the COD/TOC ratio is given by Eckenfelder (1970) for

the effluent from biologically treated domestic waste. A much higher COD/TOC ratio of 3.60 was reported by Van Hall and Stenger (1963) for municipal wastewater. A COD/TC ratio of 1.50 was also given, indicating that the municipal wastewater contained a comparatively high concentration of inorganic carbon. Although composition would be expected to be somewhat different than lobster holding water, untreated domestic wastewater exhibiting comparable COD and TOC levels is considered weak on a scale of weak, medium and strong (Metcalf and Eddy 1979).

It may be expected that the stoichiometric COD/TOC ratio of a wastewater would be approximately equivalent to the molecular ratio of oxygen to carbon ($\frac{32}{12}$= 2.66). Eckenfelder (1970) states that theorectically the ratio limits would range from zero, when the organic material is resistant to dichromate oxidation, to 5.33 for methane or slightly higher when inorganic reducing agents are present. The comparatively low COD/TOC ratio determined for the lobster holding water may be a result of resistent organic compounds. BOD and COD results may often not include many organic compounds that are partially or totally resistent to biochemical or dichromate oxidation. In most instances, all the organic carbon in these compounds is recovered in the TOC analysis.

7.3 Time-Series Experiments

The water quality parameters choosen for study during this

series of experiments include temperature, salinity, oxygen, pH, TOC, ammonia-N, nitrate-N and nitrite-N. Along with the biomass load, both temperature and salinity remained basically constant throughout each experiment. This condition was the result of the relatively short duration of each experiment (12 hours). Due to the system design both of these parameters tended to vary if monitored over a longer time period (days as opposed to hours). The temperature problem was eventually addressed and rectified based in part on recommendations developed during the work done for this thesis. The refrigeration system which was eventually installed to replace the grossly undersized unit originally in place, now maintains the holding water at a temperature of between 6°C and 8°C, a temperature which meets the established level for this parameter.

As far as the time-series experiments were concerned salinity can be considered a constant, which simplifies the analysis of the results. Generally though, salinity levels in the holding water were low when compared to open ocean concentrations which vary from about 30 to 35 o/oo. As outlined in the literature review section, these low salinity levels can have adverse effects on lobster health, and as such were a matter of concern during this work. The problem of fluctuating salinity levels is not addressed here since it was considered a practical management problem. Issues of this sort are included in a water quality manual developed during the

course of this study and presented to the managers of the lobster holding operation. Salinity levels were particularly low during the first five experiments after which a method involving the addition of bulk salt to the holding water was developed and implemented during the period covering the five remaining experiments. Salinity can be maintained at an acceptable level using this technique but the system must be monitored continuously due to salinity change resulting from evaporation and dilution.

TOC concentrations were calculated from total carbon measurements established during the time-series experiments using an empirically determined conversion factor of 0.97. Although the value of 97% organic carbon is a relatively large fraction of the carbon present it does not appear to be out of line when compared to TOC levels in other types of wastes. For instance, Bulley and Husdon (1974) determined that TOC comprised approximately 90% of the detectable carbon in swine waste. A similar value has been found for dairy manure (personal communication Dr. V. Lo). In actuality the fraction of TOC will vary depending on the material entering the lobster holding water, but if the majority of the material is lobster waste it is quite reasonable to assume that the ratio of TOC/TC will remain high.

The results from the TOC monitoring show an immediate and dramatic increase in TOC concentrations in the lobster holding water after the introduction of the animals. Two factors may

be contributing to this increase. The first is that the lobsters may be superficially carrying material, or excreting wastes with a high TOC level.

It is inevitable that some material will be transported on the exterior of the lobsters during shipment and eventually enter the holding water. Although no substantial amount of exterior fouling was ever observed on the carapaces of newly arrived lobsters, it cannot be stated conclusively that elevated TOC concentrations recorded immediately after lobster introduction are not attributable to this factor. It is also difficult to state conclusively that newly introduced lobsters are or are not excreting quantities of fecal matter substantial enough to cause this TOC change. This will depend on how recently feeding has occurred and the nature of the food consumed. No food is available to the lobsters during the approximately 24 hour shipping period from the east coast, and it is quite likely that many of the animals have been held prior to shipment without food for a number of days. Therefore, it is unlikely that large quantities of fecal matter will be generated in the holding system during the relatively short period alotted to the time-series experiments.

The second, and most probable factor contributing to the rapid increase in TOC immediately after the lobster introduction is the resuspension of previously settled material during the lobster loading activities. A substantial amount of mixing results from placing the animals in the tanks as well as from

lobster movement during this period. Lobster activity was observed to be more pronounced at higher temperatures which suggests that TOC increases should be greater at the higher experimental temperatures, assuming similar levels of residual TOC. This however was not the case, as the increase at 8°C was slightly larger than those recorded for experiments at either 13 or 19°C. A proportionately much smaller increase in TOC was recorded at 7°C (experiment 7) when the biomass load was reduced. This result illustrates the significance of the actual size of the biomass load and loading procedure, rather than the activity of the animals, in resuspending the organic material in the tanks. The increased activity, resulting from the higher temperatures, may have an affect on the settling rate of the material. At higher temperatures settling may take longer which extends the period of elevated TOC levels. There is some evidence of this occuring during the higher temperature experiments. Visually, there was no increase in water turbidity shortly after the lobster introduction over that observed during periods of normal operation.

The most significant potential effect of this TOC increase on lobster health is the associated increase in oxygen demand of the holding water. Assuming this TOC increase is in part a result of waste excretion by the lobsters, then it is possible that ammonia-N concentrations will rise due to mineralization, in the form of deamination, of proteins in the waste material. The COD equivalent as determined by the COD/TOC ratio of 1.67

would be approximately two thirds again as high as the TOC values. For example, the peak concentration of TOC at 13°C during experiment 1 was approximately 325 $mg \cdot l^{-1}$, which is equivalent to a COD concentration of 543 $mg \cdot l^{-1}$. Oxygen demand in aquaculture operations is usually expressed as BOD_5. Therefore to compare the results obtained here with published data a BOD_5/COD ratio must be estimated. It is generally known that the BOD_5/COD ratio varies from 0.4 to 0.8 for a variety of wastes (Metcalf and Eddy 1972). If a median value of 0.6 is assumed then the equivalent BOD concentration of the holding water would be 326 $mg \cdot l^{-1}$, almost identical to that determined for TOC.

Oxygen demand measured as BOD_5 for fish hatchery holding water varies from approximately 50 to 100 $mg \cdot l^{-1}$ (Brown and Nash 1981; Slone et al 1981), values which are similar to the background or steady state values measured in the lobster tanks. Maximum concentrations which were observed during the first hour of each time-series experiment are substantially higher as outlined above. It appears then that the first hour after the lobser introduction is the critical period with respect to oxygen demand.

One additional adverse effect of high TOC concentrations in seawater systems is that the dissolved component of the organic carbon tends to reduce the buffering capacity of the water by affecting the solubility of the calcium and magnesium carbonates present. This influence on buffering capacity is

addressed in more detail in the discussion on pH.

The results from the dissolved oxygen monitoring show very clearly that the greatest oxygen demand occurs within the first hour after lobster introduction. D.O. drops of approximately 1.5, 2.2 and 3.4 $mg \cdot l^{-1}$ were recorded 0.25 h after lobster introduction at 8°, 13° and 19°C respectively. Since oxygen exchange at the air-water interface is a continuous process, D.O. drops recorded during the three experiments are in a sense buffered. But since these drops are comparatively large and occur over such a short time period, errors in rate calculations should be minimal.

These changes in dissolved oxygen concentration can be expressed as a lobster respiration rate in $mg \cdot g^{-1}$ of lobster$\cdot h^{-1}$. Assuming an average system water volume of 17,500 l, an average lobster weight of 700 g, and an average of 1600 lobsters in the tanks during each experiment, calculations reveal that dissolved oxygen concentration decreased at a rate of 0.094 $mg \cdot g^{-1} \cdot h^{-1}$, 0.138 $mg \cdot g^{-1} \cdot h^{-1}$ and 0.213 $mg \cdot g^{-1}$ at 8°, 13°, and 19°C respecively during the first 15 min. of each run. These rates were calculated from actual dissolved oxygen drops which were from 10.0 to 8.5 at 8°C, 8.9 to 6.7 at 13°C and 8.1 to 4.7 at 18°C.

These values can be compared with published estimates of lobster oxygen consumption. Two empirically derived estimates exist in the literature. The first is an equation presented in Allen and Johnston (1976) based on the work of McLeese (1964):

$$M_o = K_o W^B \quad (10)$$

where M_o = quantity of oxygen consumed ($mg \cdot hr^{-1}$)

W = weight of the lobster (g)

B = an unexplained parameter derived from unpublished data

K_o = (0.0169 U - 0.0974)

U = water temperature (°C)

Using the previously established parameter values the following oxygen consumption rates can be generated from Equation 10: 0.017, 0.056 and 0.102 $mg \cdot g^{-1}$ of $lobster \cdot h^{-1}$ at 8°, 13° and 19°C respectively.

The second source of comparative data is an oxygen uptake verses temperature curve developed by Aryes and Wood (1977) for market size lobsters held under steady state environmental conditions. From this curve of oxygen consumption rates vs temperature, the oxygen consumption rates corresponding to the three experimental temperatures are 0.048, 0.068, and 0.99 $mg \cdot g^{-1}$ of $lobster \cdot h^{-1}$ for 8°, 13° and 19°C respectively. The three sets of O_2 consumption data are presented in Figure 36.

The oxygen consumption rates presented in the literature compare favourably over a wide range of temperatures. Although a similar relationship exists between oxygen consumption and temperature for the time-series experiments (i.e. O_2 consumption increases with increasing temperature) the actual rates at each temperature are approximately double the literature values. A number of explanations can be put forth

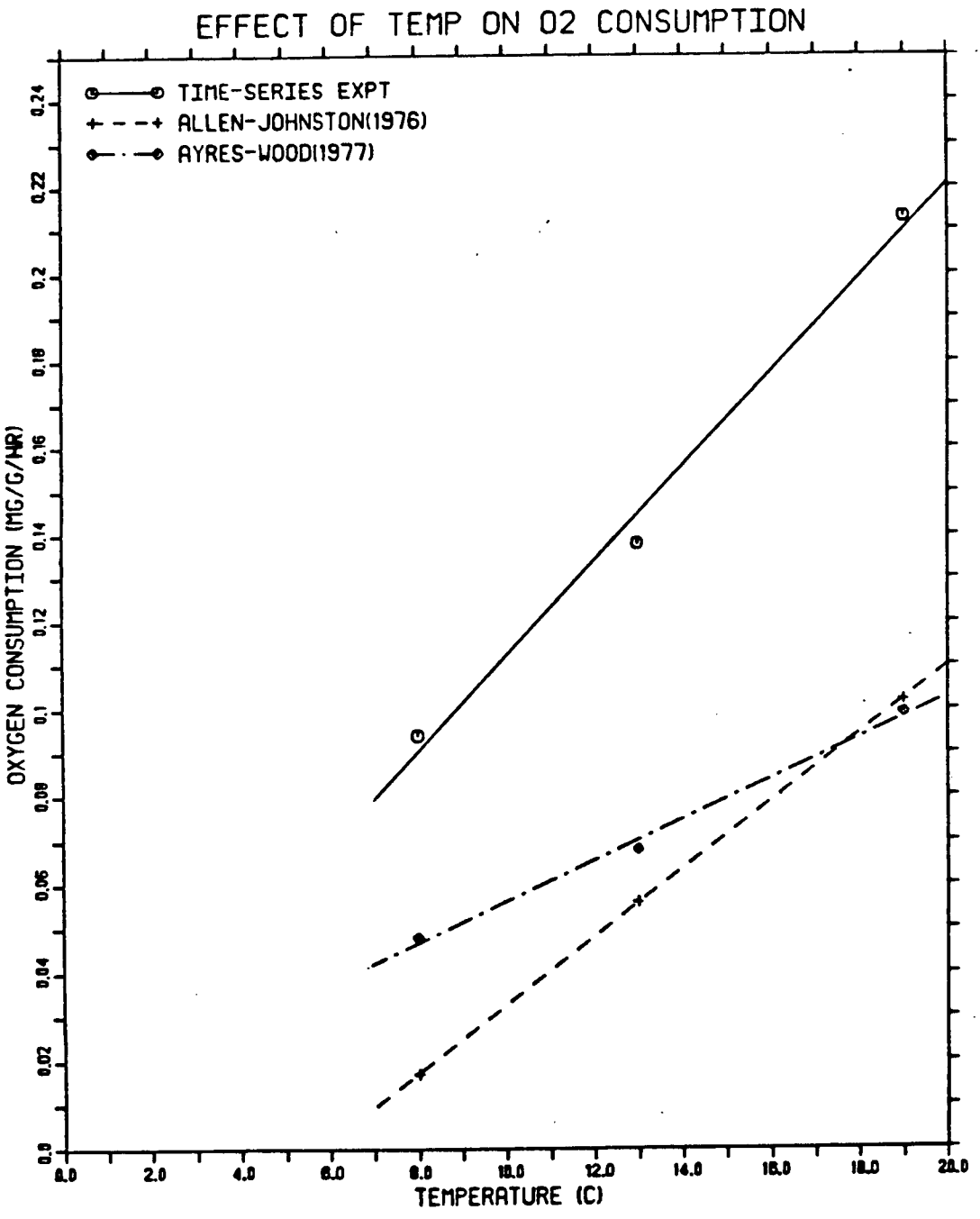

Figure 36. Effect of temperature on oxygen consumption during the first 0.25 hr of representative time-series experiments, expressed as mg of oxygen consumed per g of lobster per hr, compared to published values from two literature sources.

in this regard. First, the literature values were derived through tests with unstressed (other than temperature) animals, where as the lobsters used in the time-series experiments were potentially under considerable stress and exhibited abnormally high activity levels during the early part of each test. Both factors contribute to increased oxygen demand. As outlined in the discussion on TOC, an additional increase in oxygen consumption could result from the suspended and dissolved material present in the holding water during the period immediately after lobster introduction. These factors could be responsible for the higher oxygen demand observed during these experiments.

Although dissolved oxygen in the holding tanks never reached the potentially lethal level of 2 mg l^{-1} during any of the experiments the rapid rates at which the oxygen was consumed during the first hour of the runs should be a major consideration with respect to lobster health and storage success. It is likely that due to the added stress associated with the introduction and short-term storage process, the potentially lethal level may be considerably higher. Finally the results presented here illustrate the importance of maintaining a low holding water temperature so that oxygen carrying capacity is maximized and lobster metabolic rate is minimized.

Although potentially lethal pH levels did not occur during any of the time-series experiments, the changes that were

measured are considered significant for a number of reasons. Generally, the pH values recorded during the experiments were lower than the optimum holding range of 8.0 to 8.3. Since most experiments designed to test the effects of pH on aquatic organisms have dealt with extreme or lethal levels, very little is known about the long-term sublethal effects resulting from minor deviation from the optimal pH range. The potential synergistic effect resulting from variation in other water quality para- meters also makes it difficult to characterize and quantify chronic effects. Better known are the cause and effect relationships associated with pH and a number of biological and chemical processes that may take place in a lobster holding system. These realtionships form the basis for studying the pH changes observed during the time-series expeiments.

No significant variation in pH was observed during experiment 10 at 8°C. This observation is consistant with those made for the other parameters which also exhibit only minor variation over-time at low temperature. Biochemical processes, such as nitrification and respiration, which are influenced by pH are also effected by temperature. Low environmental temperature tends to either slow down or arrest these processes. At the higher temperatures used in experiments 1 and 2 significant pH changes did occur.

Consistent patterns of pH change emerged during experiments at both 13° and 19°C. For the first three hours of each run,

pH levels increased to a point approximately 1.5 units higher than the time 0 value. A steady decline was then observed which brought the pH down again to within 1 or 0.5 units of the time 0 level by the end of each run. It is quite probable that the initial increase in pH observed during both high temperature experiments was a result of elevated ammonia-N concentration which appeared in the holding water shortly after the lobster introductions (the ammonia-N monitoring is discussed below). The pH then declined in response to acidification resulting possibly from mineralization of organic carbon compounds, nitrification and animal respiration. In general, biological oxidation in holding systems exceeds reduction overall, resulting in a gradual decline in alkalinity and pH. The recycled seawater in the lobster holding system usually had a relatively low pH at the beginning of each experiment. The generally low pH values may be a result of these oxidation processes or they may be related to low buffering capacity of the holding water. Since salinity levels were low during the two high temperature experiments, buffering capacity may have been significantly reduced. In addition, organic carbon concentrations were high during much of the experiments and it has been shown that dissolved organics can coat the surfaces of carbonate particles thus reducing the number of ionic exchange sites (Meyers and Quin 1971; Suess 1970). A very important factor is the mediating effect of pH on aqueous chemical reactions, particularly the hydrolysis of ammonia.

Although total ammonia concentrations increased significantly during the first two hours of experiments run at 13 and 19°C, calculations showed that NH_3-N never reached toxic levels (see Table 1). NH_3-N concentrations were calculated by using the following equation derived by Whitfield (1974):

$$\% NH_3 = 100/[1 + \text{antilog } (pKa^S(T)-pH)] \quad (11)$$

where pKa^S = acid hydrolysis constant of ammonium ions in seawater

and $pKa^S(T) = pKa^S(T = 298°K) + 0.0324(298-T°K)$

NH_3-N values within the pH range 7.5 to 8.5 were obtained from tables presented in Bower and Bidwell (1978). The highest concentrations of NH_3-N calculated from the total ammonia levels presented in the results are outlined in Table 11. These values are substantially less than the acceptable lobster holding level of 1.3 mg NH_3-N$\cdot l^{-1}$.

Ammonia-N concentrations increased in a generally linear fashion during the first 0.5 h of each experiment. This was also the period during which the greatest rate increase was recorded. Ammonia-N levels increased by approximately 1.4, 4.2 and 4.1 mg$\cdot l^{-1}\cdot h^{-1}$ during the first 0.5 h of each run at 8, 13 and 19°C respectively. By incorporating the previously defined lobster biomass parameters into conversion calculations these rates can be expressed in mg of ammonia-N$\cdot g^{-1}$ of lobster$\cdot h^{-1}$. The rates corresponding to the three temperatures are 0.0219 mg g^{-1} h^{-1} at 8°C, 0.0656 mg$\cdot g^{-1}\cdot h^{-1}$ at 13°C and 0.0625 mg g^{-1} h^{-1} at 19°C. The only compara-

Table 11. Highest NH_3-N concentrations that occured during the time series experiments calculated from measured total ammonia-N values and pH.

	Temperature		
	8°C	13°C	19°C
NH_3-N ($mg \cdot l^{-1}$)	0.005	0.15	0.17
Total Ammonia-N ($mg \cdot l^{-1}$)	2.2	6.0	4.7
pH	7.1	8.1	8.1
pKa^s	9.51	9.68	9.51
Time (hr)	0.75	2.0	1.5

tive value available in the literature is that presented by Logan and Epifanio (1978). In this work ammonia excretion rates were established for larval and juvenile lobsters feeding on Artemia salina at 22°C. The relationship for both developmental stages was expresed in the equation:

$$U = -0.33 \log W - 0.37$$

where $U = \mu g\ NH_3 \cdot mg\ dry\ weight^{-1} \cdot h^{-1}$

and W = lobster weight in mg

In deriving this relationship Logan and Epifanio found that stage 7 post-larval lobsters excreted approximately 12.46 μg $NH_3 \cdot mg\ dry\ wt^{-1} \cdot h^{-1}$. A gross conversion of this value to mg ammonia-N·g wet $weight^{-1} \cdot h^{-1}$ can be made for comparison with the time-series calculations by assuming a pH of 8.0 and a wet wt./dry wt. ratio of 0.1. The value resulting from this calculation in 0.0167 $mg \cdot g^{-1} \cdot h^{-1}$.

All three of the ammonia-N rates calculated from the time-series data particularly those at 13 and 19°C, are substantially higher than the rates generated from the conversion of the Logan and Epifanio excretion data. As previously stated, there are generally two important sources of ammonia in a lobster holding of culturing system, mineralization of organic compounds by bacteria and excretion by the lobsters. Waste material adhering to the external surface of the lobster is an additional source that may be significant. Although the ammonia production rates calculated from the time-series experiments appear to be high compared to the results of Logan

and Epifanio (1978), the two sets of data are surprisingly close considering the two sets of experimental conditions. The ammonia excretion rates presented by Logan and Epifanio were determined from experiments on juvenile lobsters in a stable culture environment with a regular food supply. Although it is generally known that juvenile aquatic organisms have a proportionately higher metabolic rate and utilize available protein more efficiently than adults, it would be anticipated that ammonia excretion by adult lobsters in a holding system with a slightly lower water temperature and little or no available food should be lower. Since this did not seem to be the case during the time-series experiments one possible explanation is that a substantial portion of the ammonia generated in the holding water shortly after the lobster introduction may be a result of mineralization of organic matter. Although it is possible that some ammonia may be generated in this fashion, it is unlikely that the bacteria in the system can generate sufficient amounts of ammonia through processes such as deamination to account for the quantities that are outstanding based on the calculations made above, particularly during the short period under consideration. It is concluded therefore, that excretion by the lobsters is the major source of ammonia but that controlling TOC may effectively reduce the concentration of this compound particularly during the critical period immediately after lobster introduction.

Since a number of factors may influence ammonia-N concen-

tration in the holding water after the lobster introductions, it is difficult to determine what proportion of the reduction in ammonia-N concentration observed over time in the time-series experiments may be attributable to reduced excretion by the lobsters. Despite this problem the fact remains that a substantial load of ammonia-N appears in the holding water shortly after the introduction, and it is this dramatic increase that should be of concern in the design and maintenance of a lobster holding system. The stressful conditions and increased activity experienced by the lobsters during the first 15 or 30 minutes after the introduction may be important factors in producing this short-term increase in ammonia-N excretion. It has been shown for fish that ammonia-N excretion will often increase under stress (Hoar and Randall 1969).

Although ammonia production rates were similar at 13 and 19°C, rates were substantially less at 8°C indication that production was limited by temperature. This is not surprising since metabolic activities of all the organisms in the holding system were reduced at lower temperatures. This observation is particularly important to note with respect to lobster holding system design.

Finally, in all time-series experiments, ammonia levels reached a maximum and then began to gradually decline. This gradual reduction in ammonia concentration can be attributed to one or both of two incidental processes, volatilization or nitrification. Volatilization is analogous to the industrial

and municipal waste treatment process of air stripping. This process is pH dependent. As the pH of the wastewater increases above 7, the ammonia-ammonium equilibrium is shifted to the ammonia component which may be removed as a gas by agitating the wastewater in the presence of air. The wastewater pH is commonly adjusted to 11 prior to aeration. At 25°C and pH 11 aproximately 98% of the ammonia in the wastewater is in the un-ionized form. Although a considerable amount of aeration does take place at certain locations in the lobster holding system, pH levels tend to remain comparatively low, which in turn means that the rate of NH_3-N volatilized is also low. Nitrification could also account for the gradual reduction in ammonia-N concentrations over time in the holding system

Nitrite and nitrate concentrations were monitored during the time-series experiments to establish first whether concentrations of these compounds changed over time in the system, which would indicate some level of nitrification, and second, whether toxic levels were ever reached. Monitoring revealed that levels of nitrite and nitrate did not approach toxic concentrations during any of the time-series experiments but it is possible that sub-lethal effects may result from the levels detected in the system. Since virtually nothing has been reported regarding sub-lethal effects on concentrations it is difficult to evaluate this potential problem.

Some indication of nitrificaton is evident from the nitrite and nitrate analysis. Although minor variations in

nitrite concentration over time were recorded, particularly at 19°C, generally concentrations remained relatively stable during all the time-series experiments. Nitrate, on the other hand, gradually increased in concentration during the time-series experiments, particularly at the higher temperatures. The gradual increase in nitrate is an indication that nitrifying bacteria are present in the system oxidizing ammonia to nitrate. This observed effect of temperature on bacterial population dynamics and reaction kinetics is well documented for biological nitrification (Meade 1974; Speece 1973). It is somewhat surprising that nitrite, which is the intermediate product of nitrification, did not change signifiantly in concentration prior to this gradual nitrate increase. One explanation of this observation may be that in a well aged recycled system, the rate of ammonia oxidation by Nitrosomonas is equal to the rate of nitrite oxidation by Nitrobacter, resulting in typially low nitrite concentrations and minor nitrite variation over time (Colt and Armstrong 1981).

It appears as though temperature is the critical factor in controlling the concentration of all three nitrogenous compunds in the holding system due to its influence on the metabolic activities of both the lobsters and the heterotrophic and autotrophic bacteria present.

7.4 Silica Sand Filter Experiments

This series of experiments was carried-out to determine

whether ammonia-N in the lobster holding water was being oxidized to nitrate-N through the process of nitrification during passage through the silica sand filters.

Results from the time-series experiments indicate that nitrification rates in the silica sand filters are generally very low. At 7°C some variation in ammonia-N and nitrate-N discharge concentrations was detected but no real pattern of increasing nitrate-N and decreasing ammonia-N was observed. At the two higher temperatures limited evidence of nitrifation exists. At the 30 min mark in experiment 1 at 12°C, levels of nitrate-N began to increase in the holding water after passage through filter 2 and ammonia-N levels began to drop at approximately the same rate. A similar pattern was observed in filter 1 during experiment 2 at 17°C.

An explanation of the very low nitrification observed may be found in the conditions under which the silica sand filters operate as compared to those outlined in the literature for optimum biofilter performance.

A number of factors influence the efficiency of a biological filter, including water temperature, presence of toxic compounds in the water, pH, the concentration of dissolved oxygen, salinity, surface areas of the filtrant material, hydraulic loading (flow per unit volume of filters surface area), the residence time of the holding water in the filter, ammonia loading, and organic level in the recirculating water (Pettigrew et al. 1978; Spotte 1979). The studies of

Srna and Baggaley (1975) showed that in seawater aquaria an increase of 4°C increased ammonia and nitrite oxidation by 50% and 12% respectively. Reducing the temperature 1°C slowed the oxidation rate of ammonia by 30%, and a 1.5°C decrease slowed the rate of nitrite oxidation by 8%. It is generally accepted that increases in temperature speed up biochemical activity and a lag period is often evident in a biofilter if temprature is altered abruptly. Since no toxic compounds, such as biocides, are passed through the holding system this factor should not have influenced nitrification during the silica sand filter experiments. Srna and Baggaley (1975) determined that marine nitrifiers used in their study performed most efficiently at pH 7.45, with an effective range of 7.0-8.2. A considerable amount of oxygen is required for nitrification. Stankewich (1972) calculated the stoichiometric oxyen requirement for the conversion of NH_3-N to NO_2^--N as 3.43 kg of oxygen$\cdot kg^{-1}$ of NH_3-N oxidized. The NO_2^--N to NO_3^--N conversion requires 1.14 kg of oxygen$\cdot kg^{-1}$ of NO_2^--N oxidized. Although many species of nitrifying bacteria can tolerate saltwater and salinity changes, there is some indication that nitrification proceeds more rapidly in freshwater than in seawater systems (Kauai et al. 1964; Kuhl and Mann 1962). Available surface area in a biofilter is an imporant factor in establishing an effective community of nitrifying bacteria. Assuming that other conditions are optimum, it is generally accepted that the greater the surface area the greater the

concentration of bacteria. In a biofilter however, intersticial gaps must be large enough to allow free passage of the fluid, including any organic or inorganic particulates. Ammonia removal efficiency increases linearly as the retention time increases and appears to be independent of hydraulic load when the loading is between 1.5 and 2.5 gpm (5.68 - 7.46 $l)\cdot min^{-1}.ft^{-2}$ of filter surface area (Liao and Mayo 1974). Liao and Mayo (1974) also suggested that an ammonia loading of $2x10^{-4}$ lb ($9x10^{-5}$ kg) NH_4^+-N$\cdot ft^{-2}$ of specific medium surface area$\cdot day^{-1}$ be considered the upper limit for filter design. Finally, any organic matter present in the recirculating water encourages the growth of heterotrophic bacteria which in turn increases oxygen demand and reduces ammonia oxidation.

Based on the evidence presented above a number of factors associated with silica sand filters act to reduce their suitability as biofilters. First, although course sand is used as the filter medium, the basic reason for having the filters in line is to remove particulate matter. A large portion of this material is organic, as previously determined, and as such has a negative influence on nitrification. Second, the rentention time in the sand filters (approximately 1 min) is short and may limit the effectiveness of the filters in removing ammonia. Salinity, dissolved oxygen and pH were all maintained at suitable levels during the experiments.

Although it is almost certain that a limited amount of

nitrification is taking place in the sand filters, it appears that the reduction in the ammonia-N concentrations observed during the time-series experiments is to a large extent a result of oxidation by nitrifying bacteria in suspension or another ammonia removing process such as air stripping. In the holding system ammonia removal by either process need not be a concern provided water temperatures are kept below 10°C.

7.5 Ultraviolet Sterilizer Experiments

The use of ultraviolet (UV) irradiation, at or near 2537 A°, for the destruction of aquatic pathogenic organisms 30 μm or smaller has received a considerable amount of attention in the past. Results presented here show that the combination of pressure filtration and UV irradiation is effective in controlling bacterial levels in a lobster holding system. Although background bacterial concentrations did increase slightly with increasing temperature the generally consistent levels in the holding tank is a strong indication that the sterilization units are preforming effectively. These results are in agreement with the work reported by Burrows and Combs (1968) who stated that sterilization of fish hatchery water is best accomplished by a combination of pressure filtration and ultraviolet radiation. Herald et al. (1971) used UV irradiation to effect a 98% reduction of bacteria in a closed seawater aquarium system. Similar results were obtained by Bullock and Stuckey (1977) working on five gram-negative

bacteria pathogenic to fishes.

The influence of temperature was particuarly evident in the aquarium system. The effect of temperature on bacteria population dynamics is well documented. In this experiment a direct relationship between bacterial growth rate and temperature in the non-irradiated aquarium water was established (Figure 37). This relationship was derived by examining each of the bacterial time-series curves (Figures 32, 33, 34, 35) to locate a linear segment that extended over a two day period within the first two or three days of each experiment. From these linear segments population growth rates were calculated and plotted against their respective temperatures. Although it was based on only four points, a linear relationship is obtained with a strong correlation coefficient of 0.98 and a slope of 0.1155. This relationship was evident initially from the results of the bacterial time-series experiments, as it took approximately twice as long for the bacterial concentration in the aquarium to reach 10^4 counts$\cdot$ml^{-1} at 7°C than at 17°C. An intermediate value of 4-5 days was obtained at 12°C.

Bacterial concentrations in the holding tanks remained at approximately 10^2 counts$\cdot$ml^{-1} throughout the experiments. Hand (1977) reported that the marine intake water used in a lobster culturing system had a bacterial count ranging from 500-2000 counts per ml but the normal operating level of the system, which included a UV sterilizer was approximately 20

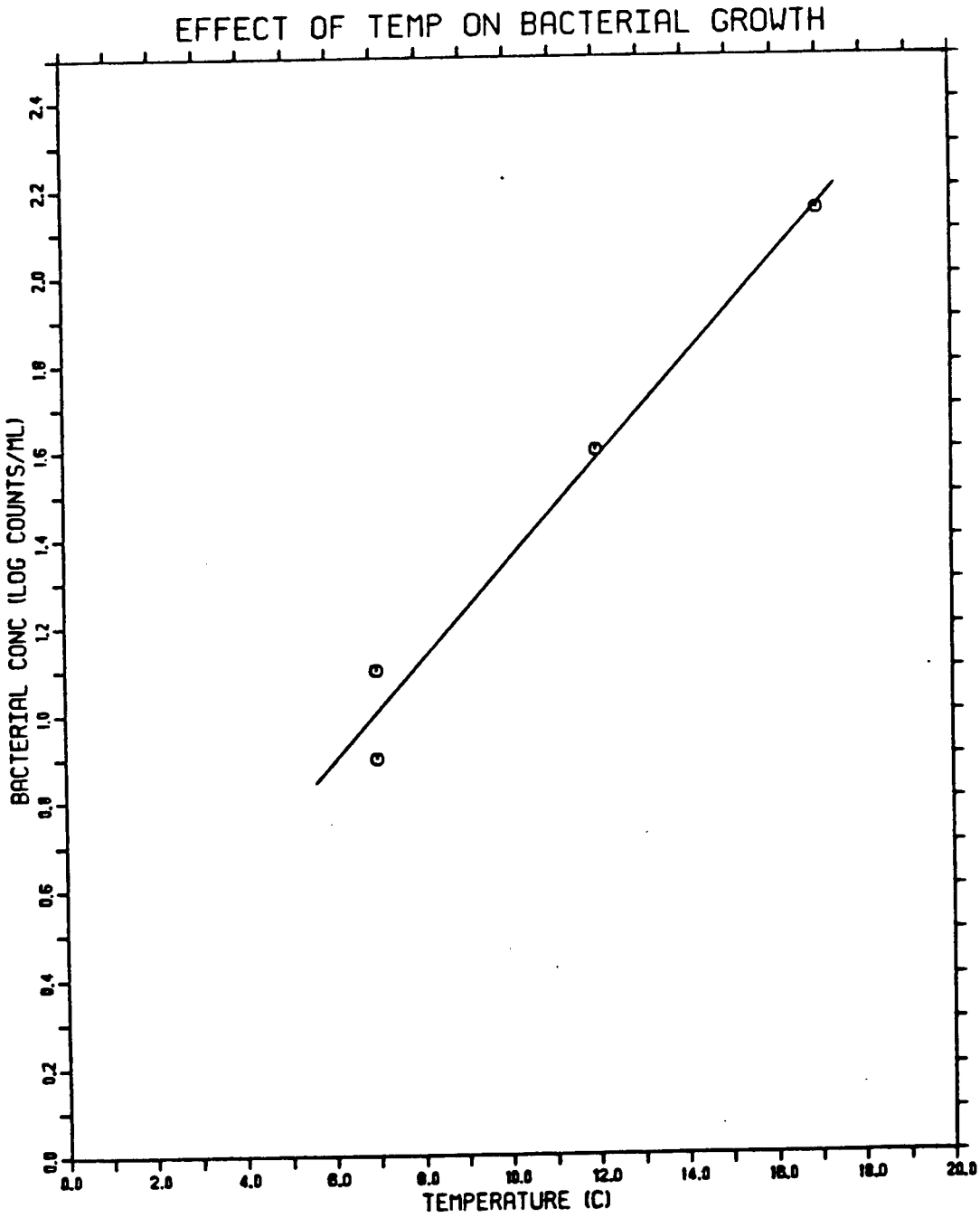

Figure 37. Effect of temperature on bacterial growth rate in the aquarium. Rates were calculated over a two day period from the linear segment of each bacterial time-series graph.

counts per ml. The slightly higher counts recorded in the system presented here may be a result of a number of factors. According to the specifications established for the system sufficient ultraviolet energy is theoretically produced to effect a 100% kill of all bacterial species that may be present. The rated output of the UV system under investigation is 60,000 $\mu Ws \cdot cm^{-2}$, compared to a required energy level of between 3,000 and 30,000 $\mu Ws \cdot cm^{-2}$ for the elimination of most bacterial species (Phillips and Hanel 1960; Kelly 1965; Koller 1965). Thus it would seem that other factors are influencing the efficiency of the UV system. The three most probable factors are the condition of the UV lamps and quartz sleeves the flow rate of the holding water past the lamps, and the turbidity of the water. Since the UV lamps and quartz sleeves are serviced regularly (once a year as per specificiations) by the holding operation managers, and the flow rate past the lamps is regulated by an accurate valve system, it is most likely that inhibition of UV penetration is attributable to suspended and dissolved material in the holding water.

The transmission of light through water follows the exponential relationship:

$$\frac{TR}{1 - RF} = e^{ad} \qquad (13)$$

where TR = transmission (%)

e = base of natural logarithm

a = coefficient of absorption (cm^{-1})

d = depth of water (cm)

RF = surface reflections (0.02 for water)

Since the average distance the UV radiation must travel in a UV sterilizer is small (approximately 10 cm) the coefficient of absorption is the significant factor in determining the percent bacterial kill in the system. The coefficient of absorption will most likely vary substantially with the filtering efficiency of the silica sand filters, which in turn varies with particulate load in the system. Data presented by Koller (1965) indicate that even small increases in the coefficient of absorption may have a significant effect on the amount of UV light reaching the microorganisms in the holding water. An increase of 0.01 in the coefficient of absorption from 0.05 to 0.06 through 10 cm of water drops the percent UV light transmission from approximately 60 to 55%. It is difficult to estimate the coefficient of absorption for the lobster holding water since it will vary sharply with lobster load and filtering efficiency. Koller (1965) assigns an absorption coefficient of 0.008 to distilled water, a value much lower than one would expect for a mean coefficient for lobster holding water. Excluding distilled water the lowest coefficient presented by Koller is 0.02 which is undoubtedly lower than lobster holding water. Even at this low coefficient

of absorption UV light transmission through 10 cm of water is reduced by approximately 15% to 25%. These figures demonstrate the importance of particulate removal prior to UV filtration and provide a possible explanation for the slightly higher than expected background levels of bacteria in the holding system.

Although the two most significant lobster diseases from a live storage point of view, Gaffkemia, and shell disease, are caused by bacteria (some forms of shell disease may be a result of fungal infection) several other less common diseases such as Lagenidum disease and Fusarium disease are fungal infections. Research indicates that fungal organisms are generally more resistant to UV irradiation than bacteria (Phillips and Hanel 1960; Kelly 1965; Koller 1965) and as such would be more likely to survive in the holding water. Although specific studies were not conducted on the presence of fungus associated with the holding water or the lobsters there were no cases of fungal diseases observed during the period of general investigation of the holding facility. A test for Gaffkemia in a shipment of lobsters produced negative results. At various times shell disease is a minor problem in the holding system but usually very few animals are infected and due in part to this study, appropriate measures, such as removing infected animals and maintaining proper levels of filtration and UV irradiation, are taken to abate the problem.

8.0 SUMMARY AND CONCLUSIONS

An overview of the work reported here reveals a number of important features associated with water quality in a short-term lobster holding system. First, a good correlation was found between the COD and TOC concentration in the lobster holding water. Since this relationship is strong it is likely that TOC is a good measure of oxygen demand in the system. The empirically determined COD/TOC ratio of 1.67 was shown to be lower than published values for a variety of waste waters. This may be due in part to organic compounds in the holding water that are resistant to the COD digestion.

TOC monitoring during the time-series experiments generally showed a dramatic increase in TOC concentrations in the holding water immediately after the lobsters were introduced into the tanks, followed by a rapid decline 15 or 30 minutes after the introduction. It was concluded that the increase in TOC was a combined result of lobster excretion, material from the external surface of the animals and resuspension of existing organics. No relationship was shown between TOC concentration and temperature but there did appear to be a biomass effect. TOC increases were not nearly as extreme when a smaller biomass was introduced into the system. Calculations with the TOC data to approximate a BOD equivalent showed that oxygen demand of the lobster holding water was 4 to 5 times higher than levels in fish hatchery water. These high oxygen demand conditions

are unduly stressful to lobsters for two reasons. First, reduced oxygen availability, and second, a considerable amount of small suspended particulate material is associated with the high TOC levels which interfers with the exchange of oxygen across the surface of the gills.

Dissolved oxygen concentrations recorded during the time-series experiments never reached potentially lethal levels, nor did any of the other water quality parameters. At the higher experimental temperatures, oxygen levels in the holding water did drop to a point that could be considered critical when other parameter levels are less than optimal. This synergistic effect, although it may be significant under certain conditions, is very difficult to quantify. A drop in oxygen concentration was observed immediately after lobster introduction, followed by a gradual increase to a steady state condition which was usually lower than the initial (time 0) concentration. A number of factors may act to reduce dissolved oxygen in the system at that point, the most significant being general oxygen demand from the water and consumption by the lobsters. Oxygen levels began to increase significantly during the time-series experiments after lobster activity in the holding tanks subsided. Rates of oxygen consumption calculated over the first 15 minutes of the experiments when compared to published values for lobster oxygen consumption showed similar trends with respect to temperature (higher consumption at higher temperature) but the time-series rates of oxygen

consumption were all higher than published values. It is concluded that the higher time-series levels are the result of increased oxygen consumption by the lobsters due to stress and a possible high oxygen demand of the water which was not a factor in the experiments reported in the literature.

Although pH was never a problem during the time-series experiments, changes in this parameter were indicative of various biochemical processes. pH generally increased during the early stage of the experiments due to the influx of ammonia. As ammonia level dropped so did pH due to processes such as nitrification and animal respiration. Due to the relatively high TOC levels that appear in the holding water shortly after lobster introduction, the buffering capacity of the holding water may have been adversely effected. Organic carbon has the capacity for reducing the solubility of sodium and calcium carbonates.

Of the three nitrogenous compounds monitored during the time-series experiments only ammonia and nitrate showed significant changes over time. Generally, ammonia concentrations increased rapidly early in the experiments at the higher temperatures due to lobster excretion and possibly mineralization of organic material. After the initial period of high stress, the lobsters most likely reduced their ammonia excretion rate, and nitrification and volatilization combined to reduce levels of this compound even further. An ammonia production rate calculated from the change in ammonia

concentration during the first 30 minutes of the time-series experiment at 19°C was higher than a rate calculated for juvenile cultured lobsters at 22°C. A number of factors can be suggested to explain this difference but the high stress conditions under which the time-series lobster were held probably contributed most significantly to the high rate of ammonia production in the system. No significant changes in nitrite concentrations were observed during any of the time-series experiments. Any nitrite produced during the nitrification process will be oxidized to nitrate rapidly thus maintaining the concentration of this compound at a relatively consistent level. The concentraton of nitrate was observed to increase gradually during the time-series experiments conducted at the two higher temperature. It is concluded that nitrification was ocuring at a low but detectable rate.

No evidence of nitrification resulted from experiments on the silica sand filter at 7°C. A number of factors are responsible for this, the most significant being the low temperature and the high flow rate through the filters. At 12 and 17°C nitrification was evident to a limited extent. The most important regulating factors are likely the high flow rate and competition from heterotrophic bacteria. At the higher temperatures a significant amount of nitrificiation may be taking place in suspension.

It is concluded that the UV sterilizer system is effective at controlling bacterial populations in the holding water,

although levels did increase slightly with increasing temperature (from approximately 10^2 counts·ml^{-1} at 7°C to about $10^{2.5}$ counts·ml^{-1} at 17°C). In the non-irradiated aquarium water bacterial populations were not controlled at any temperature but growth rates were significantly slower at lower temperatures.

Several conclusions can be drawn from the work presented here. First, it appears that short term effects of lobster introduction are the most dramatic and probably the most significant. In virtually all instances, particularly at the higher temperatures, substantial deterioration in water quality, as measured by the established water quality parameters, was observed during the first 15-30 minutes after lobster introduction. Continued monitoring for a 12 h period showed that water quality often improved dramatically after the 15 to 30 minutes critical period, but in most cases never reached the level recorded at time 0. Therefore it is this short critical period that is likely the most stressful for the lobsters during the holding period. Over the longer-term, water quality generally improved to a level at which any effects that may have resulted were insignificant compared to the short term impacts. These long term effects may be important in culturing operation but not in a short term holding system.

A second general conclusion is that temperature is the most important factor controlling water quality in the lobster holding system. Temperature directly effects the oxygen carry-

ing capacity of the water, and more importantly, the metabolic activity of the lobsters. Therefore, if temperature can be kept low (6-8°C) most of the other important water quality parameters can be maintained at suitable levels.

Although temperature control appears to be the critical factor in the design of a successful short-term holding system for lobsters, it is important to monitor a wide range of water quality parameters during operation of the system since low temperatures are not absolute insurance against deteriorating holding water quality.

Finally, although potentially lethal levels were never reached for any parameter during the critical period established during the time-series experiments, combined effects from several parameters at less than acceptable levels may prove to be fatal to stress weakened lobsters. In fact this result was observed on a number of occasions during the experiments at the higher temperatures. A number of mortalities occured which were apparently not attributable to unacceptable levels of any one water quality parameter.

9.0 RECOMMENDATIONS

A number of general recommendations regarding the design and maintenance of a short-term lobster holding system can be put forth based on the results of this study.

1. Temperature control is an essential feature in holding system design. The refrigeration unit incorporated into the design of any holding system should be capable of maintaining the holding water temperature at between 6 and 8°C.

2. To help control high TOC and particulate levels in the holding water imediately after lobster introduction, tanks should be vacuum cleaned prior to the introduction of each new shipment.

3. During the critical period immediately after introduction of the lobsters, additional oxygen should be added to the holding water to satisfy the high demand that occurs at this time.

4. To minimize deterioration of water quality upon lobster introduction it is suggested that the introduction process take place over a period of approximately one hour. This reduction in introduction rate will have a buffering effect

on water quality impacts that result from rapid introduction of lobsters into a holding system.

5. An acclimation period may be required prior to introduction of lobsters into a holding system if the difference between the shipping temperature and the holding temperature is greater than 5°C.

6. Since water quality tended to reach a more-or-less acceptable long-term steady-state level after the 30 minute critical period, it is assumed that the addition of fresh seawater at the rate of 10% of the system volume per day, was sufficient to ensure acceptable water quality in a lobster holding system over a period of several weeks. Depending on a variety of factors, such as water temperature and buffering capacity, water quality may deteriorate over a longer period, therefore it is recommended that a complete water change be carried-out on the system approximately once every month.

7. Although each individual lobster holding system will require unique design features to deal with site specific water quality problems, it is essential that a basic water quality monitoring programme be a part of the day-to-day operation of all systems.

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11.0 APPENDICES

Appendix 1. Results of TOC monitoring in mg l^{-1}, during the time-series experiments.

	Experiment #									
Time (hr)	1	2	3	4	5	6	7	8	9	10
0	18.1	36.1	25.4	101.1	26.5	57.6	25.1	34.5	19.5	48.4
0.25	204.4	261.9	310.3	241.9	195.6	224.3	164.0	461.7	241.4	385.4
0.50	222.3	244.3	305.7	256.8	220.4	386.5	89.3	489.8	304.5	405.1
0.75	316.1	201.7	272.8	268.6	231.5	291.6	43.7	451.9	276.7	261.7
1.0	184.9	179.8	224.7	287.5	222.6	205.7	45.9	369.4	201.4	144.1
1.5	170.2	185.6	206.4	256.1	191.3	211.8	38.5	195.7	169.9	139.7
2.0	166.6	164.5	139.3	224.3	176.1	225.1	33.2	181.2	144.6	117.5
2.5	161.1	143.8	91.3	185.2	155.9	198.7	27.9	177.3	101.4	118.8
3.0	84.4	150.2	82.9	168.7	138.4	169.4	36.5	186.4	93.3	105.9
4.0	86.4	138.7	84.1	177.8	115.6	181.7	39.4	169.5	56.5	102.6
5.0	42.8	121.4	78.5	191.2	96.4	176.4	32.9	171.7	50.1	112.1
6.0	46.3	126.3	75.4	176.4	105.1	144.9	40.1	168.1	52.4	101.9
7.0	36.7	109.9	74.3	165.1	86.7	150.1	35.7	156.9	46.5	98.5
8.0	38.9	84.1	83.7	154.6	101.9	139.3	29.9	159.4	56.7	87.6
9.0	50.1	77.6	80.2	149.8	68.9	127.4	34.8	145.6	60.9	94.5
10.0	40.5	89.8	75.1	159.6	76.4	115.5	39.4	151.3	55.4	96.8
11.0	38.0	81.3	81.5	150.1	56.8	111.2	36.8	149.6	52.3	101.6
12.0	36.1	75.7	78.9	145.9	61.5	104.1	39.1	141.8	48.5	89.4

Appendix 2. Results of dissolved oxygen monitoring, in mg l^{-1}, during the time-series experiments.

	Experiment #									
Time (hr)	1	2	3	4	5	6	7	8	9	10
0	8.9	8.2	8.1	7.8	8.8	9.1	10.5	10.2	10.8	10.0
0.25	6.7	4.6	4.7	3.4	4.1	7.5	8.9	9.1	8.6	8.5
0.50	5.6	2.9	3.4	2.1	5.0	7.6	8.5	9.0	8.4	7.9
0.75	5.3	3.8	3.5	2.6	5.3	7.8	8.6	9.0	8.5	7.6
1.0	5.2	4.2	3.9	3.9	5.4	8.3	8.7	9.3	8.6	8.1
1.5	5.0	4.9	4.7	4.8	5.8	8.4	8.9	9.5	8.9	7.8
2.0	5.4	5.1	4.9	4.9	6.2	8.5	9.3	9.2	8.7	7.9
2.5	6.0	5.1	5.4	5.4	6.6	8.5	9.4	9.4	8.7	8.0
3.0	6.6	5.8	5.6	5.5	6.8	8.4	9.4	9.5	8.5	8.5
4.0	6.7	5.9	5.9	5.7	6.8	8.4	9.5	9.6	8.9	8.2
5.0	6.6	6.2	5.8	6.3	7.0	8.5	9.7	9.5	8.7	8.6
6.0	6.6	6.3	5.5	6.2	7.1	8.5	9.5	9.7	8.9	8.5
7.0	6.5	6.5	5.4	6.4	7.2	8.6	9.7	9.3	8.9	8.7
8.0	6.6	6.5	5.7	6.3	7.2	8.4	9.6	9.4	9.3	8.4
9.0	6.6	6.7	5.5	6.3	7.2	8.5	9.6	9.3	9.5	8.6
10.0	6.5	6.8	5.9	6.3	7.3	8.5	9.5	9.5	9.1	8.8
11.0	6.5	6.8	5.8	6.4	7.4	8.7	9.7	9.7	9.3	8.5
12.0	6.6	7.1	5.3	6.8	7.5	8.4	9.4	9.6	9.6	8.7

Appendix 3. Results of pH monitoring during the time-series experiments.

	Experiment #									
Time (hr)	1	2	3	4	5	6	7	8	9	10
0	6.9	7.6	6.6	7.3	7.2	8.0	7.7	7.6	7.8	7.1
0.25	7.4	7.7	7.2	7.3	7.4	8.1	7.8	7.6	7.7	7.2
0.50	7.5	7.9	7.3	7.5	7.2	8.0	7.6	7.5	7.9	7.0
0.75	7.8	8.0	7.5	7.6	7.4	8.3	7.7	7.7	7.9	7.1
1.0	7.7	8.0	8.0	7.6	7.3	8.4	7.7	7.8	8.0	6.9
1.5	7.9	7.8	8.1	7.4	7.5	8.6	7.6	7.9	7.9	7.0
2.0	8.1	8.0	8.0	7.6	7.7	8.6	7.6	7.9	8.1	7.1
2.5	8.2	7.9	8.0	7.7	7.8	8.7	7.6	8.2	8.0	7.2
3.0	8.2	7.7	8.1	7.6	7.7	8.5	7.8	8.3	8.1	7.1
4.0	7.7	7.9	7.9	7.6	7.7	8.4	7.7	8.5	8.1	7.1
5.0	7.6	7.8	7.7	7.5	7.8	8.3	7.8	8.4	8.2	7.2
6.0	7.8	7.9	7.9	7.6	7.6	8.4	7.9	8.5	8.0	7.3
7.0	7.5	7.7	7.8	7.7	7.7	8.5	7.6	8.5	8.0	7.3
8.0	7.7	7.7	7.7	7.5	7.6	8.4	7.8	8.4	7.9	7.1
9.0	7.5	7.6	7.7	7.5	7.6	8.4	7.7	8.1	8.0	7.0
10.0	7.4	7.6	7.6	7.4	7.7	8.3	7.7	8.0	7.8	7.3
11.0	7.4	7.4	7.7	7.4	7.5	8.4	7.6	8.1	7.8	7.2
12.0	7.3	7.5	7.6	7.4	7.5	8.4	7.8	7.8	7.7	7.3

Appendix 4. Results of ammonia-N monitoring, in mg l^{-1}, during the time-series experiments.

	Experiment #									
Time (hr)	1	2	3	4	5	6	7	8	9	10
0	3.3	4.8	1.9	1.7	3.6	2.3	4.8	6.8	4.7	1.3
0.25	4.5	4.7	3.3	1.8	3.4	2.7	4.9	6.5	5.0	1.6
0.50	5.4	4.9	3.9	2.3	3.5	2.9	5.0	6.7	4.9	2.0
0.75	5.3	5.6	4.2	2.4	3.7	3.0	4.7	6.9	5.3	2.2
1.0	5.9	5.8	4.4	2.8	3.9	2.9	4.9	7.4	5.2	2.0
1.5	6.4	6.0	4.7	3.2	4.0	3.4	5.1	7.5	5.6	2.3
2.0	6.0	6.8	4.8	3.1	4.1	3.7	5.0	7.9	5.8	2.1
2.5	5.9	6.7	5.0	3.3	3.8	3.9	4.9	8.1	5.6	1.9
3.0	6.3	7.1	4.7	3.0	3.8	4.1	5.6	8.3	5.9	2.0
4.0	4.9	6.9	4.8	3.2	4.0	4.2	5.0	8.0	5.7	1.8
5.0	4.6	6.8	5.0	3.1	3.5	4.0	5.1	8.5	6.0	2.1
6.0	4.8	6.4	4.6	3.0	3.7	4.2	4.8	8.6	5.7	2.0
7.0	4.3	6.3	4.5	3.1	3.8	3.9	4.7	8.4	5.5	1.9
8.0	4.4	6.2	4.3	2.8	3.4	4.2	4.9	8.0	5.7	1.9
9.0	4.0	6.2	4.2	2.7	3.6	3.8	4.5	8.3	5.3	1.8
10.0	3.9	6.3	4.0	3.0	3.7	3.9	4.7	8.1	5.2	1.7
11.0	4.1	6.1	3.9	2.5	3.3	3.6	4.9	7.9	5.1	1.8
12.0	4.3	5.9	3.7	2.3	3.5	3.7	4.8	7.6	4.8	1.7

Appendix 5. Results of nitrite-N monitoring, in mg l^{-1}, during the time-series experiments.

	Experiment #									
Time (hr)	1	2	3	4	5	6	7	8	9	10
0	1.33	0.19	1.89	0.42	0.16	1.66	0.11	0.24	0.36	0.45
0.25	1.34	0.24	2.11	0.51	0.29	1.50	0.21	0.30	0.61	0.41
0.50	1.34	0.20	2.34	0.64	0.21	1.61	0.15	0.41	0.48	0.48
0.75	1.35	0.21	1.86	0.48	0.33	1.55	0.22	0.29	0.29	0.47
1.0	1.38	0.43	2.28	0.77	0.25	1.49	0.09	0.38	0.44	0.54
1.5	1.41	0.85	1.77	0.86	0.47	1.50	0.14	0.44	0.71	0.39
2.0	1.44	0.78	1.59	0.91	0.40	1.39	0.20	0.52	0.78	0.41
2.5	1.49	0.94	1.56	1.04	0.56	1.47	0.18	0.43	0.68	0.33
3.0	1.53	0.75	2.35	1.09	0.61	1.36	0.26	0.40	0.59	0.37
4.0	1.56	0.85	2.14	1.07	0.47	1.45	0.10	0.51	0.50	0.43
5.0	1.59	0.57	2.22	0.79	0.41	1.33	0.16	0.64	0.69	0.39
6.0	1.57	0.67	1.96	0.84	0.30	1.29	0.24	0.60	0.82	0.42
7.0	1.55	0.71	2.05	0.51	0.29	1.20	0.31	0.59	0.75	0.47
8.0	1.52	0.69	1.79	0.69	0.30	1.31	0.23	0.70	0.79	0.50
9.0	1.48	0.80	1.91	0.75	0.26	1.37	0.15	0.65	0.58	0.56
10.0	1.45	0.64	1.83	0.86	0.39	1.25	0.19	0.49	0.51	0.58
11.0	1.43	0.59	1.64	0.70	0.24	1.17	0.22	0.38	0.43	0.58
12.0	1.42	0.72	1.59	0.62	0.32	1.28	0.17	0.45	0.49	0.56

Appendix 6. Results of nitrate-N monitoring, in mg l^{-1}, during the time-series experiments.

	Experiment #									
Time (hr)	1	2	3	4	5	6	7	8	9	10
0	10.5	16.5	23.9	83.4	21.8	18.3	11.6	15.9	5.9	4.8
0.25	11.0	18.4	24.6	80.6	22.4	17.4	9.6	16.4	4.9	4.7
0.50	11.1	16.8	22.3	79.4	23.8	16.9	9.9	15.1	5.1	5.1
0.75	11.2	15.6	23.1	80.9	22.6	18.1	10.4	14.8	6.4	4.5
1.0	11.1	17.5	23.8	76.6	24.1	19.5	11.6	15.5	5.8	4.9
1.5	11.3	18.1	23.6	81.4	24.8	19.0	10.8	16.6	5.5	5.3
2.0	11.6	17.4	24.0	81.5	25.3	21.4	11.5	16.0	5.9	5.0
2.5	11.2	16.9	23.9	75.4	24.3	18.6	10.8	16.8	6.1	4.5
3.0	11.7	18.9	24.4	76.1	25.6	22.7	9.4	15.9	6.6	4.6
4.0	11.4	17.8	24.6	70.9	27.1	23.8	10.7	16.7	5.7	4.9
5.0	11.9	18.5	24.1	73.8	27.9	23.0	11.0	17.1	6.8	5.4
6.0	11.8	19.0	24.3	69.5	28.0	23.5	10.4	17.4	7.0	4.8
7.0	11.6	19.1	24.9	73.4	27.3	24.1	10.8	17.9	6.4	5.6
8.0	11.8	19.6	25.2	69.6	28.4	23.2	11.6	18.0	6.8	5.8
9.0	12.4	19.5	25.7	70.8	29.9	23.6	11.9	18.9	6.3	6.2
10.0	12.8	20.1	26.0	68.4	28.6	22.9	12.1	17.8	7.2	6.5
11.0	12.9	19.7	26.6	67.7	28.8	23.8	11.2	18.4	6.9	6.3
12.0	13.3	19.8	26.4	69.1	29.0	22.5	11.6	18.1	6.7	6.4