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THE CULTURE OF PENAEUS INDICUS MILNE EDWARDS
IN EXPERIMENTAL CLOSED SYSTEMS WITH
SPECIAL REFERENCE TO WATER QUALITY

by

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ABSTRACT

This study embraces two aspects of closed system culture: (a) a comparative study of four different biological filtration treatments, and (b) a quantitative determination of the nitrogen flow in a particular biological filtration system. Twenty 0,5 g juvenile Penaeus indicus Milne Edwards were cultured for approximately two months in four different kinds of sea water recirculatory systems with bacterial filtration, algal filtration, algal plus bacterial filtration and water exchange (25% every 3 days). Growth was comparable to that found in the literature for P.indicus. Nitrification occurred in all systems irrespective of the type of biological filtration employed. Ammonia, nitrite and nitrate levels were reduced by the algae, but nitrate accumulated to approximately $30 \text{ mg NO}_3^- \text{-N l}^{-1}$ in those systems without algae. Depletion of trace elements (Cu, Fe, Zn, Mn and Co) by the algae did not occur due to trace metal replenishment from the addition of food. No marked changes occurred in the concentrations of the major cations (Ca^{++} , Mg^{++} , Na^+ and K^+), but a small increase in Ca^{++} levels was detectable in those systems with oyster shell chips in the filtration medium. Only minor changes occurred in oxygen or pH values in the various systems tested. Inorganic carbon levels showed major increases in the algal filtration systems, while a slight drop occurred in the bacterial filtration systems. The increase in the algal filtration systems was thought to be due to the formation of carbonates and bicarbonates by the reaction of respiratory CO_2 with unionized ammonia and water. Suggestions are made as to how closed systems can be improved upon.

By determining the rate of excretion of P.indicus as related to size and temperature, its faecal production, and the nitrogen content of P.indicus, its food and faeces, the nitrogen flow within a system could be calculated. P.indicus was found to have an assimilation efficiency of 88,93% and a food conversion ratio of 3,49:1. Of the total dietary nitrogen input into the system, P.indicus ingested 69,02% - 19,80% of which was used for growth, 41,58% excreted and 7,64% lost as faeces. The remaining 30,98% of the total nitrogen input can be regarded as excess food nitrogen. The total nitrogen loading on the biological filtration system was found to be 80,20% of the total input of dietary nitrogen. The ability of various types of filamentous algae to remove these nitrogen compounds was determined. Suggestions are made as to the application of this information on nitrogen flow to the culture of P.indicus.

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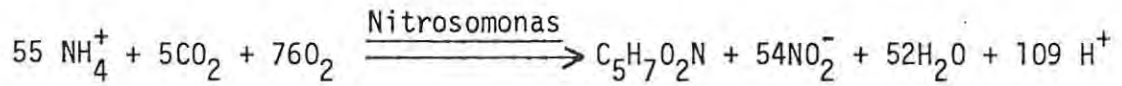
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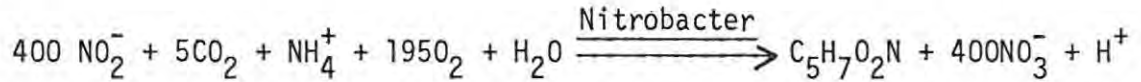
GENERAL INTRODUCTION

Advances in mariculture and marine research have placed an exigent demand on the need for marine water systems which can provide stable and controlled environments for the proper maintenance of cultured organisms. Direct utilization of sea water in open culture systems presents problems since this water is subjected to diurnal and seasonal fluctuations in temperature, salinity and turbidity, as well as possible contamination from industrial, agricultural and maritime sources. Furthermore, large mariculture farms release enormous amounts of organic waste which, if not subjected to some form of post-treatment, result in heavy eutrophication and could lead to environmental degradation of coastal areas. A closed system approach, in which water is recycled and used again, not only avoids the above problems, but also offers greater scope for water quality management.

In a closed system, metabolic end products of the cultured organisms, and the presence of accumulated excess food, rapidly change the chemistry of the culture water. These compounds, even at sublethal levels, have been found to affect growth, fecundity and resistance to disease (Spotte, 1970). Thus biological filtration systems are commonly used to transform and remineralize organic substances in the water. The filters consist of gravel beds which mechanically filter particulate organic compounds (faeces and excess food) from the water. The particulates and gravel then provide surfaces for heterotrophic and autotrophic bacterial colonization. The heterotrophs break down nitrogenous organic compounds to ammonia which is then successively oxidized to nitrite and nitrate by autotrophic nitrification. Nitrification is mediated by two highly specific groups of chemolithotrophic bacteria, the Nitrosomonas group and the Nitrobacter group. The former oxidizes ammonia to nitrite, while the latter oxidizes nitrite to nitrate according to the following equations:



and



(Haug & McCarty, 1971).

Nitrate accumulates in well-aerated closed systems and is substantially less toxic than ammonia and nitrite.

Bacterial filtration has long been used in municipal waste water treatment (Hemens & Stander, 1969; Pretorius, 1973; Parkin & McCarty, 1975), and a vast amount of literature has accumulated on the use of trickling filters (Balakrishnan & Eckenfelder, 1969a); rotating algal disc units (Pretorius, 1975); activated sludge processes (Balakrishnan & Eckenfelder, 1969b, 1970) and algal growth in lagoons for 'polishing' secondary sewage effluent (Anonymous, 1973; Toms et al., 1975). In fact, Mayo (1976) accused aquaculturalists of "re-inventing the wheel", since they have not made full use of water purification developments in the fields of sanitary and industrial engineering. However, he conceded that much of this technology is inapplicable since many methods are used (anaerobic denitrification etc.) which are incompatible with the culture of aquatic organisms.

An alternative to bacterial nitrification where toxic ammonia is oxidized to nitrate, is the direct removal of nitrogenous compounds by algae. Little is known about the effects of algae on culture water even though it has been a decorative feature of marine aquaria for decades (Atz, 1949, 1950). Shelbourne et al. (1963), and Shelbourne (1964) used illuminated fronds of Enteromorpha intestinalis Link for metabolite, CO₂ and pH control in a closed culture system. However, no data were given for the effectiveness of these algae. Alderson and Howell (1973) and Siddall (1974) have used unicellular algae for the removal of nitrogenous compounds from their culture systems. Goldman et al. (1974a, 1974b) used algae in a tertiary treatment system, while Honn and Chavin (1975) employed algae

in a quarternary sea water processing system. Most recently, Naegel (1977) combined the production of fish and algae in recirculated fresh water. The present status of algal filtration is, however, best summarized by Kinne (1976) who states that "our present knowledge of the functioning and usefulness of algal filters is very limited."

It is apparent, therefore, that we do not as yet have sufficient command over the aquatic environment. This situation needs to be rectified, and two of the more important aspects have been dealt with in this study. They are: (a) a comparison of the ability of various biological filtration systems (including algal filtration) to purify recycled sea water, and (b) a quantitative determination of the extent to which a particular animal modifies its environment, as well as the capacity of that culture system to cope with such changes. In the comparative study four different filtration treatments (algal filtration, bacterial filtration, algal plus bacterial filtration and water exchange) were compared by monitoring well known parameters of change in water quality (Part I). The biological filtration systems were stocked with Penaeus indicus Milne Edwards, and their growth was also monitored and compared (Part II). The second aspect of the study involved a quantitative determination of the flow of nitrogen in a particular culture system stocked with P.indicus (Part III). The introduction of nitrogen as food, its flow within the system, and its eventual removal by filamentous algae was examined and quantified. It is hoped that the results from this section of the work will assist culturalists in the determination of optimum culture conditions, stocking densities and feeding rates of P.indicus.

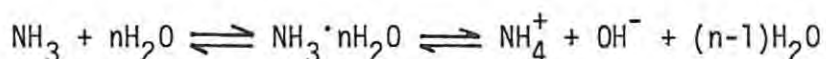
PART I: WATER QUALITY CHANGES OCCURRING WITHIN BIOLOGICAL FILTRATION SYSTEMSINTRODUCTION

At the outset of these experiments a decision had to be made as to which algae should be used and what parameters had to be measured. It was decided to utilise filamentous algae from salt marsh environments. These algae possess a large surface area for nutrient assimilation and appear to be exceptionally hardy, as they are regularly subjected to rapidly changing salinities and temperatures in nature. The algae used were of the genera Rhizoclonium Kuetzing and Chaetomorpha Kuetzing. (Identification by R.H. Simons of the Division of Sea Fisheries, Cape Town.) The following parameters of water quality were chosen: inorganic nitrogen compounds (ammonia, nitrite and nitrate), total inorganic carbon, pH, oxygen and major cation and trace metal concentrations. Phosphate and dissolved organic compounds were omitted for the following reasons:

1. Goldizen (1970) found phosphate levels never become toxic in calcareous semi-closed systems, because the phosphate is precipitated as the calcium salt.
2. A study of the accumulation of organic compounds was felt to be beyond the scope of this study.

Inorganic nitrogen compounds

The main nitrogen compounds of concern in closed aquaculture systems are ammonia, nitrite and nitrate. The ammonia in culture water originates from mineralization of organic substances by heterotrophic bacteria and from excretion by culture animals (Spotte, 1970). In aqueous solutions, unionized ammonia exists in equilibrium with the ammonium ion according to the following equation (Emerson et al., 1975):



Unionized ammonia (NH_3) is the most lethal fraction. Its relatively high lipid solubility and absence of charge enables it to diffuse quite readily across cell membranes (Fromm & Gillette, 1968). Relative proportions of NH_3 and NH_4^+ are determined primarily by the pH of the culture water. A reduction in the pH of the water from 8 to 7 results in a tenfold decrease in the quantity of unionized ammonia (Downing & Merckens, 1955). Wickins (1976a) estimated the maximum 'safe' level of unionized ammonia, which reduced the growth of seven penaeid prawn species by 1-2%, to be $0,1 \text{ mg NH}_3\text{-N l}^{-1}$.

Nitrite accumulation in closed culture systems is the result of bacterial oxidation of ammonia. Wickins (1976a) found that above $6,4 \text{ mg NO}_2^-\text{-N l}^{-1}$ the growth of Penaeus indicus Milne Edwards was reduced to approximately 50% of control animals.

Nitrate accumulation is the result of bacterial oxidation of nitrite. Wickins (1976a) found that nitrate is far less toxic than nitrite. The growth of Penaeus monodon Fabricius was not affected after 3-5 weeks exposure to concentrations of over $200 \text{ mg NO}_3^-\text{-N l}^{-1}$. Bohl (1977) mentions cessation of feeding and skin lesions occurring when trout were exposed to $800 \text{ mg NO}_3^-\text{-N l}^{-1}$. Most nitrate in culture systems exists in the form of nitric acid (Gunderson & Mountain, 1974) which reduces the pH as it accumulates.

Major cations

The concentrations of the major cations (Na^+ , K^+ , Ca^{++} and Mg^{++}) were monitored. Levels of Ca^{++} and Mg^{++} can be expected to be influenced in three ways:-

1. Precipitation with phosphate: Ca^{++} and Mg^{++} is continually lost from culture water due to precipitation with phosphates arising from accumulated excreta. This results in the loss of excess bases and eventual acidification (Saeki, 1958).

2. Precipitation with fatty acids: Berner (1968) found that during bacterial decomposition Ca^{++} is precipitated as a mixture of calcium fatty acid salts or soaps.
3. Calcium-magnesium exchange: using natural carbonate sediments of marine origin, Berner (1966) found a mole-for-mole replacement of cations in solution with those at the surfaces of the sediments. The reaction can be represented as follows:



A similar process can be expected to occur in biological filters which use shell for buffering purposes.

Most earlier work on the changes of major cations occurring in re-cycling sea water systems have taken the form of analytical comparisons of oceanic sea water and sea water removed from well established public aquaria. Oliver (1957) found that the Na^+ , K^+ and Mg^{++} concentrations in the London Aquarium differed very little from oceanic water, whereas the Ca^{++} concentration had increased to almost threefold the concentration of oceanic water. This he attributed to the presence of cockle shells in the filter bed and the periodic addition of sodium carbonate to neutralize acidity. Saeki (1963) determined the concentrations of major ions at Euoshima and Euno public aquaria and found the following: K^+ and Ca^{++} were present in higher amounts, while Mg^{++} was present in lower amounts than in natural sea water. He offered the following explanations for the changes observed - the K^+ increase was due mainly to animal excretion; the augmentation of Ca^{++} was due to the addition of lime and the decrease in Mg^{++} was due to precipitation with phosphate. Recently, Epifanio *et al.* (1973) monitored the culture water in a closed-system aquaculture pilot farm and found a decreasing trend in both Ca^{++} and Mg^{++} concentrations.

Trace metal concentrations

Although Siddall (1974) furnished no data, he stated that in his culture system a "mixed algal population (predominantly Chlorococcum species) removed all trace elements analyzed in significant amounts." Periodic harvesting of this algae gave rise to a net loss of trace elements. He stated that if the harvested algal tissue was heated to 1000°C for 3 hours and returned to the system, the important trace elements would be returned. Honn and Chavin (1975) took note of Siddall's suggestion and claimed that the problem of trace metal depletion was eliminated in their systems by combustion of the algae (Enteromorpha Link.) and replacement of the ashes. With this in mind, concentrations of certain trace metals were monitored in the first experimental trial. The trace metals chosen were copper, zinc, iron, manganese and cobalt.

Oxygen concentrations

The oxygen concentrations in closed culture systems must meet the requirements of both the cultured animals and the biological filter bed. Penaeid prawns are particularly sensitive to oxygen fluctuations. Liao (1969) found that the feeding of Penaeus japonicus Bate dropped drastically when the oxygen levels were below 65% saturation. Similarly, Egusa (1961) observed respiratory stress in moulting P.japonicus at 68% oxygen saturation. On the other hand, nitrifying bacteria are able to tolerate high oxygen tensions as well as periodic anaerobic conditions (Haug & McCarty, 1972). Schoberl and Engel (1964 - cited in Painter, 1970) found that oxygen is not limiting to the growth of Nitrobacter until the dissolved oxygen concentration falls below 2 mg.l⁻¹. Nitrosomonas is unaffected at 0,9 mg O₂.l⁻¹ at 30°C. In the presence of adequate oxygen concentrations, 50% of the oxygen demand in closed systems has been attributed to the filter bed and its attendant micro-faunal population (Hirayama, 1965).

In the present study, oxygen levels were periodically checked in the biological filtration systems. Special attention was paid to fluctuations in dissolved oxygen concentrations brought about by the respiration and photosynthesis of the filamentous algae in the algal filtration systems.

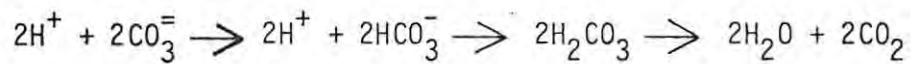
pH

The pH of a heavily stocked culture system tends to decrease to critical levels in a relatively short space of time due to the accumulation of nitrate. For example, Hirayama (1970) fed four fish (total biomass 145,9 g) excessively with 20 g of food per day and found that the pH of the water decreased from 7,5 to 4,8 in 27 days. pH's of this order are detrimental to nitrification. Haug and McCarty (1972) stated that nitrification ceases completely below a pH of 5,5. They found that the maximum rates of reaction for both Nitrosomonas and Nitrobacter occur over the pH range 7 to 9. Engel and Alexander (1958) are in agreement with this range, while Spotte (1970) recommends a narrower range of 7,5 to 8,3.

Attempts have been made to curb this drop in pH. Buffering of culture water has been practiced by the addition of crushed oyster shell to the gravel bed (Short & Olson, 1970; Skinger, 1975). Unfortunately the efficiency of this calcareous alkali reserve has been found to be impaired by the deposition of organic coatings on the shell surfaces (Siddall, 1974). Chave (1965) found that the same process occurs in the ocean.

Inorganic carbon

Closely related to pH are the levels of inorganic carbon. From the formulae of Haug and McCarty (1972), Wickens (1976a) has calculated that the oxidation of 1 mg NH_4^+ - N to nitrate requires 4,27 mg O_2 , 0,09 mg inorganic carbon and produces 0,14 mg H^+ . Besides the bacterial requirement of 0,09 mg inorganic carbon per mg NH_4^+ - N, the production of H^+ ions lowers the pH and upsets the carbonate-carbon dioxide equilibrium in the water—resulting in a further loss of inorganic carbon as carbon dioxide, as shown on the following page.



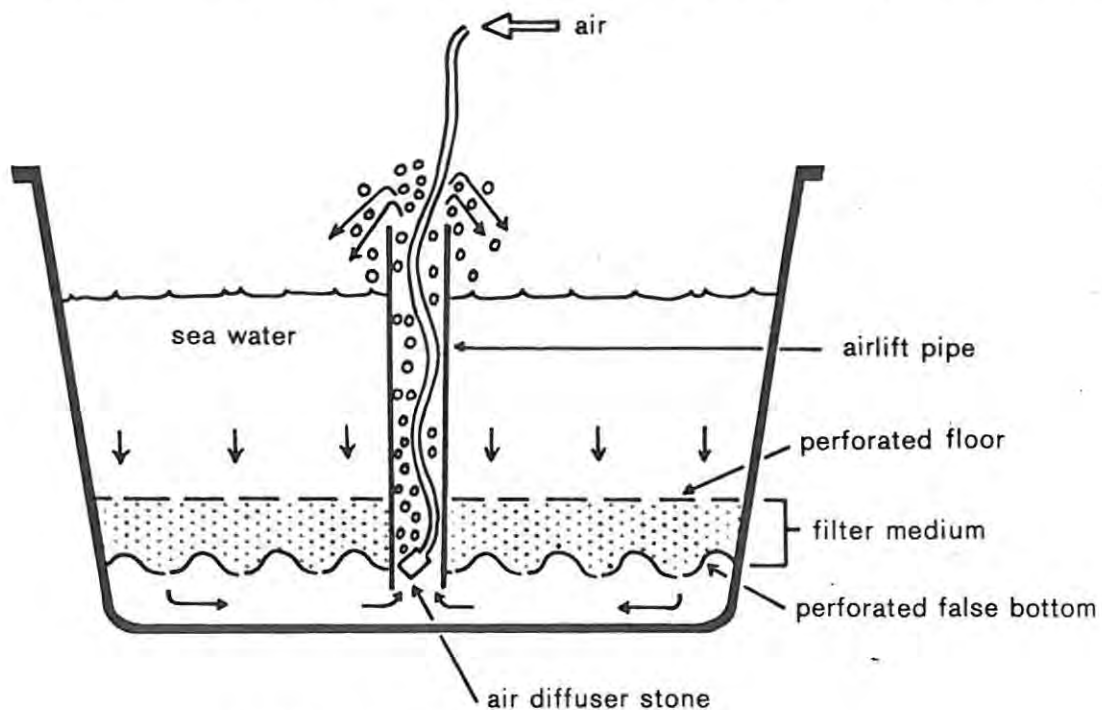
This loss of inorganic carbon can be deleterious to crustacea. Greenaway (1974) showed that the absence of external HCO_3^- reduces the net uptake of calcium in postmoult freshwater crayfish. Wickins (1976a) found that low inorganic carbon levels ($<10\text{-}12 \text{ mg } \ell^{-1}$) are likely to be lethal to prawns.

MATERIALS AND METHODS

(i) Design of biological filtration systems

Sea water has the ability to dissolve a wide variety of toxic substances (Atz, 1964) such as dioctyl phthalate plasticizer which leaches from new plastics in contact with water (Carmignani & Bennett, 1976). Most aquatic animals are extremely sensitive to such substances and therefore an attempt was made to construct the biological filtration systems from inert or leached materials. All tanks (780 mm x 580 mm x 480 mm black polyethylene; 125 litre capacity), polyvinylchloride piping, glass reinforced plastic (GRP) and perspex used in this study were soaked for at least 10 days in fresh water before use. Four types of systems were constructed:

Bacterial filtration systems (Fig. 1). These tanks were equipped with sub-gravel filters of the type used by Spotte (1970). Perforated false



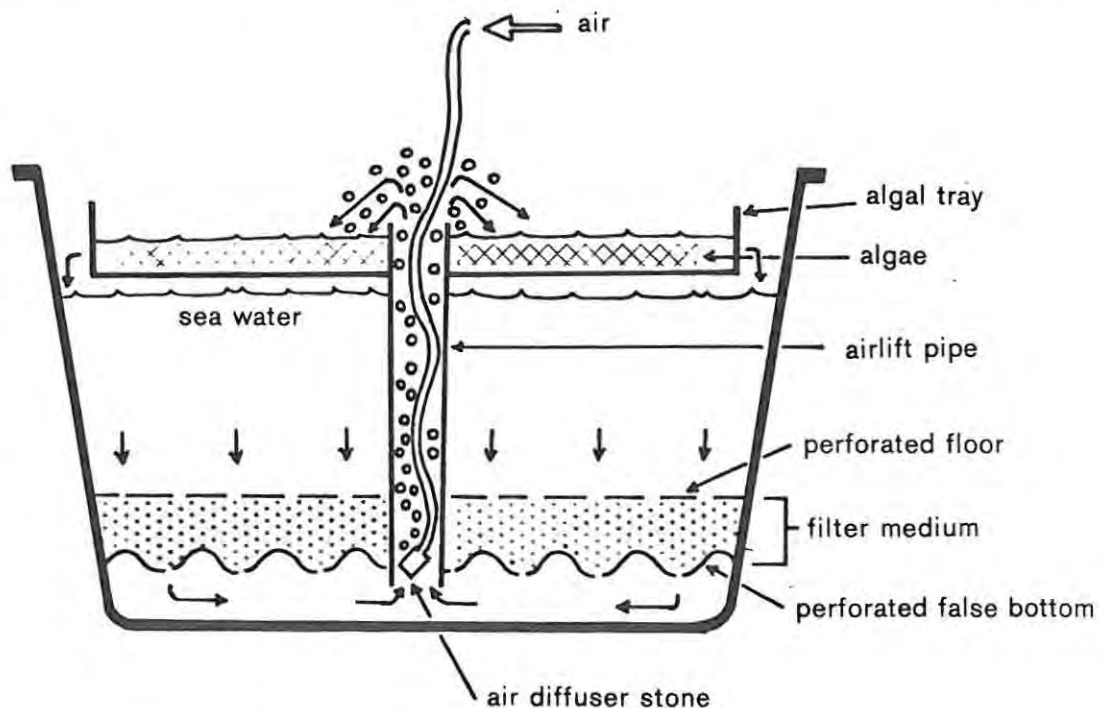
BACTERIAL FILTRATION

Figure 1: Diagrammatic representation of a cross-section through a bacterial filtration system used in the experimental trials. Arrows indicate the direction of water flow.

bottoms of GRP sheeting supported a filter medium made up of 16 kg of quartzite gravel chips (mean diameter 5 mm) and 4 kg crushed oyster shell (mean chip size 2,5 mm). Quartzite gravel chips provided a large surface area for the growth of heterotrophic and nitrifying bacteria while the oyster shell was included to buffer the culture water against pH changes.

Airlifts were used to circulate water through the biological filters. They were made of vertical lengths of polyvinylchloride piping (28 mm diameter) which extended from below the subgravel filter to above the water surface. A regulated flow of air was injected into the piping using diffuser stones, and the consequent water displacement produced a circulation through the filter medium as arrowed in Figure 1. The rate of flow through the system was about 2 litres min^{-1} .

Algal and Bacterial filtration systems (Fig. 2). These filtration systems were identical to the bacterial filtration systems, except that in addition the seawater flowing from the top of the airlift pipes passed through



ALGAL AND BACTERIAL FILTRATION

Figure 2: Diagrammatic representation of a cross-section through an algal and bacterial filtration system used in the first experimental trial. Arrows indicate the direction of water flow.

710 mm x 540 mm fibreglass trays containing filamentous algae. The trays were constructed as shown in Figure 3 and contained 120 g drained wet weight Rhizoclonium sp. at the beginning of the experiment. Growth of

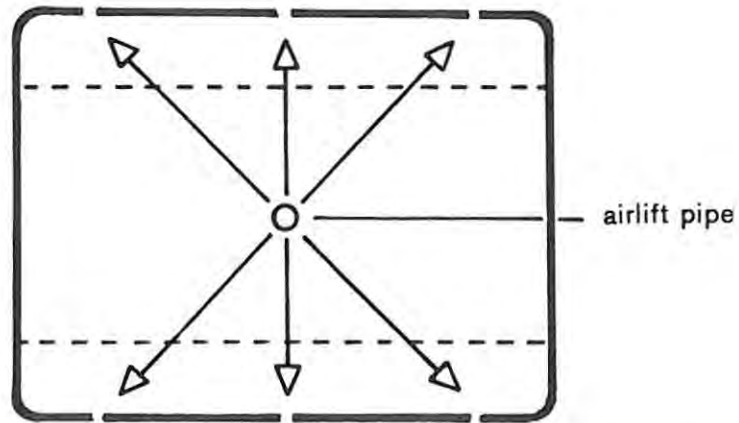
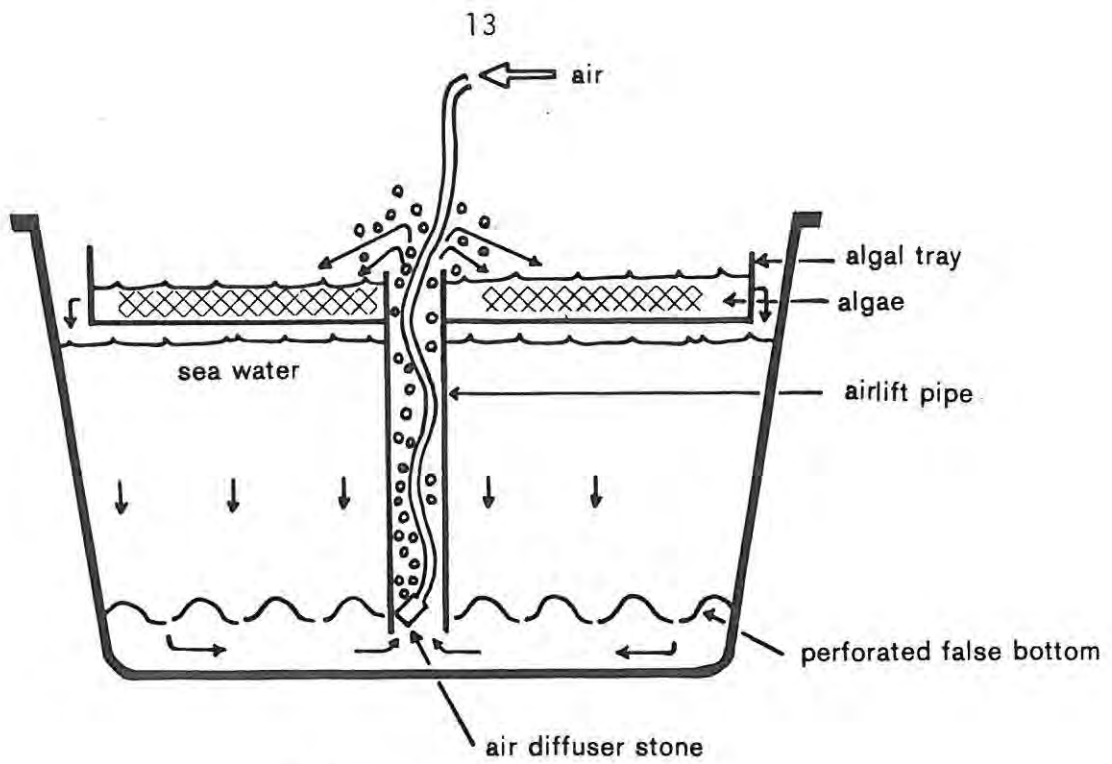


Figure 3: Plan of an algal tray used in the first experimental trial. Dotted lines represent polyvinylchloride mesh between which filamentous algae was placed. Arrows indicate direction of water flow.

the filamentous algae was not monitored, but harvesting was periodically carried out when it appeared that the algae was becoming very dense.

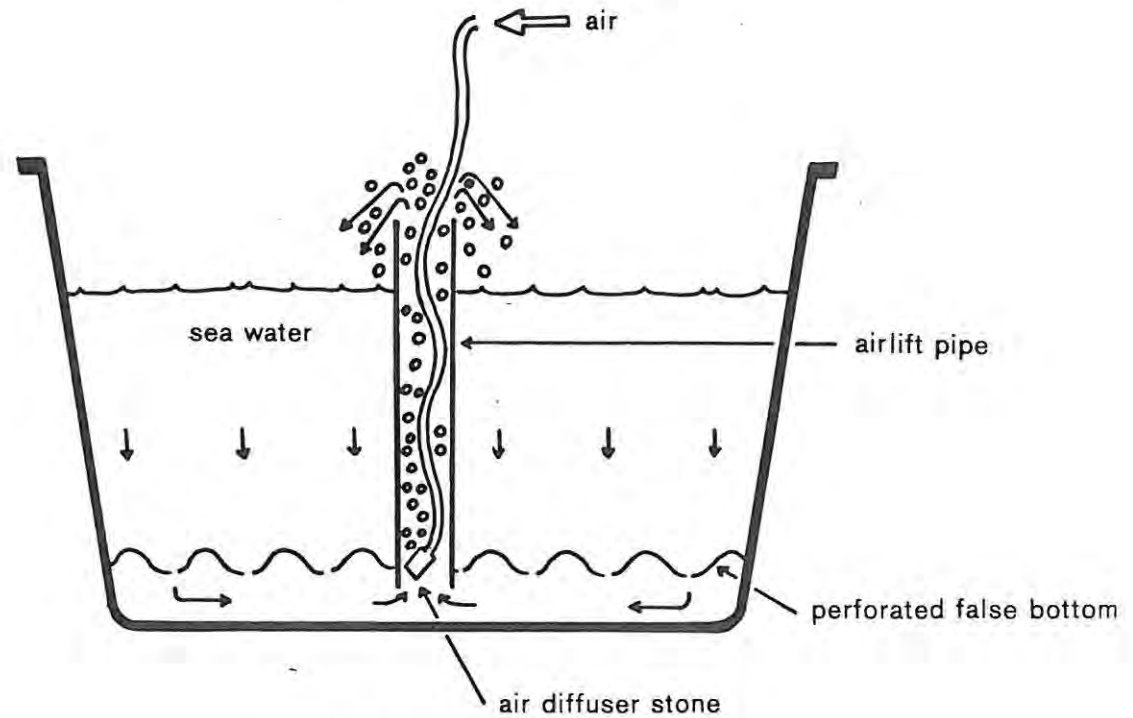
Algal filtration systems (Fig. 4). In these filtration systems the filamentous algal trays represented the sole means of biological filtration, no gravel filter medium was present. The trays contained 120 g Rhizoclonium which was periodically harvested.

Water exchange systems (Fig. 5). These systems were designed to serve as controls. They had neither algae nor a gravel filter medium. Twenty-five percent of the seawater in the tanks was exchanged every 3 days.



ALGAL FILTRATION

Figure 4: Diagrammatic representation of a cross-section through an algal filtration system used in the first experimental trial. Arrows indicate the direction of water flow.



WATER EXCHANGE

Figure 5: Diagrammatic representation of a cross-section through a water exchange system used in the first experimental trial. Arrows indicate direction of water flow.

Two experimental trials were run. In the first trial each of the above four biological filtration systems were set up in duplicate. The parameters measured were:

Ammonia concentration

Nitrite concentration

Major cation concentrations (Ca^{++} , Mg^{++} , Na^+ and K^+)

Trace metal concentrations (Cu, Fe, Zn, Mn and Co)

Oxygen concentration

pH

Total inorganic carbon concentration

Growth of P.indicus

The second experimental trial was run primarily to obtain data on nitrate concentrations, the analysis of which was unsatisfactory in the first experimental trial. For this reason, only the bacterial filtration systems and the algal filtration systems were set up to monitor nitrate uptake. In addition, a few small modifications were made. The algal trays contained 450 g wet weight Chaetomorpha sp. since Rhizoclonium was unavailable. Water flow within the trays was directed by perspex baffles (Fig. 6) to ensure a more uniform flow over the filamentous algae.

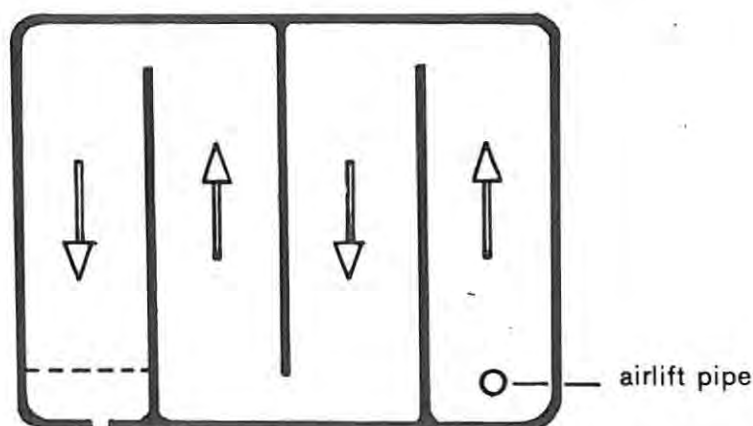


Figure 6: Plan of an algal tray used in the second experimental trial. The dotted line represents polyvinylchloride mesh which prevented the filamentous algae from spilling out of the overflow. Arrows indicate direction of water flow.

Periodic harvesting was once again undertaken and the same parameters were monitored as in the first experimental trial, except for trace elements and major cations.

Both experimental trials lasted approximately 2 months and were carried out in a constant environment room set at 28°C and with a 12:12 hour light-dark regime. The incident light received by the algae measured 3100-3800 microeinsteins $\text{m}^{-2} \text{sec}^{-1}$, while that received by the prawns measured 1000-2000 lux. In the first experimental trial two problems occurred: (a) Sudden light change at dawn stressed the prawns and they injured their carapaces by jumping and colliding against the algal trays, and (b) a few prawns were lost due to 'jumpouts'. These problems were avoided in the second experimental trial by the installation of a light-dimmer system and by provision of screens below the algal trays.

(ii) Material

Juvenile P.indicus, netted in the estuary, were airfreighted from the Fisheries Development Corporation Prawn Research Unit in Amatikulu, Northern Natal to Rhodes University in sealed polyethylene bags, filled with 10 l sea water and 20 l pure oxygen. Each bag contained 200 prawns. The bags were in turn placed in insulated containers. The whole journey took less than 12 hours and mortalities were negligible. Upon arrival the prawns were acclimated for one week in three 500 litre subgravel filter re-circulation systems before experiments. All experiments were initiated with 0,5 g wet weight prawns, since at this stage they readily accept formulated pellets and move into an exponential growth phase (H.F.B. Champion, pers.comm.).

The biological filtration systems were stocked with 20 juveniles per tank (i.e. 28 g wet weight m^{-2}). This initial biomass loading was chosen since it could be expected to rise to 200 g wet weight m^{-2} at the end of the experiments when the prawns reached a 4 g size. This biomass loading can

be considered as semi-intensive in culture, but would not overload the biological filter systems and necessitate culling during experiments.

The prawns were fed twice a day with a pelleted ration at a rate of 2.5% of their wet weight. "Higashi-Maru" prawn pellets, imported from Japan, were used as the sole food source.

(iii) Determination of Na^+ , Ca^{++} , K^+ and Mg^{++} .

Water samples were taken every 2 weeks from the various biological filtration systems during the first experimental trial. The following dilutions were prepared with glass distilled water:

Ca^{++}	-	1:10
K^+	-	1:100
Na^+	-	1:1000
Mg^{++}	-	1:1000

All samples were measured on a Varian Techtron Type AA-4 Atomic Absorption Spectrophotometer. The spectrophotometer was calibrated each time with freshly prepared 0, 10, 20, 30, 40 and 50 $\mu\text{g ml}^{-1}$ standard solutions of Ca^{++} , Mg^{++} , Na^+ and K^+ . To suppress interferences, Ca^{++} and Mg^{++} samples were measured using a nitrous oxide-acetylene flame, while Na^+ and K^+ samples were measured using an air-acetylene flame as recommended in the 'Varian Sea Water Manual'. Results were plotted as the % change in concentration of the culture water cations from their initial concentration in fresh sea water.

(iv) Extraction and determination of Cu, Fe, Zn, Mn and Co.

Samples were taken approximately every 2 weeks from each tank during the first experimental trial. To avoid the possible sources of contamination mentioned by Robertson (1968), high-density one litre polyethylene bottles which had been treated for 24 hours with 5N HCl were used for sample collection. Trace metal extractions were carried out using the sodium

diethyldithiocarbamate-chloroform extraction as outlined by Watling and Watling (1976). In this method 1 litre of sample was transferred into a 2 litre globular separating funnel. After the addition of a buffer-chelating reagent (sodium acetate trihydrate, glacial acetic acid and sodium diethyldithiocarbamate) the sample was vigorously shaken for 5 minutes to extract the trace metals. Chloroform was then added and the mixture shaken vigorously for a further 5 minutes to dissolve the buffered chelate plus trace metals. The organic phase was then allowed to separate and then drawn off into a stoppered 250 ml Erlenmyer flask. This chloroform extraction was repeated twice. The organic complex in the combined extract was then destroyed with concentrated nitric acid before slowly evaporating the solution to dryness on a hot plate. The remaining residue was dissolved in a 10 ml aliquot of N-hydrochloric acid when required for atomic absorption analysis. All samples were measured on a Varian Techtron Type AA-4 Atomic Absorption Spectrophotometer using a $100 \mu\text{g ml}^{-1}$ mixed standard of Cu, Fe, Zn, Mn and Co.

(v) Oxygen and pH determinations

The oxygen content of the biological filtration systems was measured using a Radiometer BMS 3 Blood Gas Analyzer. Three aspects were examined:

1. Oxygen levels were compared in the bacterial and algal filtration systems by measuring the percentage saturation at about midday in the algal trays, the main animal compartment, and below the false bottoms. This was repeated on five occasions and the results were averaged.
2. Diurnal fluctuations of oxygen content in the algal filtration systems were measured at the inlet and outlet of the algal trays used in the second experimental trial. Measurements were taken every 3 hours for 48 hours.

3. Periodic spot checks of oxygen concentrations were made in all biological filtration systems throughout both experimental trials.

pH was monitored every week in all biological filtrations systems in both experimental trials, using a Tacussel Electronique U8N pH-meter. In addition, the diurnal fluctuation in pH in the algal filtration systems was measured at the inlet and outlet of the algal trays. Measurements were taken every 3-6 hours for 48 hours.

(vi) Determination of total inorganic carbon

Total alkalinity (expressed as $\text{meq. CaCO}_3 \ell^{-1}$) was determined according to the method of Mackereth (1963). Total alkalinity, pH, temperature and salinity were then substituted into the equations of Mook and Koene (1975) and Edmond and Gieskes (1970) to calculate total inorganic carbon. For further details, see Allanson and Rudd (in press). These determinations were done every 7 to 10 days in both experimental trials.

(vii) Determination of ammonia and nitrite

Ammonia analyses were carried out according to the Solarzano (1969) method. The blue colour of indophenol obtained by the reaction at high pH of ammonia, phenol and hypochlorite absorbs strongly at 640 $\text{m}\mu$. The colour intensity is correlated with NH_4^+ -N concentration ($n = 22$; $r = 0,995$ for the range $0-0,5 \text{ mg NH}_4^+$ -N ℓ^{-1}).

Nitrite analyses were carried out according to the Strickland and Parsons (1968) method. In this colour reaction, the nitrite ion is diazotized with sulphanilamide and then coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride. The dye absorbs strongly at 543 $\text{m}\mu$ and absorption is correlated with NO_2^- -N concentration ($n = 32$; $r = 0,998$ for the range $0-0,3 \text{ mg NO}_2^-$ -N ℓ^{-1}).

(viii) Determination of nitrate

The determination of nitrate in sea water is much more difficult than in fresh water due to the interference of the chloride ion (Mullins & Riley, 1955). Smith (1977) conducted interlaboratory comparison studies of water analysis methods in South Africa. He found large differences (coefficient of variation 20,9-47,9%) in nitrate determinations on identical samples. In this study, attempts to obtain nitrate concentrations in the first experimental trial met with limited success. Three methods were tried. Two involved hydrazine reduction (Armstrong, 1963; Mullin & Riley, 1955), and one reduction by spongy cadmium (J. Talling, pers.comm.). The problem appeared to be due to the excessive amounts of copper in the culture systems (see Results: Major cation and trace metal concentrations). Although copper is used to catalyze the reaction, Mullin and Riley (1955) found that excessive amounts cause a visible precipitation of cuprous oxide. These precipitates were observed while attempting nitrate analyses in the later stages of the first experimental trial. In the second experimental trial, copper contamination was prevented (described later) and nitrate was successfully determined by combining the reduction method of Mullin and Riley (1955) and the nitrite analysis technique of Strickland and Parsons (1968).

RESULTS

(i) Major cation and trace metal concentrations

Figure 7 shows that no significant changes occurred in the Na^+ , K^+ , Ca^{++} and Mg^{++} concentrations in any of the biological filtration systems tested. Most of the changes fell within 5% of the initial concentrations in normal sea water. The Ca^{++} levels were slightly higher in the algal plus bacterial filtration systems (A + B) and the bacterial filtration systems (B) when compared with the water exchange (WE) and algal filtration

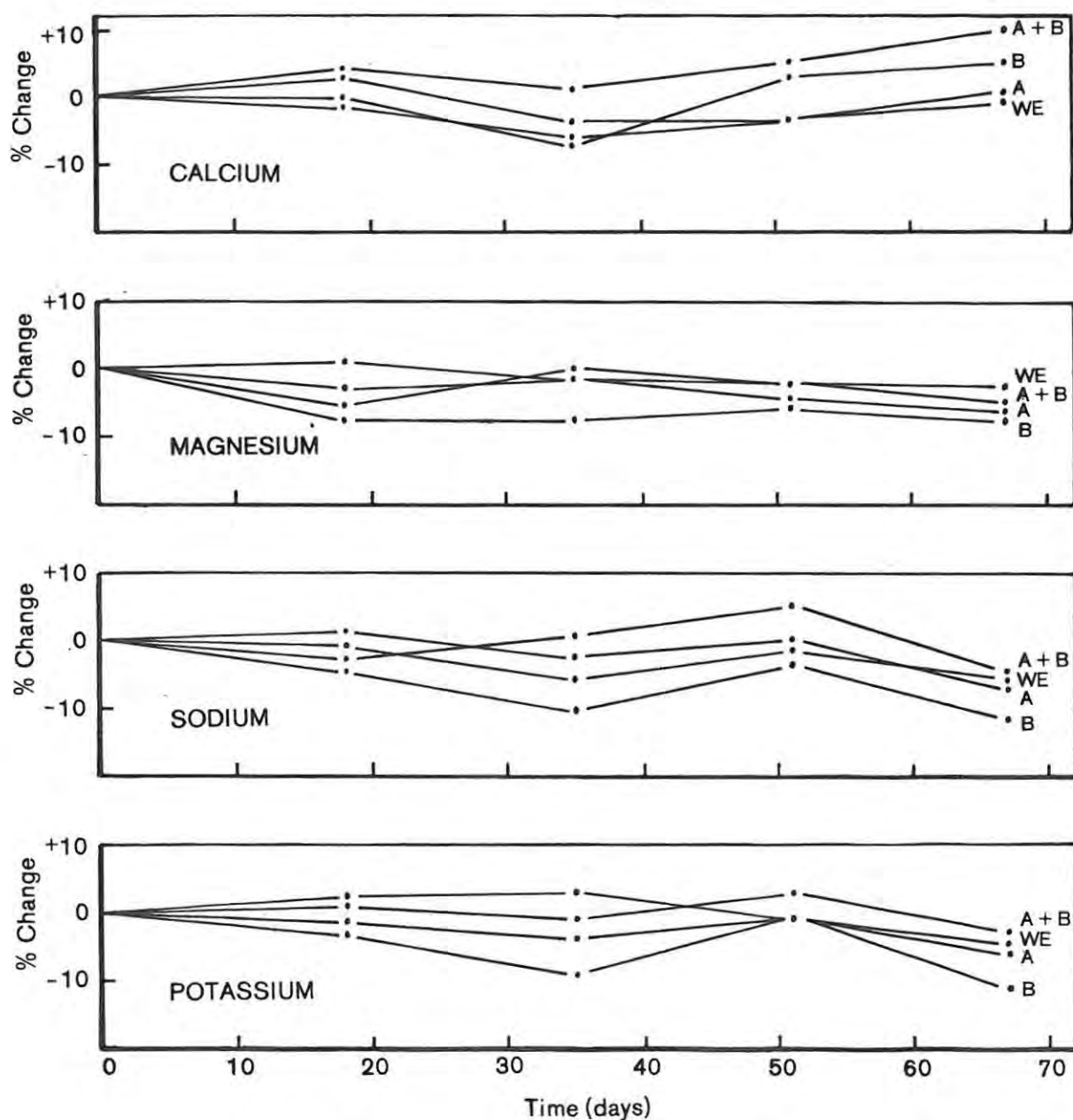


Figure 7: Percentage change from natural sea water of major cations in the closed system recirculation tanks of the first experimental trial. B = bacterial filtration; A + B = algal plus bacterial filtration; A = algal filtration; WE = water exchange.

systems (A). This is possibly due to the presence of oyster shell chips in the first two filtration systems.

Figure 8 shows the changes in trace metal concentrations in the various biological filtration systems in the first experimental trial. The

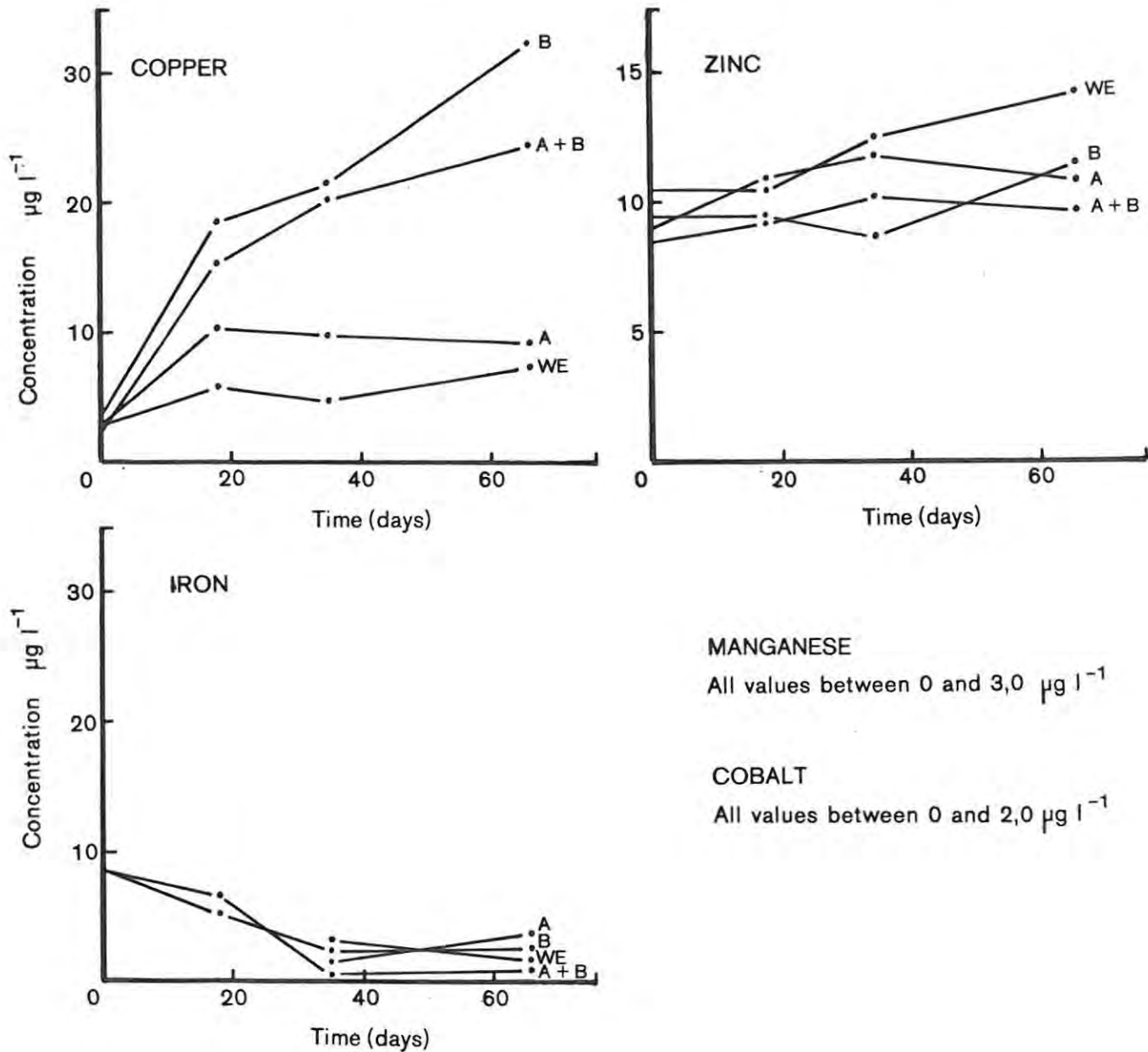


Figure 8: Changes in trace metal concentrations in the closed recirculation tanks of the first experimental trial.

B = bacterial filtration; A + B = algal plus bacterial filtration;
A = algal filtration; WE = water exchange.

average values of the two replicate systems have been plotted. Mn and Co have not been plotted due to the low range of concentrations recorded.

Mn concentrations ranged between 0 and $3 \mu\text{g l}^{-1}$. Co concentrations ranged between 0 and $2 \mu\text{g l}^{-1}$. There appears to be a slight increase in Zn concentrations and a small depletion of Fe. However, it must be noted that these changes occurred in all the systems analyzed, irrespective of whether filamentous algae was present or not. The only significant changes were found in the amount of copper present in the various biological filtration systems. The concentration of Cu in the bacterial filtration systems (B) increased from $3,2 \mu\text{g Cu l}^{-1}$ to $32,3 \mu\text{g Cu l}^{-1}$. This Cu build-up was curbed in those systems containing filamentous algae. The algal and bacterial filtration systems (A + B) increased from $2,0 \mu\text{g Cu l}^{-1}$ to $24,5 \mu\text{g Cu l}^{-1}$, while the algal filtration systems (A) increased from $1,6 \mu\text{g Cu l}^{-1}$ to $9,0 \mu\text{g Cu l}^{-1}$. The systems in which sea water was exchanged (WE) increased from $2,6 \mu\text{g Cu l}^{-1}$ to $6,1 \mu\text{g Cu l}^{-1}$. In this latter case the constant replenishment of sea water prevented a large Cu build-up.

This increase in Cu concentration was probably due to condensation moisture in the copper piping used in the air reticulation system of the laboratory building. A "Compair" Model M4 automatic water-ejector and filter was installed into the airline system for the second experimental trial. Cu levels were determined at the beginning and end of this experiment and no significant increase was found in any of the biological filtration systems tested.

(ii) Oxygen and pH fluctuations

The average oxygen content found in the various compartments of the bacterial and algal filtration systems is shown in Figures 9 and 10. In the bacterial filtration systems (Fig. 9) the average % saturation of sea water emanating from the airlift pump (area A) was 117% saturation. This dropped slightly to 111% saturation in the animal compartment (area B) and down to 92% saturation below the gravel filter (area C).

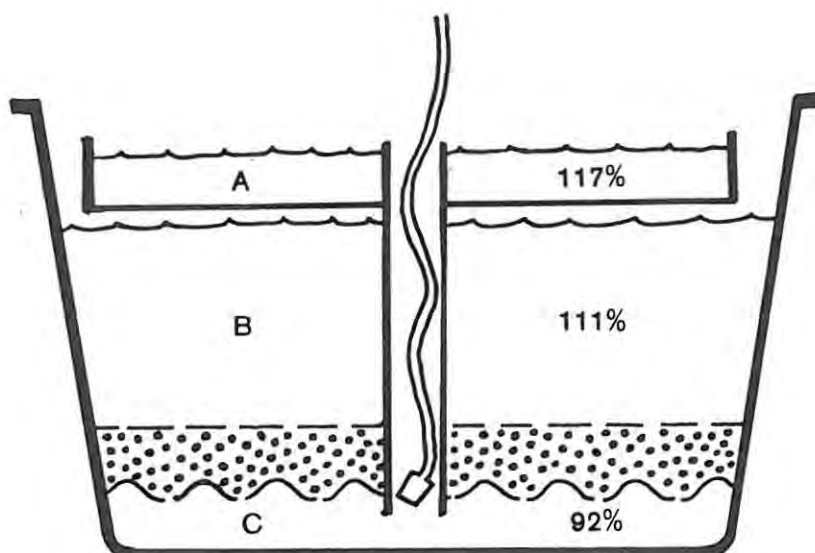


Figure 9: Diagrammatic representation of a cross-section through a bacterial filtration system showing the average % oxygen saturation found in the various compartments (A, B and C) in the second experimental trial.

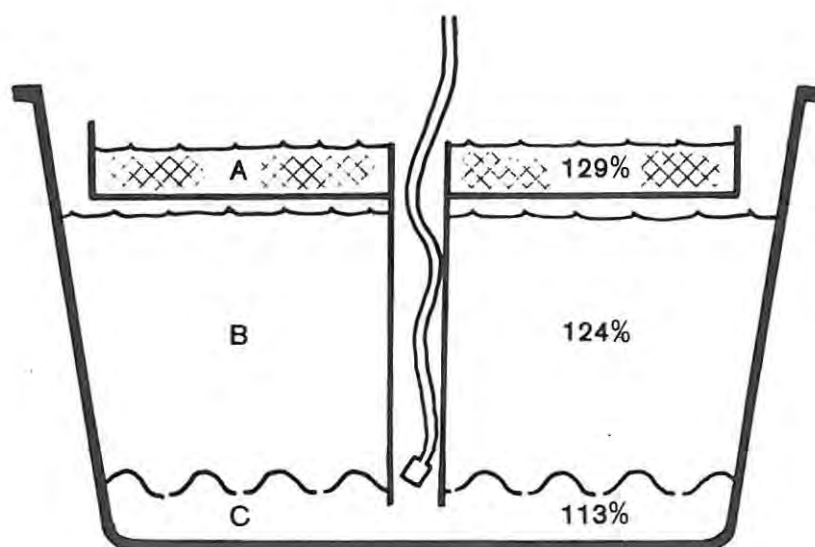


Figure 10: Diagrammatic representation of a cross-section through an algal filtration system showing the average % oxygen saturation found in various compartments (A, B and C) in the second experimental trial.

In the algal filtration systems (Fig. 10) the oxygen content in all compartments was higher: 129% saturated in the algal trays (area A); 124% saturated in the animal compartments (area B) and 113% saturated below the false bottoms (area C).

Diurnal fluctuations in oxygen content in the algal filtrations systems are shown in Figure 11. It can be seen that the oxygen concentrations at the outlet of the trays are marginally higher than those at the inlet during periods of illumination. The reverse occurred in periods of

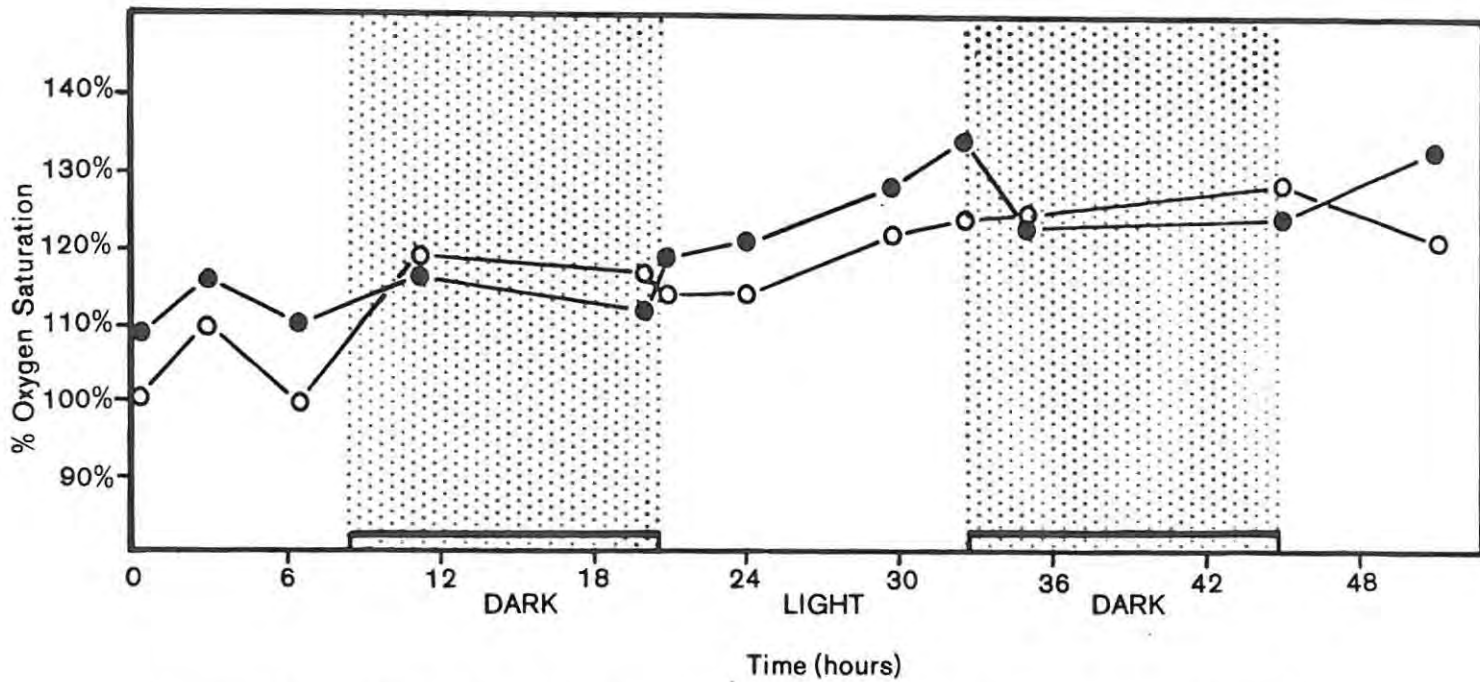


Figure 11: Light-dark fluctuations in percentage oxygen saturation in algal tray inlets (-o-) and outlets (- ● -) measured over a 52 hour period in the second experimental trial.

darkness. Bubbles of gas (presumably oxygen) were also evolved by the filamentous algae during the day.

Periodic spot checks carried out in all the filtration systems throughout the two experimental trials showed that dissolved oxygen concentrations to which prawns were exposed (compartment B), were always in excess of 100% saturation.

No excessive changes in pH were found in either the first or second experimental trial. All pH values in the first experimental trial fell into the range 7,4 - 8,3. In the second experimental trial both the algal and bacterial filtration systems showed a slight drop in pH from

8,18 to 7,65. All pH values fell within the range of the diurnal fluctuations found in the algal filtration systems in the second experimental trial. These are illustrated in Figure 12. This figure

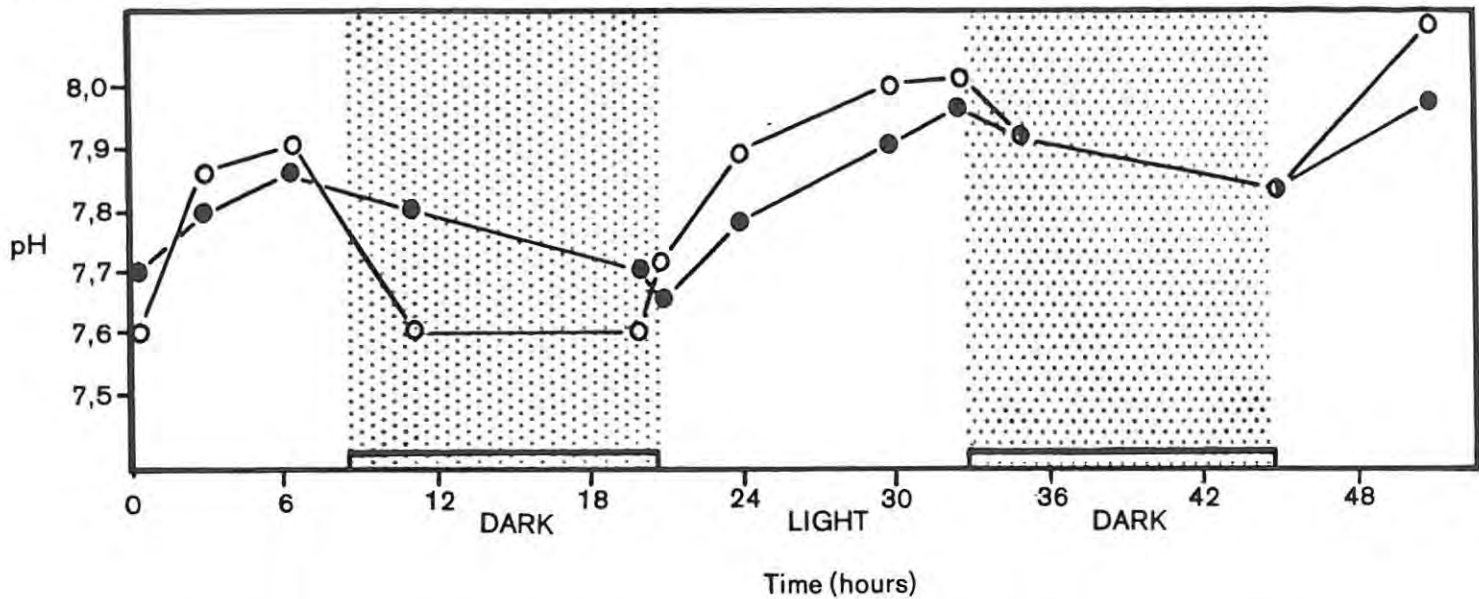


Figure 12: Light-dark fluctuations in pH in algal tray inlets (●) and outlets (○) measured over a 52 hour period in the second experimental trial.

shows that during the period of light, when the algae is photosynthesizing, the pH at the algal tray outlet is marginally higher than at the inlet. In periods of darkness, the reverse is true.

(iii) Total inorganic carbon levels

Figure 13 shows the changes in total inorganic carbon content which occurred in the first experimental trial. The water exchange and algal and bacterial filtration systems showed only minimal changes and have been omitted from this figure. Bacterial filtration systems showed a slight drop, while there was a marked increase in inorganic carbon levels (from 31,9 mg inorganic carbon ℓ^{-1} to 47,7 mg inorganic carbon ℓ^{-1}) in the algal filtration systems.

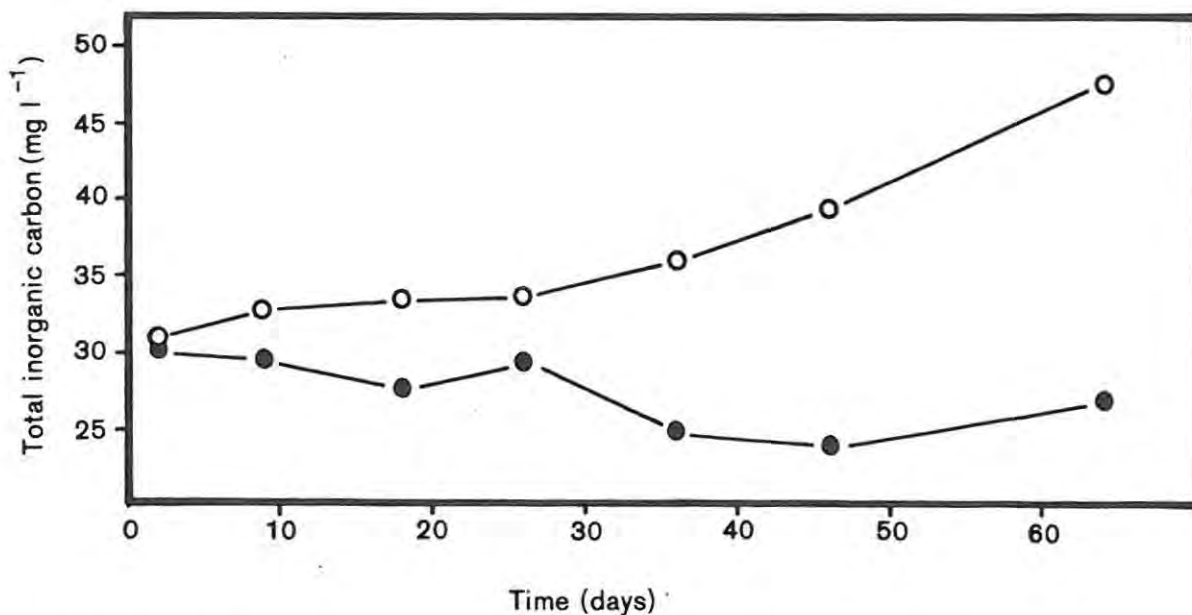


Figure 13: Total inorganic carbon levels in the algal filtration systems (-o-) and bacterial filtration systems (-●-) during the first experimental trial.

In the second experimental trial (Fig. 14) similar results were obtained. The inorganic carbon concentration dropped in the bacterial filtration

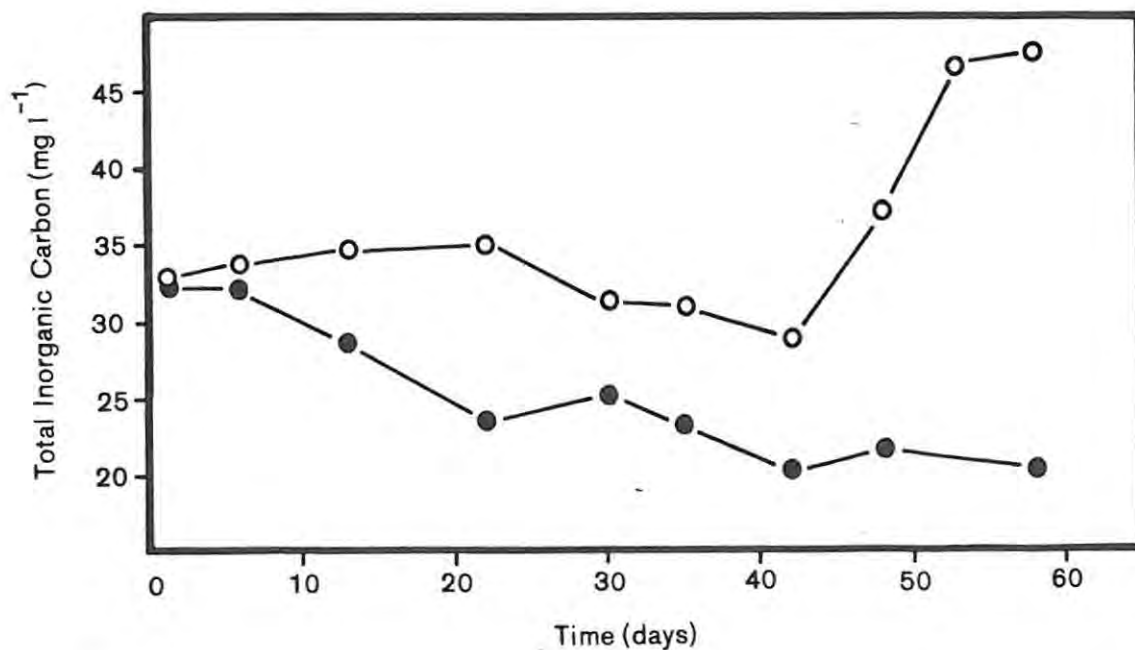


Figure 14: Total inorganic carbon levels in the algal filtration systems (-o-) and bacterial filtration systems (-●-) during the second experimental trial.

systems (from 32,4 mg inorganic carbon ℓ^{-1} to 20,2 mg inorganic carbon ℓ^{-1}). In the algal filtration systems the inorganic carbon concentration remained relatively constant until the 42nd day, when it started to increase rapidly from 28,9 mg inorganic carbon ℓ^{-1} to 47,5 mg inorganic carbon ℓ^{-1} on the 58th day.

(iv) Nitrification and nitrate accumulation

Trial 1:- Ammonia: In all systems the duplication of results was good. The maximum concentrations of ammonia were lowest in the systems with algal plus bacterial filtration (Fig. 15b), followed by the algal filtration systems (Fig. 15c) and the bacterial filtration systems (Fig. 15a). The highest levels of ammonia attained, occurred in the systems with water exchange (Fig. 15d). In those tanks which had bacterial filtration (Fig. 15 a and b), the decline in ammonia levels was far more rapid (11-15 days) than those which had algae only, or exchanged water (21-28 days - Fig. 15 c and d). The ammonia levels after stabilization (i.e. after 30 days), also remained significantly lower (Students 't' test, $P < 0,001$) in the systems which had bacterial filtration (Fig. 15 a and b) than in those systems which had algal filtration only or water exchange (Fig. 15 c and d).

Trial 1:- Nitrite: Once again, the duplication of results in replicate tanks was good. In none of the filtration systems tested did the nitrite concentrations exceed 1 mg $\text{NO}_2^- \text{-N } \ell^{-1}$. In the bacterial filtration systems (Fig. 16a) and the algal plus bacterial filtration systems (Fig. 16b) - i.e. in those systems with gravel - the nitrite levels stabilized after 30 days. In the algal filtration systems (Fig. 16c) and those systems in which water was exchanged (Fig. 16d) the nitrite levels stabilized at a much higher level after approximately 15 days. They remained at this level for the full duration of the experiment. A comparison of the nitrite levels after stabilization

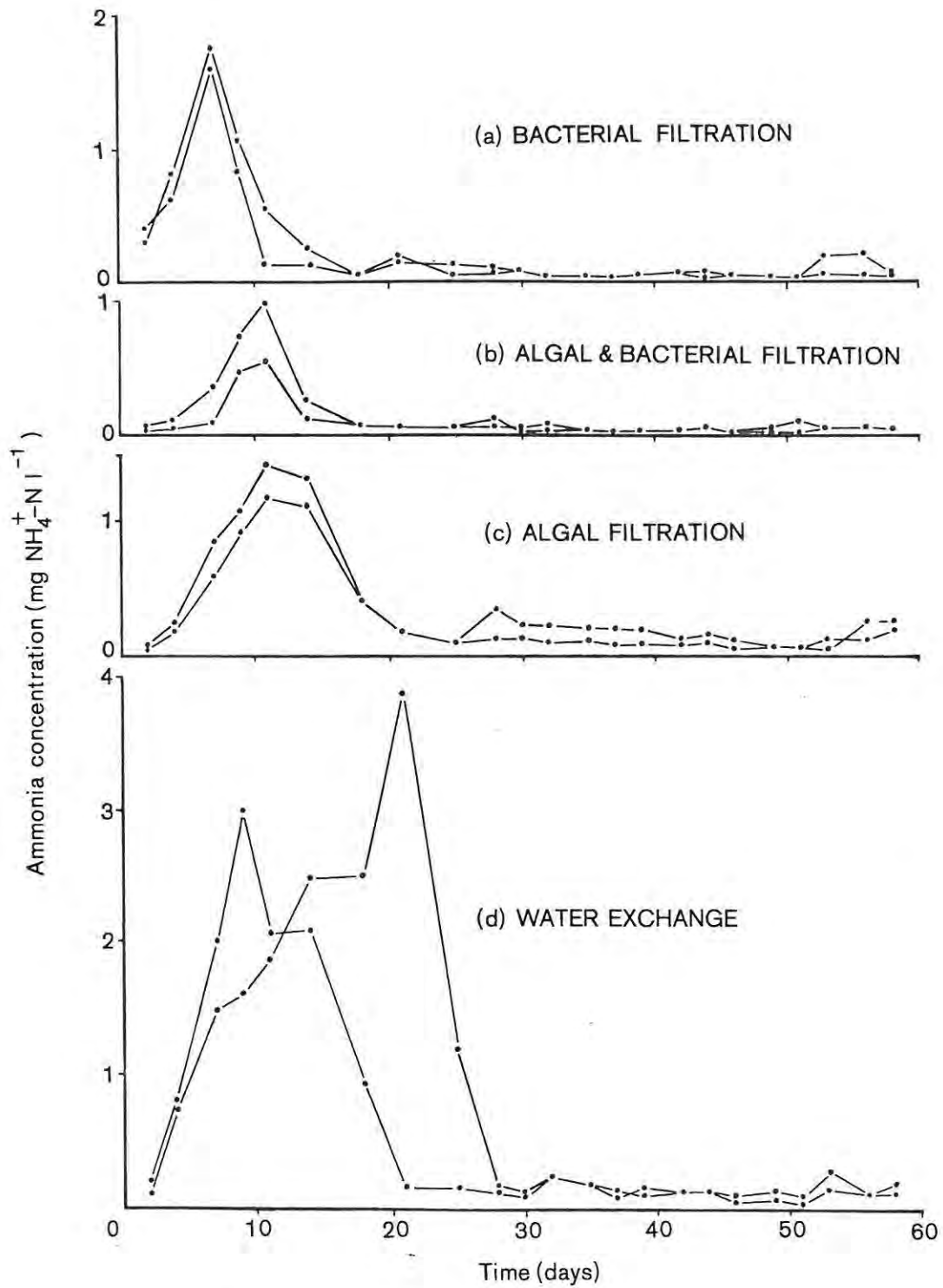


Figure 15: Ammonia concentrations in the four biological filtration systems (a, b, c and d) during the first experimental trial. The graphs show the results of two replicates in each case.

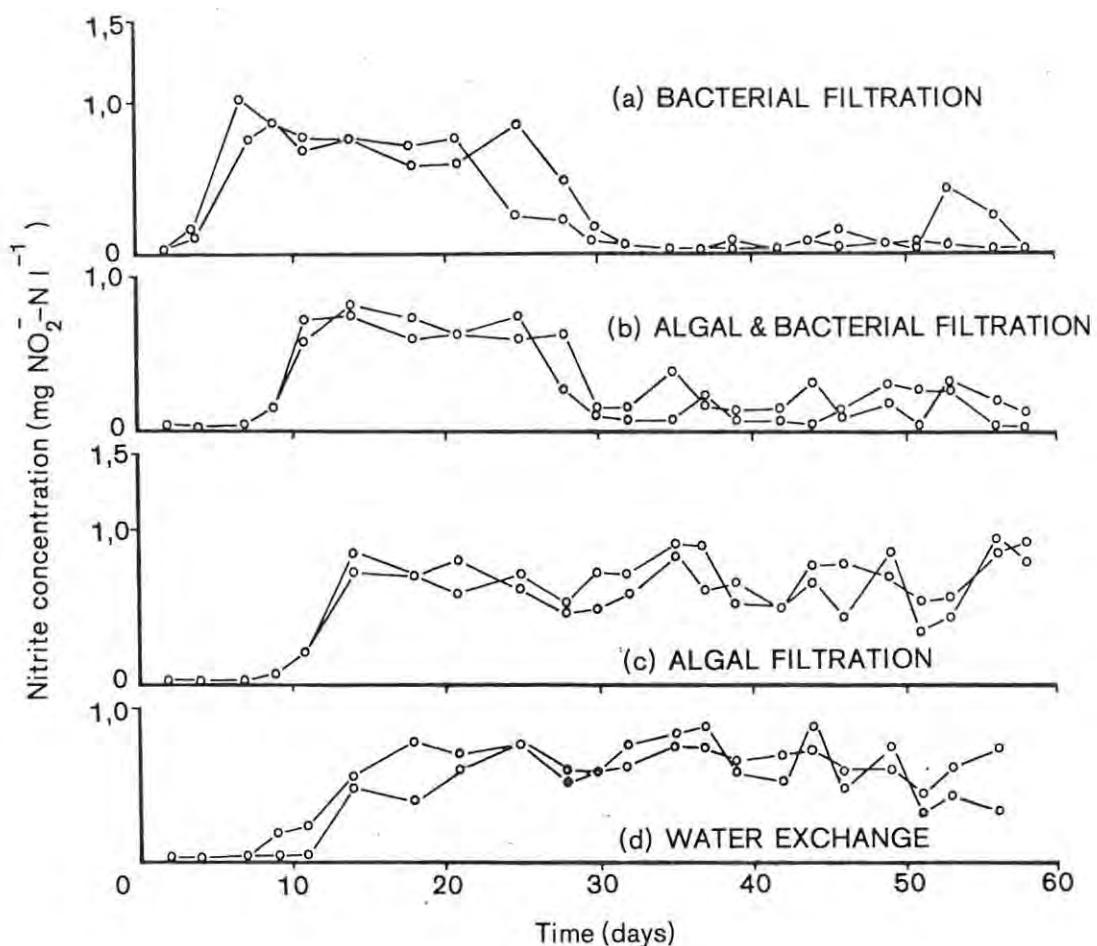


Figure 16: Nitrite concentrations in the four biological filtration systems (a, b, c and d) during the first experimental trial.

in the bacterial filtration systems (Fig. 16a) with the nitrite stabilization levels in the algal and bacterial filtration systems (Fig. 16b) seems to indicate that the presence of algae in the latter systems had a harmful effect on the ability of the gravel filters to oxidize nitrite.

Trial 2:- Ammonia, nitrite and nitrate: Although the second experimental trial was designed primarily to supply information on nitrate accumulation and removal by algae, the results on nitrification of ammonia and nitrite warrant a few comments. The maximum levels of ammonia during nitrification in the bacterial filtration systems

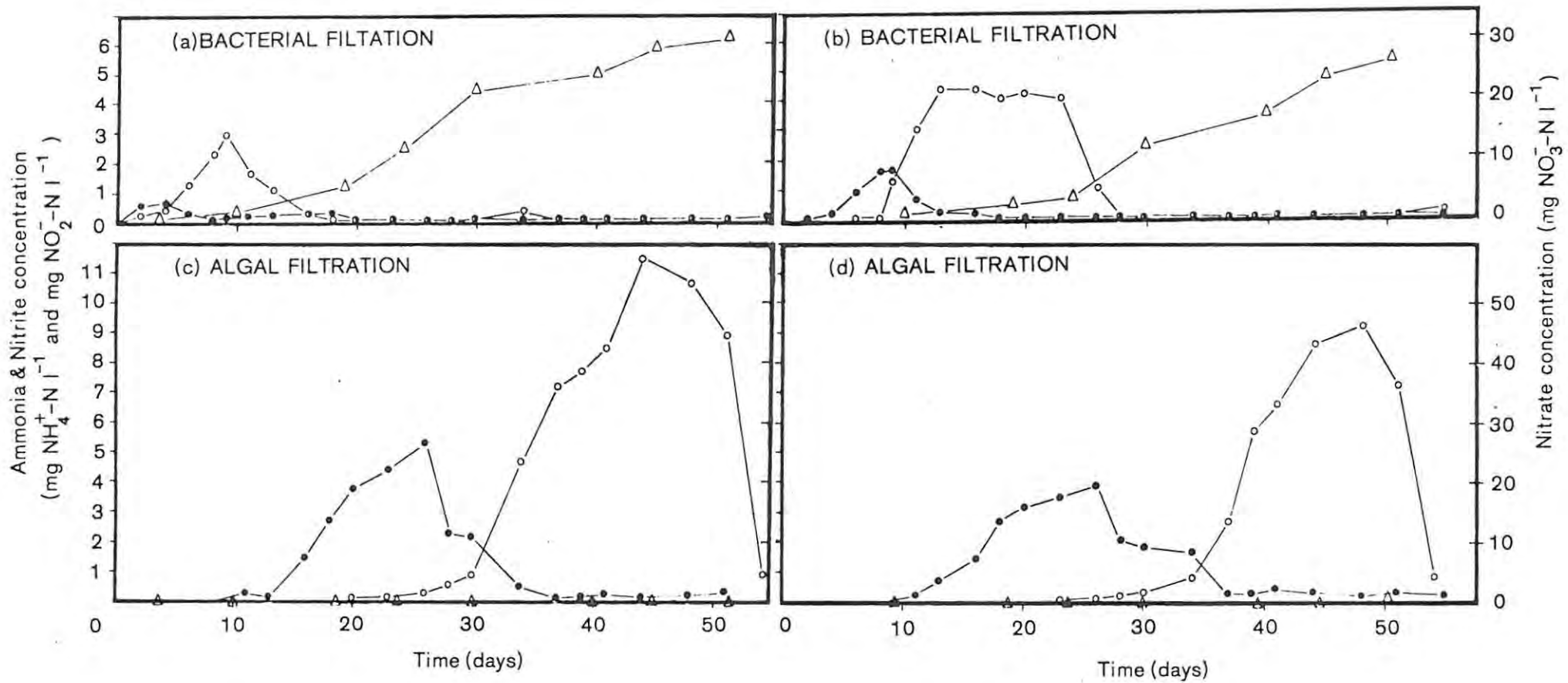


Figure 17: Ammonia (-●-), nitrite (-○-) and nitrate (-△-) concentrations in the bacterial filtration and algal filtration systems during the second experimental trial.

(Fig. 17 a and b) are far lower than in the algal filtration systems (Fig. 17 c and d). Similarly, the maximum levels of nitrite during nitrification in the bacterial filtration systems are lower than in the algal filtration systems. Furthermore, the establishment of nitrification was completed in 18 to 28 days in the two bacterial filtration systems, whereas nitrification required the full duration of the experiment (55 days) in the algal filtration systems. Nitrate accumulated in the bacterial filtration systems (Fig. 17 a and b) and reached concentrations of $26,2 \text{ mg NO}_3^- \text{-N l}^{-1}$ and $30,9 \text{ mg NO}_3^- \text{-N l}^{-1}$. In the algal filtration systems (Fig. 17 c and d) no nitrate was detectable throughout the course of the experiment.

(v) Algal condition

After the introduction of algae from the field into the filtration systems a marked change occurred in pigmentation of the filaments and they developed a dark green colour in 3-4 days. A few observations seemed to indicate that the filamentous algae tended to lose condition during the course of both experimental trials. These were:

1. Less regular need to harvest as the experimental trials progressed.
2. A gradual fall-off in the amount of gas bubbles trapped in the filaments - indicating a reduction in the amount of photosynthesis?
3. The presence of decaying sections of algae.
4. A certain amount of fragmentation of the algal filaments in the latter part of both experimental trials.

The lack of any form of mechanical filtration was noticeable in the systems which had algal filtration only. Excess food and faecal material, which had been stirred up by the prawns, was carried up the airlift pipes and deposited in the algal trays. A layer of this detrital matter

eventually covered the bottom of practically the whole algal tray and its decomposition was probably the cause of the eventual decay of some sections of the filamentous algae.

In the second experimental trial the algal harvest was dried at 80°C to constant weight. This dry weight was then converted back to wet weight using a conversion factor (x8,499) calculated from 6 separate drying experiments. A comparison of the wet weight of algae removed during the experiment, with the wet weight introduced initially, showed that the algae in the two algal filtration systems increased their biomass by 55,0 and 68,5%.

DISCUSSION

The trace metal concentrations obtained in the first experimental trial (Fig. 8) indicate that minimal trace metal depletion took place in the biological filtration systems containing algae. This is in contrast to the findings of Siddall (1974) who found depletion of all trace metals analyzed. It must be added, however, that the biological filtration systems in these experiments were in operation for less than half the duration of the system used by Siddall (1974) - 67 days as opposed to 156 days. Nevertheless, it is felt that a sufficient replenishment of trace elements occurs with the introduction of food.

As regards the major cation concentrations, we have seen from the results (Fig. 7) that no significant changes occurred in any of the biological filtration systems over the 67 days of monitoring. The changes found by earlier workers (Oliver, 1957; Saeki, 1963) were in public aquaria which had been established and running for years rather than months. Major cation changes therefore do not present a problem in short-term closed system culturing.

Oxygen levels in the bacterial filtration systems are surprisingly high - ranging from 92% saturation to 129% saturation. Although aeration using airstones is not the most efficient device to use (Scott, 1972), it may be that the length of the airlift pipe could be partly responsible for the efficiency of aeration in these filtration systems. It is known that the longer the airlift, the more efficient it is (Miklosz, 1970). However, the super-saturation of oxygen found in these systems cannot be accounted for.

The presence of algae had two effects on the oxygen content of the culture system:

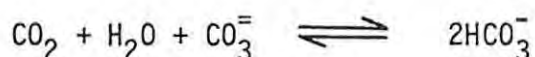
1. The oxygen values in the algal filtration systems were elevated (Fig. 10). This is in agreement with the results of Alderson and Howell (1973) who recorded oxygen values of 125% saturation during the light period in rearing tanks containing unicellular algal populations.
2. A marginal increase in % oxygen saturation was detectable during the day, but not at night (Fig. 11), and can be explained by the photosynthetic activities of the algae during the day.

These two algal induced changes in oxygen concentration were to be expected. These results simply quantify those changes. The slight upward trend in dissolved oxygen concentrations shown in Figure 11 was, however, unexpected. This trend cannot be explained but may be part of a longer term cycle.

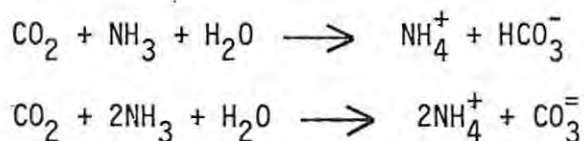
In the bacterial filtration systems a fairly large drop in oxygen content occurred after the culture water had passed through the gravel beds (Fig. 9 - from 111% to 92% saturation). This is confirmation of the large oxygen demand of the micro-organisms of the filter bed. However, in neither the algal nor the bacterial filtration systems did the oxygen concentration appear in any way limiting to the animals cultured. The use of algae for biological filtration purposes therefore presents no problems in this respect.

The concentrations of inorganic carbon in the filtration systems never dropped to a level ($< 10-12$ mg inorganic carbon ℓ^{-1}) where they could have been lethal to the prawns (Wickins, 1976a). On the contrary, in both the first and second experimental trials, there was a marked increase in the total inorganic carbon concentrations in the algal filtration systems (Figs 13 and 14). As explained in the methods, this increase in inorganic carbon was monitored as an increase in titratable base. On

the available information, an explanation for this increase in base is difficult to find. The algae in these filtration systems were often found to contain discoloured patches which appeared to be decaying. Liptrot (1978) found that the decay of Enteromorpha mats in Swartvlei was a causative factor in the rise of inorganic carbon throughout the estuary in periods of closure. This implies an available source of mineral carbonates with which the free or "aggressive" CO_2 arising from decomposition can react to form bicarbonate as described by Golterman (1969):



Although mineral carbonates in estuaries are usually abundant, this is not the case in the algal filtration systems used in these experiments. An alternative explanation for the observed increase in inorganic carbon (and hence titratable base) might be that the CO_2 produced by the respiration of the algae and micro-organisms reacts with water and unionized ammonia to produce carbonate and bicarbonate ions as described by Berner (1968):



The absence of marked pH changes in the experimental trials can be attributed to the low stocking densities used as well as the buffering capacity of sea water. The presence of algae did not give rise to any marked pH changes. This is in agreement with Siddall (1974) who states that "algal growth was not successful in returning pH to normal levels." The light-dark reversals in pH at the algal tray inlets and outlets (Fig. 12) were, as expected, due to algal removal of CO_2 during illuminated periods and its release during periods of darkness.

Calculations of the unionized ammonia values (NH_3 -N) from the total ammonia values (NH_4^{+} -N) in the first experimental trial are given in Table 1.

Table 1: Maximum values of total ammonia which occurred in the various biological filtration systems from the first experimental trial and the corresponding amount present as unionized ammonia calculated according to Whitfield (1974).

Filtration System	mg NH_4^+ -N ℓ^{-1}	mg NH_3 -N ℓ^{-1}
Algal and Bacterial	0,56	0,025
	0,97	0,045
Algal	1,43	0,062
	1,18	0,051
Bacterial	1,57	0,068
	1,76	0,051
Water Exchange	2,99	0,088
	3,87	0,055

It will be noticed that all unionized ammonia values were within the 'safe' level of $0,1 \text{ mg NH}_3 \text{ -N } \ell^{-1}$ found by Wickins (1976a) for penaeid species. Similar calculations for the second experimental trial are shown in Table 2.

Table 2: Maximum values of total ammonia in biological filtration systems from the second experimental trial and the corresponding amount present as unionized ammonia calculated according to Whitfield (1974).

Filtration System	mg NH_4^+ -N ℓ^{-1}	mg NH_3 -N ℓ^{-1}
Algal	3,93	0,202
	5,3	0,273
Bacterial	1,74	0,100
	0,62	0,036

The unionized ammonia concentrations in these algal filtration systems did exceed the $0,1 \text{ mg NH}_3\text{-N l}^{-1}$ 'safe' level of Wickins (1976a).

The time of exposure to these levels was very short (Fig. 17), and the consequent effect on the growth of the prawns is debatable. This will be discussed in Part II.

The capacity of the systems with gravel (Fig. 15 a and b) to undergo nitrification in a shorter time period, and to maintain lower ammonia stabilization levels than those systems without gravel (Fig. 15 c and d) was due to the greater surface area offered for bacterial attachment by the gravel chips. Surprisingly, the latter two systems in which water was exchanged or algae only were used, also show stabilization of ammonia levels. This indicates that at low stocking densities, the tank surfaces and accumulating detrital particles themselves provided sufficient surface area for Nitrosomonas populations to oxidize all ammonia produced within the system.

The stabilization of nitrite levels in the bacterial filtration and the algal plus bacterial filtration systems (Fig. 16 a and b), and the failure of these levels to decrease in the algal filtration and water exchange systems (Fig. 16 c and d) appears to be indicative of two things:

1. The failure of the nitrite levels to decrease in the algal filtration systems suggests that the algae might not possess a large capacity for nitrite uptake.
2. The failure of the nitrite levels to decrease in the water exchange systems, suggests that although the surface area of the tanks were sufficient for Nitrosomonas populations to oxidize ammonia, they were not sufficient for the Nitrobacter populations to oxidize nitrite.

The stabilization levels of nitrite in Figure 16a are lower than in Figure 16b. The only difference between these two filtration systems was that the latter had algae in addition to gravel. It therefore appears that the algae might reduce the ability of the filter bed bacteria to oxidize nitrite. Although further research is required, the possibility exists that algal antibiosis might be hindering the establishment of Nitrobacter populations. The influence of algal antibiosis on the ecology of marine micro-organisms has been dealt with by Sieburth (1968). In this context it is interesting to note that the stabilization of the nitrite in the algal filtration systems of the second experimental trial took the full duration of the experiment (Fig. 17).

In the second experimental trial, initial uptake of ammonia and nitrite by the filamentous algae was sufficient to prevent the accumulation of these compounds (Fig. 17). The accumulation of ammonia after approximately 10 days could have been due to one or more of the following explanations:-

1. Decomposition of organic matter and excretion by prawns had progressed to a level where the algae could no longer cope with the ammonia production.
2. After an initial period of 'luxury consumption' (Fogg, 1975) of ammonia by the algae, it became saturated and ammonia started to accumulate.
3. Loss of condition by the algae.

Nitrate did not accumulate in the algal filtration systems (Fig. 17 c and d). It might be argued that nitrate accumulation would only have started after the maximum levels of nitrite had been reached. However, in the bacterial filtration systems nitrate began to accumulate after a few days (Fig. 17 a and b). It must be concluded that the filamentous algae effectively removed all nitrate produced in the algal filtration systems.

Table 3 summarizes the results obtained in the preceding portion of this study.

Table 3: Summary of results on the changes in water quality obtained in the various biological filtration systems. +++ = large increase; ++ = moderate increase; + ; small increase; o = change negligible or absent; - = small decrease; -- = moderate decrease; NT = levels not toxic; T = toxic levels; * = for short period only.

Filtration Method → Parameters ↓	Bacterial Filtration	Algal Filtration	Algal + Bacterial Filtration	Water Exchange
Exptl. Trial 1 Maximum unionized NH ₃	N.T.	N.T.	N.T.	N.T.
Exptl. Trial 2 Maximum unionized NH ₃	N.T.	T*		
Exptl. Trial 1 Maximum Nitrite	N.T.	N.T.	N.T.	N.T.
Exptl. Trial 2 Maximum Nitrite	N.T.	N.T.		
Exptl. Trial 2 Maximum Nitrate	+++	o		
Ca ⁺⁺	+	o	+	o
Mg ⁺⁺	o	o	o	o
Na ⁺	-	-	-	-
K ⁺	-	-	-	-
Cu: Exptl. Trial 1	+++	+	+++	+
Cu: Exptl. Trial 2	o	o		
Fe	-	-	-	-
Zn	+	+	+	+
Mn	o	o	o	o
Co	o	o	o	o
Oxygen	+	++		
pH: Exptl. Trial 1	o	o	o	o
pH: Exptl. Trial 2	-	-	-	-
Exptl. Trial 1 Total inorganic C	-	+++	o	o
Exptl. Trial 2 Total inorganic C	--	+++		

Four noticeable changes occurred:-

1. Unionized ammonia exceeded the $0,1 \text{ mg NH}_3 \text{-N } \ell^{-1}$ level in the algal filtration systems during the second experimental trial.
2. Nitrate levels increased substantially in the bacterial filtration systems during the second experimental trial.
3. Copper contaminated the bacterial filtration and algal plus bacterial filtration systems during the first experimental trial.
4. Total inorganic carbon levels increased substantially in the algal filtration systems during both experimental trials.

The high unionized ammonia concentrations occurred over a short duration (< 9 days). The copper contamination was successfully avoided in the second experimental trial. Therefore, only the increased nitrate levels in the bacterial filtration systems and the increased inorganic carbon levels in the algal filtration systems constitute any real difference between the various biological filtration systems. It thus appears that during short term biological filtration (up to 2 months), different biological filtration treatments offer more or less the same water quality. Bacterial filtration, however, can be regarded as the most suitable since a system of water exchange is labour intensive, while those methods involving the use of algae give rise to problems. Under the light regime used ($3100\text{-}3800 \text{ microeinsteins m}^{-2} \text{ sec}^{-1}$), the algae was unable to assimilate all nitrogen compounds produced, and steadily lost condition. The apparent increase in pigmentation developed by the algae seems to confirm that light intensity was the main reason for the decline in condition of the algae. Further disadvantages of the use of algal filtration systems were the absence of any form of mechanical filtration for particulate removal from culture water, as well as the possibility of algal antibiosis hindering the establishment of Nitrobacter populations.

PART II: GROWTH OF PENAEUS INDICUS IN BIOLOGICAL FILTRATION SYSTEMS

INTRODUCTION

In Part I of this study, various parameters of water quality were used to compare the efficiency of four different biological filtration methods. It was concluded that, apart from a few minor differences, the various biological filtration systems produced more or less the same water quality during the two month experimental trials. In this section, the growth and survival of P.indicus obtained during those experimental trials is reported. The results are used to compare the filtration systems and to determine how the filtration systems compare with other experimental systems reported in the literature.

MATERIAL AND METHODS

Growth and survival of prawns of roughly the same size (0,5 g) was determined every two weeks for the full duration of both experimental trials. P.indicus were netted, counted and held temporarily in small containers of aerated sea water during weight determinations. Prior to weighing, excess surface water was removed from each prawn with absorbent paper towelling. The prawns were weighed to three decimal places on a top-loading balance.

RESULTS

The growth data obtained for P.indicus in the first experimental trial are listed in Table 4.

Table 4: Fortnightly wet weight (g) and percentage survival of P.indicus cultured in the four biological filtration systems during the first experimental trial.

Biological Filtration System	Time									
	Initial		2 weeks		4 weeks		6 weeks		8 weeks	
	Mean wet weight (g)	Mean wet weight (g)	Survival	Mean wet weight (g)	Survival	Mean wet weight (g)	Survival	Mean wet weight (g)	Survival	
Bacterial filtration	0,50	1,38	100%	2,54	65%	3,61	65%	4,41	60%	
	0,50	1,35	95%	2,23	70%	3,68	55%	4,25	50%	
Algal filtration	0,50	1,12	100%	1,71	95%	2,52	95%	3,05	95%	
	0,50	1,24	100%	2,30	100%	2,92	100%	3,34	100%	
Water exchange	0,50	1,47	95%	2,50	70%	3,25	55%	3,79	45%	
	0,50	1,39	100%	2,21	85%	2,88	85%	3,35	80%	
Algal plus bacterial filtration	0,50	1,24	95%	2,34	90%	3,30	75%	3,98	70%	
	0,50	1,12	100%	1,97	100%	2,47	85%	3,15	65%	

The mean fortnightly wet weights of the prawns in the bacterial filtration systems were compared with those in the other biological filtration systems. Using Students 't' test, the following results were obtained:

- 2 weeks - $P > 0,01$
- 4 weeks - $P > 0,01$
- 6 weeks - $P < 0,001$
- 8 weeks - $P < 0,001$

At the 1% level of significance it can be seen that from the sixth week onwards, the mean wet weight of the prawns in the bacterial filtration systems was significantly larger than those in the other biological filtration systems.

Chi-square tests were applied to the fortnightly survival data on prawns in the various biological filtration systems in the first experimental trial. The following results were obtained:

2 weeks - $P > 0,99$
4 weeks - $P > 0,05$
6 weeks - $P < 0,02$
8 weeks - $P < 0,01$

A significant difference (at the 2% level) in rates of survival in the different biological filtration systems was detectable after 6 weeks. Lowest rates of survival occurred in the bacterial filtration systems, while the algal filtration systems had the highest rates of survival. The water exchange and algal plus bacterial filtration systems had intermediate rates of survival.

Growth data obtained for P.indicus in the second experimental trial are listed in Table 5. (See over).

Students 't' and chi-squared tests were applied to the fortnightly growth and survival data. No significant differences were found in the growth or survival of P.indicus cultured in the algal or bacterial filtration systems.

Table 5: Fortnightly wet weight (g) and percentage survival of P.indicus cultured in the algal filtration and bacterial filtration systems during the second experimental trial.

Biological Filtration System	Time									
	Initial		2 weeks		4 weeks		6 weeks		8 weeks	
	Mean wet weight (g)	Survival	Mean wet weight (g)	Survival	Mean wet weight (g)	Survival	Mean wet weight (g)	Survival	Mean wet weight (g)	Survival
Bacterial filtration	0,50	100%	1,10	100%	1,91	100%	2,67	100%	3,34	100%
	0,48	90%	0,99	90%	1,86	90%	2,53	90%	3,38	75%
Algal filtration	0,54	90%	1,00	90%	2,00	90%	3,06	90%	3,30	90%
	0,49	100%	1,11	100%	1,97	100%	2,93	95%	3,22	90%

DISCUSSION

Statistical analyses of the data show that, while P.indicus in the bacterial filtration systems of the first experimental trial grew significantly larger than the prawns in the other systems from the sixth week onwards, the trend in survival was exactly the opposite (Table 4). In other words, from the sixth week onwards, P.indicus showed poorer survival rates in the bacterial filtration systems when compared with the other systems. The resultant change in population densities could explain the differences found in the growth results. This seems to be confirmed by the results of the second experimental trial, where no significant difference was found in the survival rates, and the growth rates were similar (Table 5). It therefore appears that it is not possible to make direct comparisons between various biological filtration systems with different rates of survival due to the effects of stocking density changes.

A comparison of the rates of growth for the first two weeks of each trial shows a difference. In the second experimental trial (Table 5) growth was slower (mean wet weight 0,99 - 1,11 g) than in the first experimental trial (Table 4 - mean wet weight 1,12 - 1,39 g). A possible reason is that the estuarine prawns obtained for the second experimental trial had shell lesions and damaged antennal scales which appeared to be secondarily infected with chitinoclastic bacteria (Anderson & Conroy, 1968; Cook & Lofton, 1973; Delves-Broughton & Poupard, 1976). The disappearance of the shell disease during the experiment and the increase in growth rates by the 4th week is indicative that the water quality in these filtration systems was suitable for prawn growth.

In section I (Table 2; Fig. 17) it was found that during the third and fourth weeks of the second experimental trial, unionized ammonia levels in the algal filtration systems exceeded the $0,1 \text{ mg NH}_3\text{-N l}^{-1}$ 'safe' level

recommended by Wickins (1976a) for penaeids. A comparison of the growth and survival data of the two filtration systems after the fourth week (Table 5) shows that the temporarily increased levels of unionized ammonia had no appreciable effect on the algal filtration prawns.

The growth achieved in these systems requires comparison with similar results found in the literature. This has been attempted and is shown in Table 6. Due to the marked differences in growth rates found in different species of penaeids (Forster & Beard, 1974), this comparison has been restricted to the culture of P.indicus. The growth of P.indicus in the

Table 6: Growth and survival of Penaeus indicus in laboratory experimental systems. All systems were semi-open (receiving replacement sea water every week), except for the systems of the first experimental trial in this study, which were closed recirculation systems.

Reference	Mean wet weight (g)			Stocking Density	Survival			
	Initial	3 weeks	5 weeks		8 weeks	3 weeks	5 weeks	8 weeks
Forster & Beard, 1974	0,26			4,90	25 m ⁻²			36
Forster & Beard, 1974	0,23			4,31	147 m ⁻²			35
Colvin, 1976a	0,95	3,06			10 per 100 l tank	100		
Colvin, 1976a	0,56		3,57		10 per 100 l tank			97
Colvin, 1976b	0,61		3,79		10 per 100 l tank			90
Champion (pers.comm.)	0,5			4,3				
1st Experimental trial	0,5			4,41	54 m ⁻²			60
1st Experimental trial	0,5			4,25	54 m ⁻²			50

first experimental trial compares favourably with figures obtained by Forster and Beard (1974) and Champion (pers.comm.). The 50 and 60% survival obtained in the first experimental trial was greater than the 35 and 36% survival obtained by Forster and Beard (1974) even though prawns were lost as 'jump-outs'. However, Colvin (1976a and b) obtained higher growth and survival values than those obtained in this study. A probable reason for this could be the substantially lower stocking densities used by Colvin (1976 a and b). In general, Table 6 shows that the growth and survival rates of P.indicus obtained in these closed system cultures are equal to those obtained in other semi-open culture systems. Good management of closed system cultures therefore appears to obviate the need to continually supplement recirculation systems with fresh sea water.

PART III: NITROGEN FLOW IN BIOLOGICAL FILTRATION SYSTEMS

INTRODUCTION

In Part I of this study it was shown that parameters of water quality such as the concentrations of oxygen, major cations, trace metals and the pH are not likely to pose any real problems in short-term closed system culturing. The major changes that occurred in these systems concerned the nitrogen compounds, ammonia, nitrite and nitrate. It is essential to understand which factors influence the build-up of nitrogen compounds in these systems for good water quality management and the rational design of nitrification facilities. The quantity of nitrogen entering the system and the amount which is removed must be known. Furthermore, a knowledge of the cycling of nitrogen within the biological filtration system will be of great assistance in determining stocking densities and feeding rates, and also in assessing the contribution of excess food on ammonia concentrations.

Input of nitrogen into the system

The sole primary source of nitrogen input into a culture system is through the introduction of food. In this study, prawns were fed at a fixed daily rate of 5% of their biomass. Determination of the nitrogen content of the pelleted ration therefore enabled calculation of nitrogen input due to feeding.

Cycling of nitrogen within the system

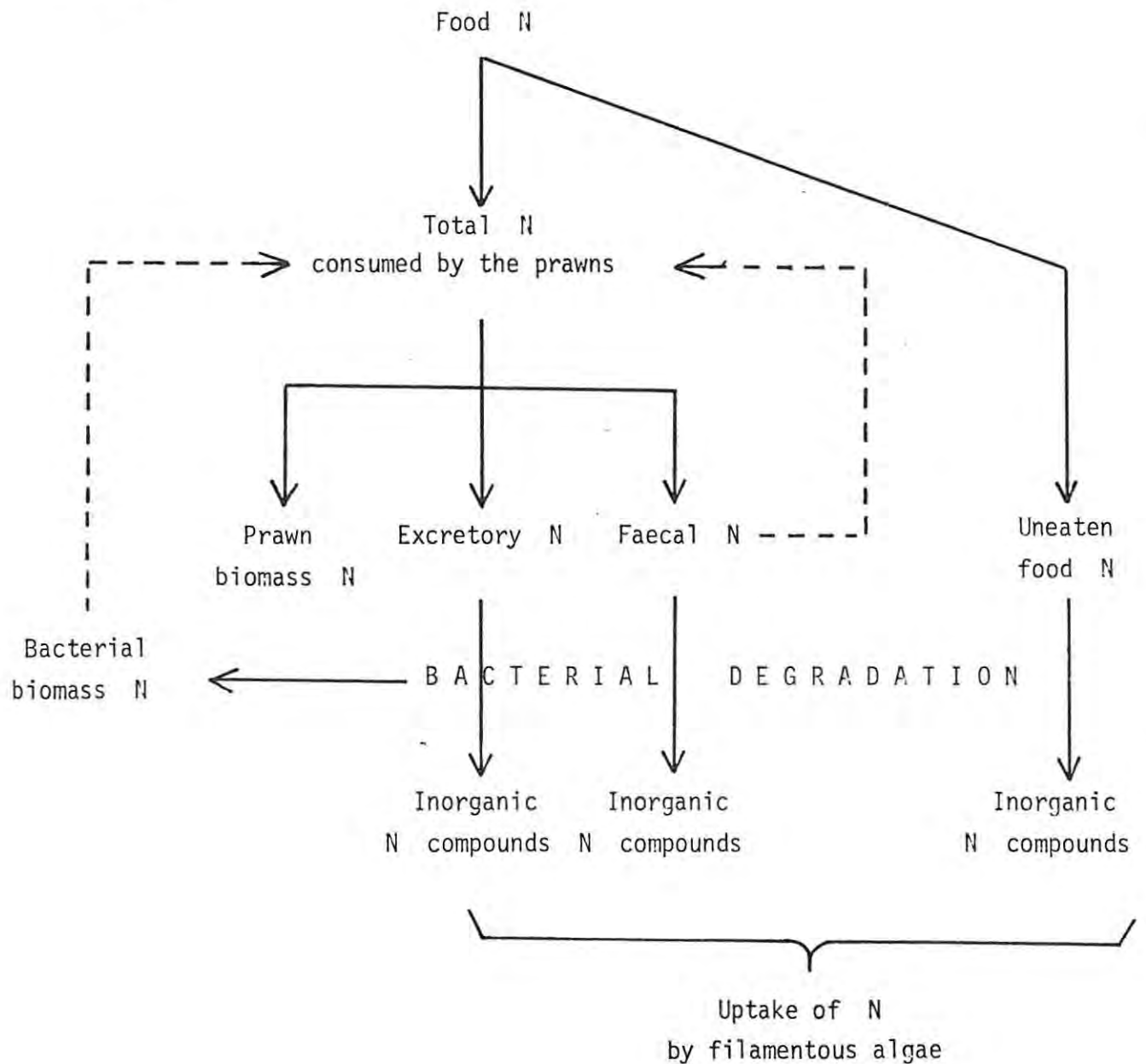
Nitrogen entering a closed system as food will either be consumed by the prawns or lost as excess food. Wickins (1976b) points out that during the external trituration process of penaeids, much of the food is scattered into the water. This excess food is then degraded by bacteria while existing as detritus within the system. Detritus, and even faecal pellets (Johannes & Satomi, 1966), are often re-processed by penaeids

(Dall, 1968). Nitrogen is also taken up by growing bacterial populations. While most nitrogen consumed by the prawns is assimilated, a certain proportion is lost in the faeces. Some assimilated nitrogen is excreted as metabolic waste.

Nitrogen removal from the systems

All nitrogen degraded by bacteria in a closed system will exist eventually as inorganic nitrogen compounds. The sole means of removal of this nitrogen is by the use of algae which are periodically harvested.

These pathways of nitrogen (N) can be diagrammatically represented as follows:-



It would be extremely difficult to accurately quantify the above pathways of nitrogen. In order to gain an insight into the relative importance of the major nitrogen pathways within a closed system culture, it is necessary to make two simplifying assumptions:

1. The nitrogen bound up as bacterial biomass is negligibly small in comparison to the total amount of nitrogen passing through the system.
2. All nitrogen that enters the system and is not actually bound up as prawn biomass, is eventually broken down by bacterial action to inorganic nitrogen compounds (ammonia, nitrite and nitrate).

The former assumption is fairly reasonable, because the nitrogen demand by bacteria is significant only during the establishment of nitrification. Quastel and Scholefield (1951) have shown population densities of nitrifying bacteria to be relatively constant and independent of available energy sources. Increases in the metabolic activities of individual cells, rather than increases in cell numbers, compensate for fluctuations in energy sources. The second assumption is certainly applicable to long-term culture systems, but in short-term culture systems there might be a time lapse between the introduction of organic nitrogen and its complete conversion to inorganic derivatives. As the duration of this time lapse is unknown, the experiments were continued until a steady state had been reached with respect to the intermediates in the degradation process (ammonia and nitrite).

Having made these assumptions, it will be possible to quantify the relative importance of the various pathways by determination of:

1. Total food nitrogen added to the system.
2. Nitrogen content of P.indicus.
3. Faecal production of P.indicus.

4. Total nitrogen excretion of P.indicus.
5. Uptake of nitrogen compounds (ammonia, nitrite and nitrate) by algae.

This information enables calculation of the amount of nitrogen available for bacterial degradation (excretory N + faecal N + uneaten food N), or the nitrogen loading on the system due to uneaten food [Total food N - (prawn biomass N + faecal N + excretory N)]. In a similar way, the assimilation efficiency and food conversion ratio of P.indicus can be calculated.

MATERIAL AND METHODS

(i) Kjeldahl and ammonia determinations

Total organic nitrogen was determined by the micro-Kjeldahl method. Ammonia determinations were carried out by titration with 0,01N HCl after steam distillation in a Parnas-Wagner apparatus. Both methods are described by Golterman (1969). The accuracy of micro-Kjeldahl digestion was checked using glycine as an organic standard. For 30 replicate samples it was found to be accurate to 99,4% (S.D. 2,0). The accuracy of the distillation technique was checked using $(\text{NH}_4)_2 \text{SO}_4$ as an inorganic standard. For 10 samples it was found to be accurate to 99,4% (S.D. 3,2).

(ii) Nitrogen excretion in *Penaeus indicus*

Total nitrogen and ammonia nitrogen excreted by *P. indicus* over a 24 hour period (12:12 light-dark photoperiod) was determined at 28^oC (n = 29), 24^oC (n = 21), 20^oC (n = 22) and 16^oC (n = 20). Prawns of 1-12 g wet weight, were netted from the Amatikulu and Fish River estuaries. A few prawns larger than 12 g wet weight were used in the 28^oC experiments, and these were either obtained by trawl off the northern Natal coast or netted in the Fish River estuary. All prawns used in these experiments were at the intermoult stage, since at this stage the oscillations of nitrogen output are minimal in most Crustacea (Needham, 1956).

Prawns were held in large volume bacterial filtration systems in the laboratory for a minimum period of 2 weeks before use. During this time they were fed ad libitum with "Higashi Maru" prawn pellets. Prawns were acclimated for 48 hours to the various temperatures before determining their excretion rates. In order to avoid extraneous sources of nitrogen, the prawns were not fed during the experiments. This short period of starvation is unlikely to have affected their rate of nitrogen excretion as Butler et al. (1969) found that *Calanus* with a full gut excreted 7,42 $\mu\text{g-N mg dry weight}^{-1} \text{ day}^{-1}$, while individuals with an empty gut excreted 7,45

$\mu\text{g-N mg dry weight}^{-1} \text{ day}^{-1}$. Prawns were washed with distilled water before and after the experiment to remove any adhering excretory substances.

Depending on the size of prawns used, experiments were carried out in glass containers with between 300 and 1000 ml of Whatman GF/C filtered sea water. Aeration air was initially bubbled through concentrated H_2SO_4 to remove volatile bases (Sharma, 1966; Horne, 1968). As a precautionary measure, air leaving the containers was passed through a solution of boric acid and mixed indicator (0,5% bromocresol green and 0,1% methyl red in 95% ethanol) to trap any volatile ammonia that might be lost to the air. In these experiments, ammonia collected in the terminal boric acid trap was insufficient to change the colour of the indicator and the loss from this source was therefore regarded as being negligible. After the experiments, aliquots of the sea water in which the prawns were held, were filtered through Whatman GF/C filter paper to remove faecal material, and then analyzed for total nitrogen and ammonia nitrogen.

Although an experimental period of 24h has been used for a variety of aquatic animals (Needham, 1957; Corner & Newell, 1967; Janssens & Cohen, 1968), it has been severely criticized. Webb and Johannes (1969) found that after 24 hours bacterial populations in their 75 ml containers had reached a density of $0,5 \times 10^6 \text{ ml}^{-1}$ and removed 30% of the dissolved amino acids. They also pointed out that because marine bacteria assimilate ammonia, the possibility exists that published values of ammonia release might be too low. To minimize ambiguities due to microbial activity, control experiments were conducted without animals. Fixed amounts of ammonia ($2,5 \text{ mg NH}_4^+ \text{-N l}^{-1}$) were incubated for 24 hours at 28°C . A few prawn faecal pellets were added as a bacterial seed. In some containers malachite green (Horne, 1968), and in others acetate buffer (Staddon, 1959), was used to prevent the establishment of micro-organisms. The final ammonia concentrations were compared with untreated controls.

(iii) Faecal production of *P.indicus*

Twenty *P.indicus*, ranging in size from 1,99 to 26,73 g wet weight, were used to measure faecal production over a 24 hour period (12:12 light-dark photoperiod). They were fed with pelleted ration 1 h before commencement of experiments. During the experiments prawns were held in containers of aerated, Whatman GF/C filtered sea water. After 24 hours the faeces were filtered from the sea water, dried at 80°C, and weighed. Since sand may comprise a recognizable dietary component of faeces (personal observation), all prawns used in these experiments were taken from a laboratory tank with a coarse gravel chip substratum.

(iv) Nitrogen content of formulated ration, *P.indicus* and faecal pellets

Two separate samples of "Higashi-Maru" prawn pellets were dried at 80°C and then ground to a fine powder with a pestle and mortar. Ten determinations of total nitrogen were carried out on each sample using the micro-Kjeldahl method. Ten *P.indicus* ranging in size from 0,5 to 5,0 g wet weight were dried at 80°C and similarly analyzed for total nitrogen content. Approximately 30 faecal pellets collected from a number of prawns of varying sizes were pooled, dried at 80°C, powdered, and also analyzed for total nitrogen.

(v) Nitrogen uptake by filamentous algae

To simulate conditions in the biological filtration systems, experiments were carried out using sea water from a bacterial filtration system which had been established for 2 months. Two litres of this water was filtered, spiked with NH_4Cl , NaNO_2 or KNO_3 , and 12 to 18 g wet weight of filamentous algae was added. Duplicate controls, containing no algae, were run to determine the proportion of the nitrogenous compounds lost to the air. Ammonia and nitrite uptake was monitored every $\frac{1}{2}$ to 1 hour using the analytical techniques described in Section I. Uptake of nitrate was measured at 6 hourly intervals during periods of light and dark. The

dry weight of all algae used was determined at completion of the experiments.

Great difficulty was experienced with nitrate uptake experiments which required a longer time period (+ 3 days). In many cases no uptake of nitrate seemed to occur, and the results given show the maximal rates of uptake obtained. The reason for the failure of the algae to take up nitrate is thought to be their condition.

The types of filamentous algae used in these experiments were either Chaetomorpha sp., Enteromorpha sp., or Rhizoclonium sp. - depending on availability.

(vi) The effect of excess feeding on ammonia concentrations

Four bacterial filtration systems were set up as described in Part I. All were stocked with 20 P.indicus, each weighing approximately 2,3 g wet weight. They were fed at a rate of 5% of their total biomass per day. After the establishment of nitrification (about 30 days), the feeding regime was changed to either 5, 10, 15 or 20% of animal biomass per day for a 7 day period, after which feeding reverted back to the 5% regime. Ammonia analyses were carried out 3 times a week as described in Part I.

RESULTS

(i) Excretion and faecal production of *P.indicus*

A linear relationship was found to occur between the wet weight of *P.indicus* (size range 1 to 12 g) and total amount of nitrogen excreted at each temperature tested (Fig. 18). The correlation is significant at the 1% level in all cases. The relationship is probably not linear over a large

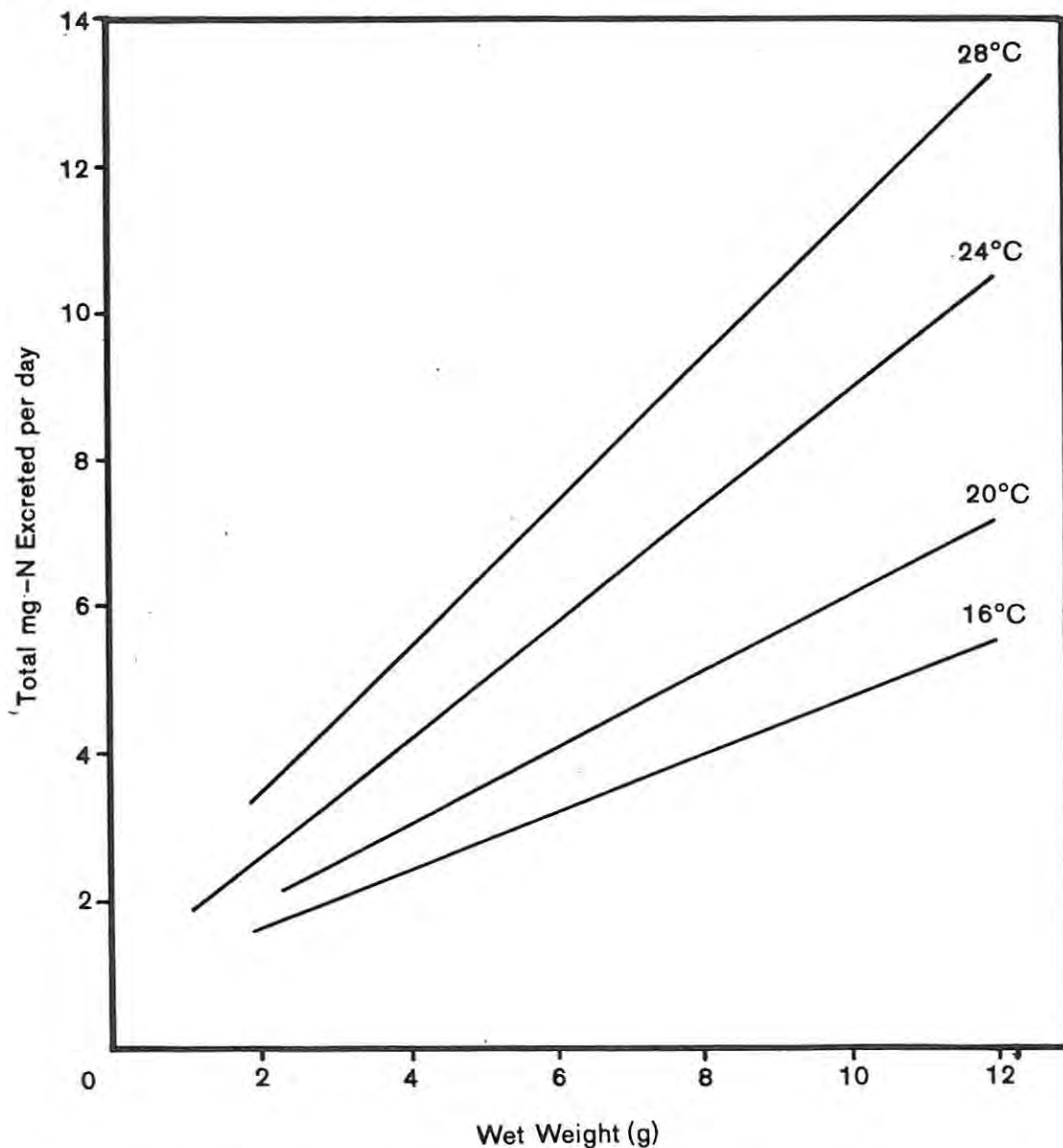


Figure 18: Excretion of *Penaeus indicus* at 28⁰C ($y = 0,9767x + 1,5255$; $n = 29$; $r = 0,93$), 24⁰C ($y = 0,7887x + 1,0673$; $n = 21$; $r = 0,57$), 20⁰C ($y = 0,5140x + 0,9848$; $n = 22$; $r = 0,83$) and 16⁰C ($y = 0,3880x + 0,887$; $n = 20$; $r = 0,86$), where x is the wet weight in grams of 1-12 g *P.indicus* and y is the total mg nitrogen excreted per day.

size range as shown in Fig. 19, where the results of determination of nitrogen production by estuarine prawns larger than 12 g clearly depart from the linear relationship found for smaller individuals. The figure also shows that large prawns of marine origin excrete more nitrogen per day at 28°C than those of estuarine origin. Ammonia made up 72,6% (S.D. 1,41) of the total nitrogen excreted at 28°C (n = 39).

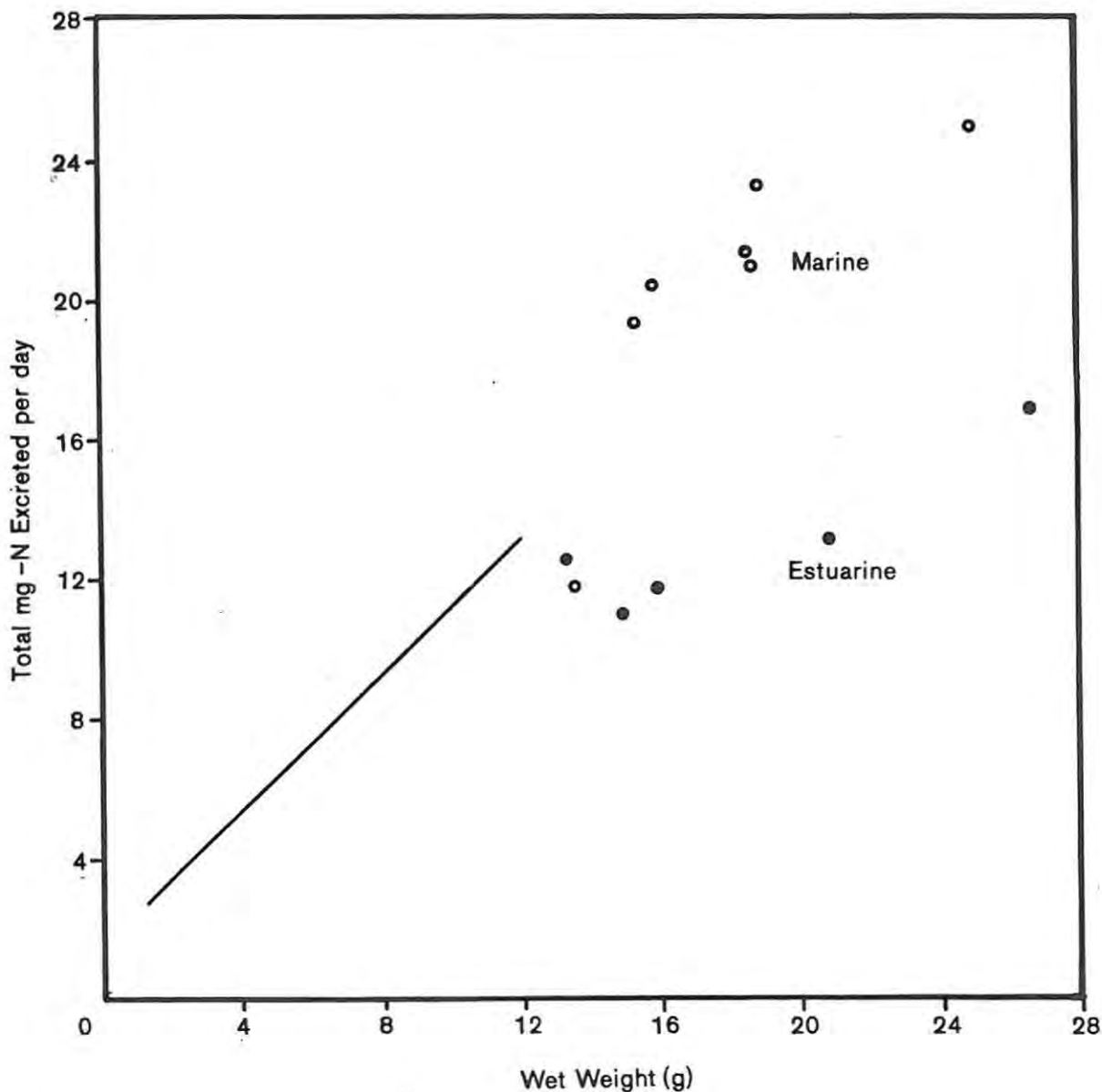


Figure 19: Excretion in mg total -N day⁻¹ of Penaeus indicus of marine (open circles) and estuarine (closed circles) origin at 28°C. Also included is the regression line for the excretion of 29 estuarine animals of 1 - 12 g wet weight (from Fig. 18).

Controlled experiments showed that ammonia was lost during the 24h duration of these excretion experiments (Table 7). The average final concentration in the four untreated cultures ($2,094 \text{ mg NH}_4^+ \text{-N } \ell^{-1}$) is 12,57% lower than the average final concentration in the treated cultures ($2,395 \text{ mg NH}_4^+ \text{-N } \ell^{-1}$). This difference can be attributed to a loss of ammonia due to oxidation by nitrifying bacteria. A correction factor of 12,57% was therefore applied to all excretion results when later used in calculations.

Table 7: Initial and final concentrations of ammonia in treated and untreated 500 ml samples of sea water spiked with $2,5 \text{ mg NH}_4^+ \text{-N } \ell^{-1}$, inoculated with bacteria, and incubated for 24 hours at 28°C .

Treatment	Initial concentration ($\text{mg NH}_4^+ \text{-N } \ell^{-1}$)	Final concentration ($\text{mg NH}_4^+ \text{-N } \ell^{-1}$)	Average final concentration ($\text{mg NH}_4^+ \text{-N } \ell^{-1}$)
Malachite Green	2,5	2,47	
Malachite Green	2,5	2,25	2,395
Acetate buffer	2,5	2,41	
Acetate buffer	2,5	2,45	
No Treatment			
A	2,5	2,00	
B	2,5	2,25	2,094
C	2,5	2,125	
D	2,5	2,00	

The faecal production of *P.indicus* at 28°C is shown in Fig. 20. The reason for the large degree of variability is not known. The regression equation for the line drawn is $y = 1,528x + 10,1761$, where x is the wet weight of the prawns and y is the amount of faeces produced in mg dry weight per day ($n = 20, r = 0,72$).

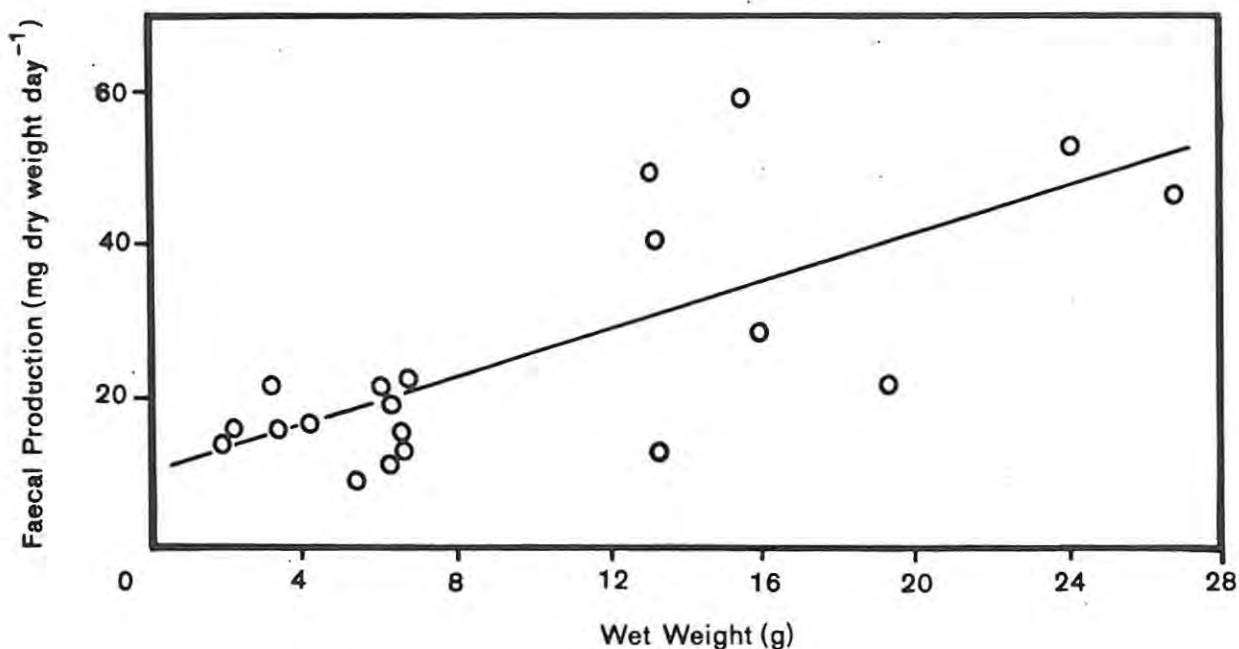


Figure 20: Faecal production of Penaeus indicus at 28°C as related to wet weight (N = 20; $r = 0,72$).

(ii) Nitrogen content of P.indicus, faecal pellets and formulated ration

Table 8 gives the total nitrogen content of P.indicus, faecal pellets and "Higashi-Maru" formulated ration.

Table 8: Total nitrogen content of P.indicus, faecal pellets and formulated ration.

	n	Total nitrogen mg -N g dry weight ⁻¹	S.D.
<u>P.indicus</u>	15	125,5	10,19
Faecal pellets	17	66,3	7,68
Formulated ration	20	89,6	6,10

(iii) Nitrogen uptake by filamentous algae

The uptake of ammonia by both Chaetomorpha sp. and Enteromorpha sp. is shown in Figure 21. No release of ammonia occurred during the period

of darkness. During periods of illumination, the maximal rates of ammonia uptake, corrected for losses shown by the control, were respectively 1,898 and 1,732 $\text{mg NH}_4^+ \text{-N h}^{-1} \text{ g dry weight}^{-1}$ Enteromorpha and Chaetomorpha sp.

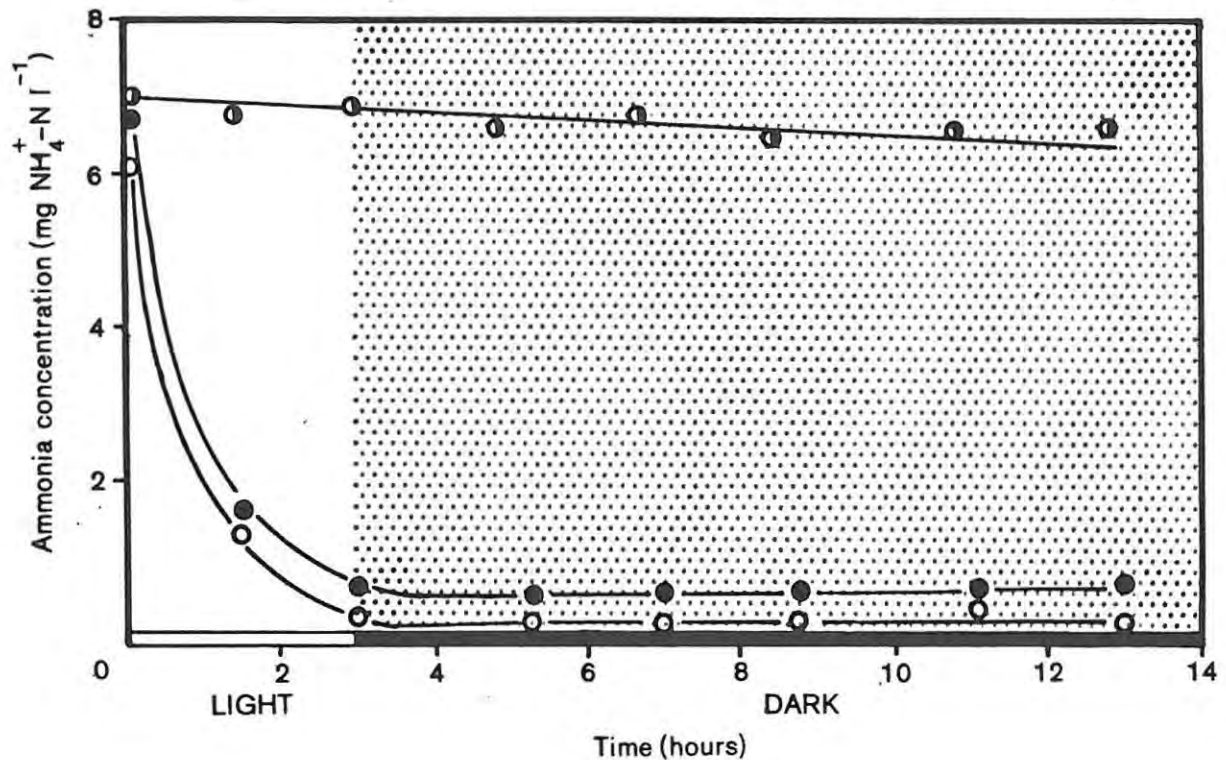


Figure 21: Ammonia concentrations during an uptake experiment by Chaetomorpha sp. and Enteromorpha sp. in light (c. 3800 microeinsteins. $\text{m}^{-2} \text{sec}^{-1}$) and dark at 28°C. (- ● -) = 1,796 g dry weight Enteromorpha spp.; (- o -) = 1,848 g dry weight Chaetomorpha spp.; (- ● -) = mean of two controls without algae.

The uptake of nitrite by Chaetomorpha sp. is shown in Figure 22. The rates of uptake of nitrite were respectively 0,26 and 0,30 $\text{mg NO}_2^- \text{-N h}^{-1} \text{ g dry weight}^{-1}$ for the two replicates. Nitrite loss in the control was undetectable. Nitrite was not removed by Chaetomorpha sp. during periods of darkness.

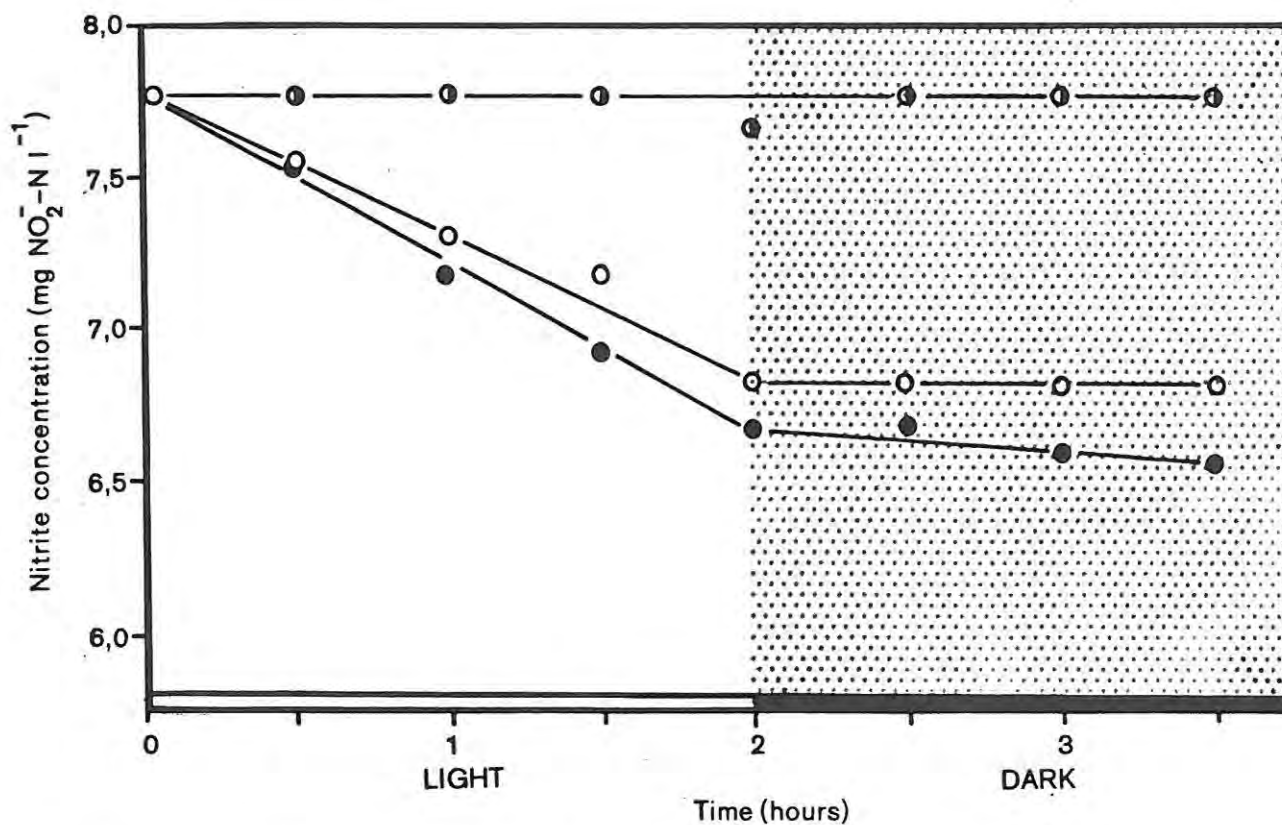


Figure 22: Nitrite concentrations during an uptake experiment by *Chaetomorpha* sp. in light (c. 3800 microeinsteins $m^{-2} sec^{-1}$) and dark at $28^{\circ}C$. (- ● -) = 1,842 g dry weight algae; (- o -) = 1,591 g dry weight algae; (- ● -) = mean of two controls without algae.

Nitrate uptake by filamentous algae is shown in Figure 23. No nitrate loss occurred in the control, while rates of removal were 1,91 and 3,67 $mg NO_3^- - N g dry weight^{-1} day^{-1}$ for the *Rhizoclonium* sp. and *Enteromorpha* sp. respectively.

There are indications that nitrate is assimilated during periods of darkness.

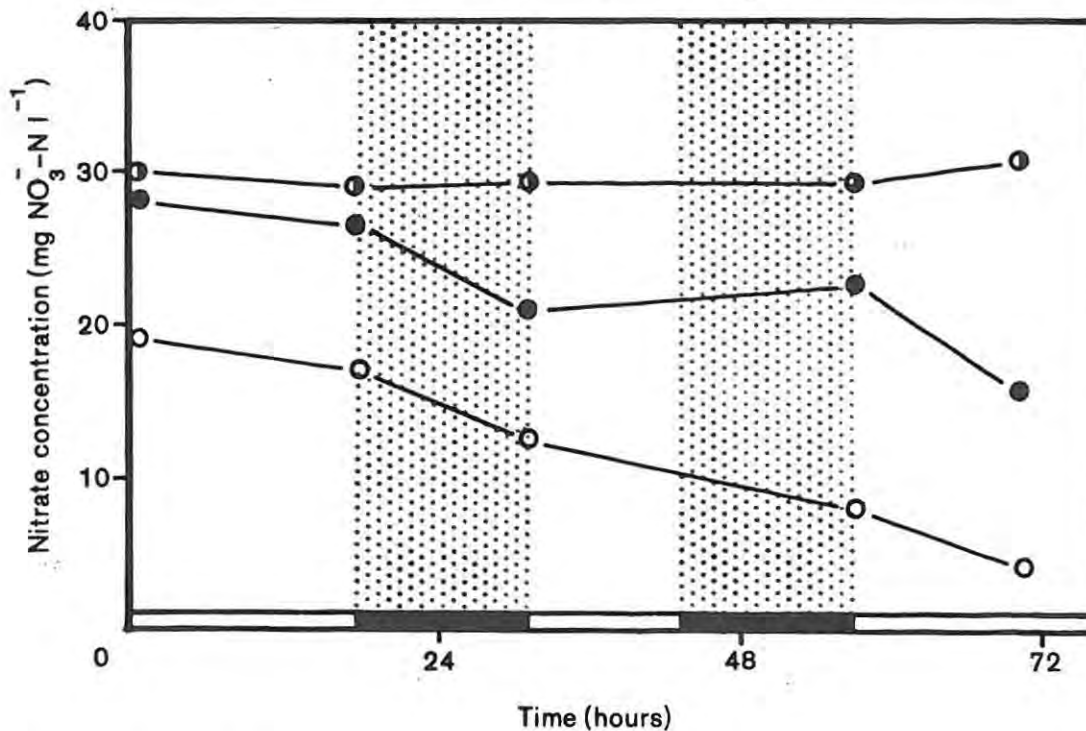


Figure 23: Nitrate concentrations during an uptake experiment by *Enteromorpha* sp. and *Rhizoclonium* sp. in light (c. 3800 microeinsteins $\text{m}^{-2} \text{sec}^{-1}$) and dark at 28°C. (- ● -) = 1,098 g dry weight *Enteromorpha* sp.; (- ○ -) = 2,58 g dry weight *Rhizoclonium* sp.; (- ○ -) = mean of two controls without algae; shaded areas = dark cycle.

(iv) The effect of excess feeding on ammonia concentrations

At a ration of 5% of the prawn biomass the ammonia concentration remained stable (Fig. 24). At 10 and 15%, the ammonia concentration increased and reached between 0,4 and 0,5 $\text{mg NH}_4^+ \text{-N l}^{-1}$ in 7 days. At a feeding rate of 20% of their biomass, there was a dramatic increase in ammonia concentration after the 5th day, rising to 6,1 $\text{mg NH}_4^+ \text{-N l}^{-1}$ on the 7th day. The water in the filtration system had also acquired a murky appearance after the third day of excess feeding while all other tanks remained clear. Ammonia levels in the filtration systems receiving the 10 and 15% feeding rates stabilized after reversion back to the 5% feeding regime, whereas the 20% system did not.

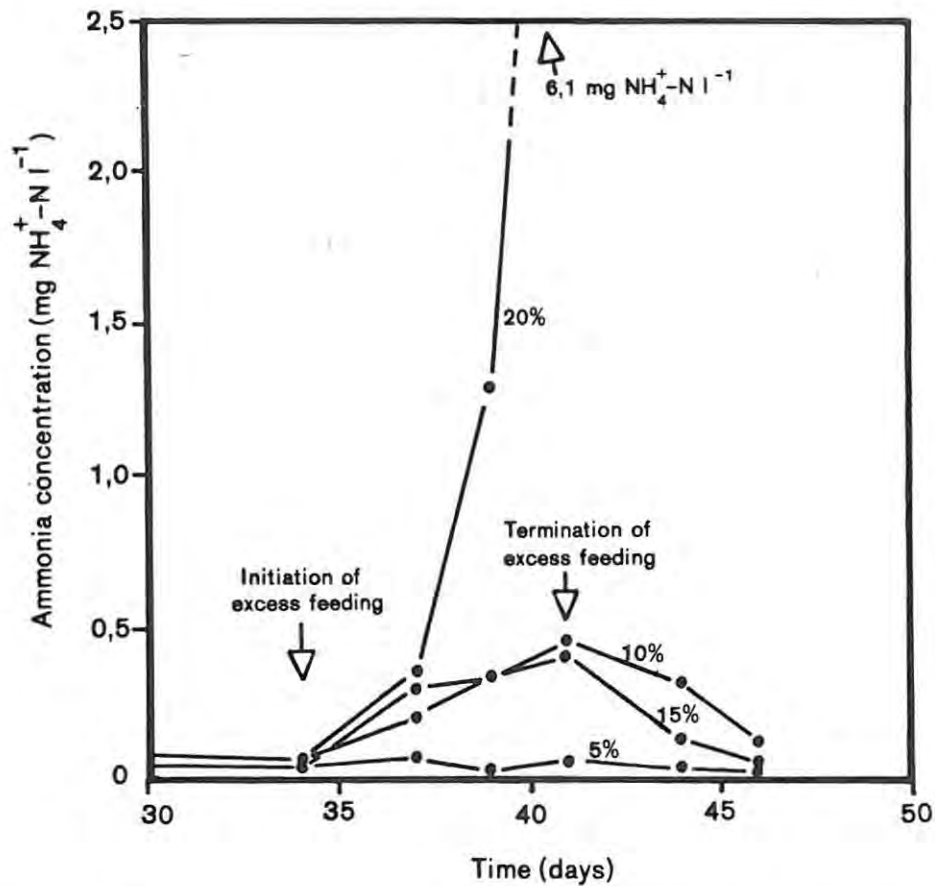


Figure 24: Ammonia concentrations in four biological filtration systems stocked with Penaeus indicus. The prawns were fed at daily rates of 5, 10, 15 and 20% of their biomass from the 34th to the 41st day. On all other days all systems received food at a rate of 5% of the biomass of the prawns.

(v) Calculations

The data obtained in this section of the study enables calculation to be made of the relative contribution of N to the system by:

1. Excretion of prawns
2. Faecal production of prawns
3. Uneaten food.

Using nitrogen as an indicator, estimates can also be made for the percentage assimilation of food and the food conversion ratio of P.indicus. For the purpose of these calculations a hypothetical case will be used to facilitate calculations. Let us consider a biological filtration system

stocked with twenty 0,5 g wet weight prawns which grow linearly to 4,0 g wet weight in 60 days, and are fed at a rate of 5% of their biomass daily. This is a situation not far removed from the results given in Table 4 (Part II).

Total nitrogen input into the system

The amount of pelleted ration introduced into the system throughout the 60 day period is the summation of 5% of the prawn daily weight. The summation (S_n) of the daily weight of an individual prawn can be calculated using the formula for adding up an arithmetic series of the form:

$$a + (a + d) + (a + 2d) + \dots + (A + (n - 1) d)$$

The formula is

$$\begin{aligned} S_n &= \frac{n}{2} (2a + (n - 1) d) \\ &= \frac{n}{2} (a + [a + (n - 1) d]) \\ &= \frac{n}{2} (\text{1st term} + \text{last term}) \end{aligned}$$

where a = initial weight

$$\begin{aligned} d &= \text{daily increment in wet weight (g)} \\ &= \frac{4,0 - 0,5}{n - 1} \\ &= 0,0593 \end{aligned}$$

Which is equivalent to

$$\begin{aligned} S_n &= \frac{n}{2} (\text{1st term} + \text{last term}) \\ &= \frac{60}{2} (0,5 + 4,0) \\ &= 135,0 \text{ g} \end{aligned}$$

The summation of the daily wet weights of 20 prawns would then be

$$\begin{aligned} &20 \times 135,0 \text{ g} \\ &= 2700,0 \text{ g} \end{aligned}$$

The amount of formulated ration fed is therefore

$$2700,0 \times \frac{5}{100} = 135,0 \text{ g}$$

From Table 8 the formulated ration contains 89,6 mg N g dry weight⁻¹.

Therefore, total N input into the system during

$$\begin{aligned} 60 \text{ days} &= 135,0 \times 89,6 \text{ mg N} \\ &= 12096,0 \text{ mg N.} \end{aligned}$$

Percentage loading due to prawn excretion

The regression equation for the total nitrogen excretion of P.indicus at 28°C (Fig. 18) is

$$y = 0,9767x + 1,5255$$

This equation can be used to calculate the first and last term for an arithmetic series equation.

$$\begin{aligned} \text{First term (0,5 g prawn)} &= 0,9767 \times 0,5 + 1,5255 \\ &= 2,0139 \end{aligned}$$

$$\begin{aligned} \text{Last term (4,0 g prawn)} &= 0,9767 \times 4,0 + 1,5255 \\ &= 5,4323 \end{aligned}$$

The total nitrogen excreted by 1 prawn in 60 days

$$\begin{aligned} &= \frac{60}{2} (2,0139 + 5,4323) \\ &= 223,386 \text{ mg N} \end{aligned}$$

For 20 prawns this would be 20 x 227,109 mg N

$$= 4467,72 \text{ mg N}$$

From Table 7 this would be an underestimate. To correct for bacterial degradation of nitrogen compounds during the experiments another 12,57% should be added to this. The corrected total excretion of 20 prawns over 60 days therefore is 5029,31 mg N. Thus the percentage nitrogen

$$\begin{aligned} \text{loading due to prawn excretion is } &\frac{5029,31}{12096,0} \times \frac{100}{1} \\ &= 41,58\% \end{aligned}$$

Percentage loading due to faecal production of prawns

The regression equation for the faecal production of P.indicus at 28°C (Fig. 20) is

$$y = 1,528x + 10,1761$$

Therefore, the first and last terms can be calculated as above, and are 10,9401 and 12,2881. The total faeces produced by 1 prawn during the 60 day experiment = $\frac{60}{2} (10,9401 + 12,2881)$
 = 696,85 mg dry weight.

For 20 prawns this would be 20 x 696,85
 = 13937,0 mg dry weight faeces
 = 13,937 g dry weight faeces.

From Table 8 the faecal pellets contain 66,3 mg N g dry weight⁻¹.

Therefore the total N produced as faeces during the 60 days

$$\begin{aligned} &= 13,937 \times 66,3 \\ &= 923,837 \text{ mg N} \end{aligned}$$

The percentage nitrogen loading on the system due to faecal production

$$\begin{aligned} \text{is } &\frac{923,837}{12096,0} \times \frac{100}{1} \\ &= 7,64\% \end{aligned}$$

Before being able to calculate the percentage N loading due to excess food the proportion of the total N input bound as prawn flesh is required.

Percentage nitrogen taken up by prawns

The total wet weight increase of the prawns is 20x(4,0-0,5) g.
 = 70 g

A conversion factor worked out from the wet and dry weights of 100 prawns was found to be

$$\text{Dry weight} = 0,2726x \text{ wet weight (S.D. 0,014)}$$

Therefore the total dry weight increase of the prawns is 0,2726 x 70
 = 19,082 g

According to the data presented in Table 8, P.indicus contains

$$125,5 \text{ mg N g dry weight}^{-1}$$

The total N bound as prawn flesh is

$$19,082 \times 125,5 \text{ mg N}$$

$$= 2395,17 \text{ mg N}$$

$$\begin{aligned} \text{Therefore the percentage nitrogen bound as prawn flesh} &= \frac{2395,17}{12096,0} \times \frac{100}{1} \\ &= 19,80\% \end{aligned}$$

Percentage loading on system due to excess food

Nitrogen in the food pellets must either be consumed by the prawns and thus appear in prawn flesh, excretory ammonia or faeces, or it must enter the system as uneaten food. The percentage nitrogen loading due to excess food would then be = % Total N input - (% N biomass of prawns + % excretory N + % faecal N)

$$= 100 - (19,80 + 41,58 + 7,64)$$

$$= 30,98\%$$

Assimilation efficiency of P.indicus

Nitrogen can be used as an indicator to calculate the assimilation efficiency of P.indicus. All N ingested is used either for growth (biomass N), metabolism (excretion N) or is passed through the animal (faecal N).

$$\begin{aligned} \% \text{ Assimilation} &= \frac{\text{biomass N} + \text{excretion N}}{\text{biomass N} + \text{excretion N} + \text{faecal N}} \\ &= \frac{19,80 + 41,58}{19,80 + 41,58 + 7,64} \\ &= 88,93\% \end{aligned}$$

Food conversion ratio of P.indicus

The food conversion ratio of P.indicus may be considered as the ratio of ingested nitrogen to bound nitrogen.

$$\begin{aligned} \text{Food conversion ratio} &= \text{biomass N} + \text{faecal N} + \text{excretory N} : \text{biomass N} \\ &= 69,02 : 19,80 \\ &= 3,49 : 1 \end{aligned}$$

DISCUSSION

A strong linear relationship was found between the total nitrogen excreted and the wet weight of 1-12 g P.indicus at 28°C, 24°C, 20°C and 16°C (Fig. 18). The departure from linearity in the excretion rates at 28°C of larger prawns (Fig. 19), invites some speculation. The excretion rates of large prawns of marine origin is shown to be much higher than estuarine prawns of the same size. This could be due to, (a) marine prawns being more active than estuarine prawns, or (b) an excretion deficiency in large estuarine prawns. As regards this latter possibility, it might be argued that large estuarine prawns are defective in some way, since most penaeids migrate to sea before this size is reached. However, caution must be exercised in the interpretation of this difference between the excretion of large estuarine and marine prawns, since the data on which it is based is very sparse.

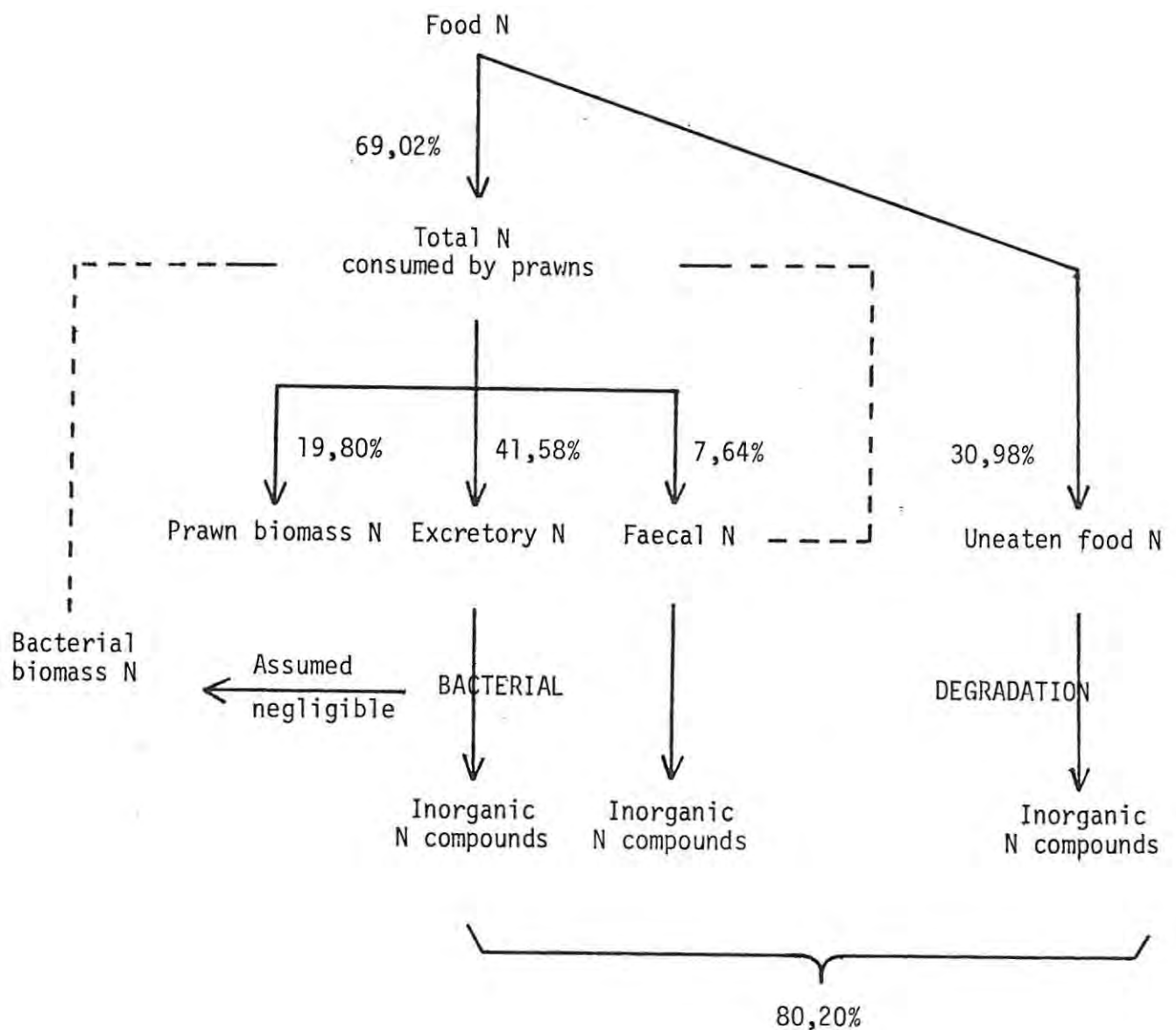
Wickins (1976b) states that except for some unpublished data of J.R.M. Forster, little accurate data on the rates of nitrogen excretion in penaeid prawns exists in the literature. In the absence of more suitable data he estimates that a 5 g prawn may excrete 1,0 mg total $\text{NH}_4^+ \text{-N g}^{-1} \text{ day}^{-1}$. From the results presented in this study (Fig. 18), an excretion rate of 1,2 mg total $\text{-N g}^{-1} \text{ day}^{-1}$ is achieved by a 5 g prawn. Corrected for bacterial degradation during the experiments (Table 7), this would be equivalent to 1,35 mg total $\text{-N g}^{-1} \text{ day}^{-1}$. Since it was found that 72,6% of this total nitrogen was excreted as ammonia at 28°C, this would be equivalent to an excretion rate of 0,98 mg total $\text{NH}_4^+ \text{-N g}^{-1} \text{ day}^{-1}$ for a 5 g P.indicus. The estimated value (1,0 mg total $\text{NH}_4^+ \text{-N g}^{-1} \text{ day}^{-1}$) of Wickins (1976b) corresponds well with this figure.

Faecal production of P.indicus is shown in Fig. 20. As can be expected in faecal production rate determinations for different individuals, the points are somewhat scattered (correlation coefficient $r = 0,72$). The

use of the regression equation in the subsequent calculations should, however, give rise to very little error, since the nitrogen content of P.indicus faeces (Table 8) was found to be very low (66,3 mg-N g dry weight). The low nitrogen content of the faecal material can be explained by the high assimilation efficiency (88,93%) of P.indicus for dietary nitrogen (calculated in this study).

Figures 21 and 22 show that, while Chaetomorpha sp. appears to readily assimilate ammonia ($1,732 \text{ mg NH}_4^+ \text{-N h}^{-1} \text{ g dry weight}^{-1}$), it assimilates nitrite at a much slower rate ($0,26 - 0,3 \text{ mg NO}_2^- \text{-N h}^{-1} \text{ g dry weight}^{-1}$). This probably accounts for the observed failure of the nitrite levels to decrease and stabilize in the algal filtration systems used in Part I (Figs 16c and 17 c and d). Enteromorpha sp. removes nitrate at a rate of $3,67 \text{ mg NO}_3^- \text{-N g dry weight}^{-1} \text{ day}^{-1}$ (Fig. 23). At this rate of removal, it can be calculated that if 450 g wet weight of Enteromorpha sp. were added to the bacterial filtration system in Fig. 17a (Part I), it would take ± 20 days to remove the $30,9 \text{ mg NO}_3^- \text{-N l}^{-1}$ present in the system. This fact would be a point in favour of periodic addition of filamentous algae to bacterial filtration systems, when nitrate levels reach excessive concentrations.

Using the results obtained in the calculations in this part of the study, the various pathways of nitrogen flow in a closed system culture of P.indicus have been quantified (see following page). The most striking aspect of these pathways is the small percentage of dietary nitrogen (19,80%) utilized for the growth of P.indicus. The remaining 80,20% of the total nitrogen introduced as food, constitutes a loading on the biological filtration system used. If not removed by algal filtration, this nitrogen will accumulate in the system as nitrate, thus decreasing the pH and eventually resulting in mortalities. Of this 80,20%, it can be seen that the nitrogen loading due to uneaten food constitutes 30,98%,



three quarters the loading due to the excretion of *P.indicus* (41,58%). This stresses the importance of avoiding over-feeding during closed system culture operations. The experimental results (Fig. 24) show that an established bacterial filtration system can only cope with a certain amount of excess feeding over a short period. Excess feeding for 7 days at rates of 10% and 15% of prawn biomass per day, gave rise to an ammonia loading that was below the maximum carrying capacity of the system, and equilibrium after termination of these higher feeding regimes was attained within a few days. However, a higher feeding rate of 20% of the biomass per day pushed the system beyond its maximum carrying capacity and resulted

in permanently increased ammonia levels.

The following comparisons of the results obtained in this section of the study can be made with similar results in the literature:

1. Other penaeids have been found to have assimilation efficiencies in the range of the 88,93% found for P.indicus. Feeding Metapenaeus benettae Racek and Dall with C¹⁴ labelled bacteria, Moriarty (1976) obtained assimilation efficiencies ranging from 84-96%. Ting (1970 - cited in Wickins, 1976b) found that Penaeus monodon Fabricius has an assimilation efficiency for dietary nitrogen that is > 80%.
2. In this study it was found that 80,20% of the total nitrogen input into the system is available for decomposition, while 19,80% is utilized in prawn growth. If a correlation between nitrogen metabolism and oxygen consumption can be assumed, then these results are remarkably similar to those of Shigueno (1972 - cited in Wickins, 1976b) who found that the micro-organisms in pond water and bottom sand remove 84,2% of the oxygen utilized, while the fish and prawns in ponds account for the remaining 15,8%.
3. The food conversion ratio of 3,49:1 obtained in this study for P.indicus compares favourably with a number of authors quoted by Wickins (1976b) who found typical food conversion ratios for penaeids, fed dried foods at 5-15% of their biomass per day, to range between 1,6:1 and 8:1.

From the above similarities it appears that the assumptions made in this part of the study with regard to the calculations are valid, and that the figures obtained approximate the nitrogen flow characteristics within closed system cultures of penaeid prawns.

GENERAL DISCUSSION AND CONCLUSION

This study has provided information on the excretion rates of P.indicus as related to size and temperature. It has been found by regression analysis that a linear relationship exists between the wet weight of 1 - 12 g P.indicus and the total amount of nitrogen excreted at 16^oC, 20^oC, 24^oC and 28^oC (Fig. 18). Furthermore, it has also been found that the percentage ammonia of the total nitrogen excreted is remarkably constant at 28^oC (72,57%, S.D. 1,41). This information, together with that on faecal production, assimilation efficiency (88,93%) and the food conversion ratio (3,49:1) of P.indicus will be of great value for energetic studies on this animal. The excretion data can also be used in the commercial culture of P.indicus. Once the culturist has determined the optimum nitrogen loading for his culture systems (which can be done by artificially loading the systems with fixed amounts of nitrogen and monitoring ammonia levels), these regression equations can be used to calculate stocking densities of the size of prawns he wishes to culture at any particular temperature between 16^oC and 28^oC.

The calculations concerning the nitrogen cycling within a biological filtration system have demonstrated a few important points as regards closed system culture of aquatic animals. It was found that only a small percentage of the total dietary nitrogen (19,80%) is utilized for the growth of P.indicus, while the remaining 80,20% constitutes a loading on the biological filtration system used. Expressed differently, this may be seen as the total nitrogen loading on the system being equivalent to (Total nitrogen input into the system - Nitrogen assimilated as animal tissue). Realization of this fact enables this information to be implemented in the closed system culture of any animal. By simple determinations of nitrogen content of the culture animal and its food, the nitrogen loading on the system for an expected growth rate can be

calculated. Culture systems can thus be designed to cope with this nitrogen loading.

A further important point has been found by the analysis and quantification of nitrogen flow within these closed system cultures. This is the need to avoid excess feeding. Of the 80,20% nitrogen loading on the biological filtration system, 41,88% was found to be due to excretion and 7,64% to faecal production. These are unavoidable by-products of achieving the growth of P.indicus obtained. The remaining 30,98% comprises the nitrogen loading on the system due to excess food. This is a large percentage of the total dietary nitrogen that is introduced into the system, but nevertheless one that can be minimized by correct culture practices. Overfeeding should be avoided. Furthermore, formulated dietary ingredients should have binding agents that minimize wastage by fragmentation and leaching. It must be realized that this figure is only applicable to P.indicus fed "Higashi Maru" prawn pellets. Penaeids are wasteful feeders and the situation as regards the culture of other aquatic animals could well be entirely different.

In Part I of this study it was concluded that the four biological filtration systems tested offered more or less the same water quality. The only real differences were found to be elevated nitrate levels in the bacterial filtration systems, and elevated inorganic carbon levels in the algal filtration systems. In Part II of the study it was found that no major differences in the growth of P.indicus occurred in the various biological filtration systems tested. The differences in growth that were found in the first experimental trial were not repeated in the second experimental trial. It was concluded that the differences found in the first experimental trial could be attributed to stocking density effects. Thus the growth achieved was a direct reflection of the water quality found in the systems. This is encouraging, since it indicates that the water quality parameters chosen for this study adequately determined the

suitability of the water for growth of P.indicus.

The results obtained in this study, as well as some general observations, indicate that the biological filtration systems used in this work operated satisfactorily. Firstly, the rate of growth of P.indicus in these experimental trials compare favourably with similar data for semi-open systems. Secondly, the ability of the prawns to overcome their shell disease in the second experimental trial, suggests that the water quality was, in general, of a high standard. However, it is felt that these systems could still be improved. Since special attention has been paid to the use of algal filters, their potential will be discussed before improvements are recommended.

The following advantages resulted from the use of algae:

1. Increased levels of inorganic carbon were found in the algal filtration systems in both experimental trials (Figs 13 & 14). Since these increases could not be due to algal decay an alternative explanation was offered. It was suggested that respiratory CO_2 reacts with water and unionized ammonia to produce carbonate and bicarbonate ions as described by Berner (1968). Should this explanation be correct, it would confer a major advantage upon the use of algae for biological filtration purposes. Toxic unionized ammonia would be rendered less harmful, and poor growth and survival due to depressed levels of inorganic carbon (Wickins, 1976a) would be avoided.
2. The accidental copper contamination from condensation moisture in the copper piping of the air reticulation system illustrated a further advantage of algal filtration. The resultant copper levels in the bacterial filtration systems showed a tenfold increase whereas in the algal

- filtration systems this increase did not occur (Fig. 8).
3. The algae also gave rise to elevated oxygen levels in those systems in which it was used (Fig. 10). Bubbles of gas (presumably oxygen) were evolved by the algae during the day, and at no stage did oxygen levels drop below 100% saturation in the algal filtration systems. In the bacterial filtration systems the large oxygen demand by micro-organisms in the gravel bed decreased oxygen concentrations to 92% saturation below the gravel filter (Fig. 9).
 4. Filamentous algae successfully depressed the build-up of ammonia during the establishment of nitrification (Fig. 15).

The most serious disadvantage of algal filtration was the continual loss of condition of the algae. Although filamentous algae used in the second experimental trial initially successfully removed all ammonia, nitrite and nitrate, after 10 days (Fig. 17c and d) ammonia and nitrite began to accumulate in these systems. Furthermore, as the experiments progressed, the algae produced less and less gas bubbles, did not require as frequent harvesting, and decaying sections were noticed. Two reasons can be offered for this loss of condition. Firstly, the lack of mechanical filtration in the algal filtration systems resulted in excess food and faecal material being deposited in the algal trays. Its decomposition was probably the cause of the eventual decay of some sections of the algae. Secondly, the illumination provided in these experiments ($3100 - 3800 \text{ microeinsteins m}^{-2} \text{ sec}^{-1}$) appeared to be insufficient for optimum growth.

A further disadvantage of algal filtration was an apparent antibiotic effect of the algae on nitrifying bacterial populations: In the first experimental trial (Fig. 16a) stabilization levels of nitrite in the bacterial filtration systems were lower than those in the algal plus

bacterial filtration systems (Fig. 16b). In both experimental trials the bacterial filtration systems (Figs. 15 & 17) completed nitrification far sooner than the algal filtration systems.

Considering the above advantages and disadvantages of algal filtration, it appears that the optimum means of biological filtration would be the use of bacterial filtration systems, to which algae are periodically added. In this way the disadvantages of algae (loss of condition of algae; lack of mechanical filtration; inability to completely remove nitrite) could be avoided, while the advantages (nitrate removal; increased inorganic carbon levels; detoxification of unionized ammonia; removal of contaminatory substances such as copper) could still be retained.

Furthermore, when algae is periodically used for filtration purposes, the algal filters should be physically separated from the culture tanks. In this way it will be possible to supply the algae with continuous lighting or even full sunlight, while the cultured animals can be supplied with whatever light regime is optimal for their growth. This improved system could be operated for at least a period of two months without any major changes in water quality. After two months, however, replacement sea water should be added. In the discussion in Part III it was calculated that a two month accumulation of nitrate, (such as was found in Fig. 17a), could be removed by 450 g wet weight Enteromorpha sp. in ± 20 days. Algal additions should therefore be restricted to 3 weeks every two months.

A system such as this would enable the culturist to obtain good growth results as well as provide him with all the advantages of closed system culture, such as:

1. Independence from natural fluctuations in water quality.
2. Independence from environmental pollution.
3. Saving on labour.

4. Saving on heating costs (closed systems only require temperature maintainance as opposed to open and semi-open systems where energy is required to heat all sea water additions from ambient to 28⁰C).
5. Close control on feeding, harvesting and disease.

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