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D. S. Heales

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G.P.O. BOX 1538, HOBART, TAS. 7001, AUSTRALIA

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Water Quality Changes during the Conditioning of Small, Closed Seawater Systems

D.S. Heales

Division of Fisheries Research
CSIRO Marine Laboratories
P.O. Box 120, Cleveland, Qld 4163

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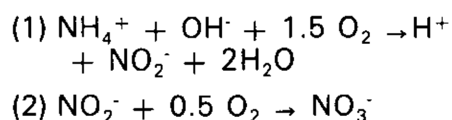
Abstract

Mineralisation and nitrification processes were monitored during the conditioning period of replicate recirculating seawater systems A and B. High quality water was achieved by a combination of bacteriological and mechanical filtration, activated charcoal adsorption, foam separation and U.V. sterilisation. These two systems were conditioned over a 12-week period using *Penaeus merguiensis* as conditioning animals. Detritus from faecal matter and food increased ammonia levels immediately. At two weeks, nitrite levels increased and ammonia fell. Nitrite levels remained high until week 8 in system A and week 10 in system B. Nitrate levels rose slowly throughout the experiment. After conditioning, nitrogen levels in both systems remained well below 0.1, 1.0 and 50 mg l^{-1} for ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen respectively, the upper safe limits at which growth and fecundity of tropical prawns are not affected. Increasing nitrate-nitrogen levels present problems for long-term experiments and some water replacement is necessary. Little variation in dissolved O_2 and pH levels was observed during the conditioning period.

Introduction

Small recirculating seawater systems are often used in experimental situations where fine control over temperature and salinity is necessary. The main problem with these systems, however, is that waste food and animal excretory products rapidly accumulate and the compounds that are formed adversely affect animal growth, fecundity and resistance to disease (Spotte, 1970, 1979). Ammonia and nitrite are the most toxic compounds. Mineralisation, the first stage in this process, is the breakdown of nitrogenous organic compounds to inorganic substances by heterotrophic bacteria. Ammonia is the main end product and exists in two forms: highly toxic (ammonia) and the less toxic ammonium ion (NH_4^+). The percentage of each form depends primarily on pH with the ammonium ion predominant. The next stage, nitrification, is the biochemical oxidation of ammonia to nitrate. Ammonia is oxidized to nitrite by autotrophic bacteria, mainly *Nitrosomona spp*. Nitrite is then oxidised to nitrate by a second bacterial group, *Nitrobacter spp*. These nitrification reactions have been summarised by Spotte (1979):



(1) $NH_4^+ + OH^- + 1.5\ O_2 \rightarrow H^+ + NO_2^- + 2H_2O$

(2) $NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$

Once a system is conditioned, both processes occur simultaneously.

Wickins (1976) found that the growth rate of penaeid prawns was reduced by 1-2% after exposure to 0.1 mg l^{-1} ammonia-nitrogen and that of *Penaeus*

indicus was reduced by 50% after exposure to 6.4 mg l^{-1} nitrite-nitrogen. No growth rate reduction was found in *Penaeus monodon* after 3-5 weeks of exposure to 200 mg l^{-1} nitrate-nitrogen. The safe limits found for inorganic nitrogen compounds were 0.1, 1.0 and 50 mg l^{-1} for NH_3-N, NO_2-N and NO_3-N respectively (Wickins and Beard, 1978). The aims of the present experiment were to monitor the changes in water quality that occurred while establishing a recirculating seawater system and to determine the suitability of the system for culturing juvenile penaeid prawns.

Materials and Methods

Two identical water reticulation systems (systems A and B) were constructed in the laboratory. The total capacity of each system was 450 l of which 300 l (67%) were available for prawn culture. The remaining 150 l was in transit through the water treatment section at any instant. All plumbing was PVC. Culture tanks were rectangular polythene of 25 l or 7 l capacity. Each water treatment system consisted of a bacterial filter, a granulated charcoal filter, a protein skimming tower, a U.V. sterilising light and two glass-wool filters.

Each bacterial filter consisted of a 25 l polythene tank in which P.V.C. pipe offcuts provided approximately 3.25 m^2 surface area for bacterial growth. The carrying capacity of the bacterial filter was calculated from excretion, nitrification and hydraulic loading rates (Wickins and Beard, 1978). The capacity was doubled to ensure safety within the expected animal load limits. A flow rate of 25 l h^{-1} recycled the total volume every 18 hours.

One litre of activated charcoal pellets per system provided approximately 0.4 m^2 adsorption surface area inside a filter jacket. The charcoal was removed from system B after 30 days due to charcoal dust clogging aerator nozzles. A 0.5 m protein skimmer was installed before the main header tank in order to strip and separate excess proteins remaining in the water. An ultra-violet light source was fitted immediately after the main header tank in order to reduce bacterial numbers. No metal parts were used inside the system. Water lost through evaporation was replaced by glass-distilled water.

Water samples for NH_3, NO_2 and NO_3 analysis were taken from each system at least weekly and analysed as follows. Total ammonia-nitrogen ($NH_3 + NH_4^+$) levels were estimated by indophenol blue colour absorption at 640 nm as described by Dal Pont *et al.* (1974) and recorded as total NH_4-N mg l-1 (Wickins and Beard, 1978). NO_2-N levels were estimated by a modified Strickland and Parsons (1968) method as described by Major *et al.* (1972). Sulphanilamide was used to diazotize the nitrite ion (NO_2^-), which then coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride. The colour formed absorbed strongly at 543 nm. NO_3-N levels were determined by cadmium reduction to nitrite and standard nitrite estimation (Major *et al.*, 1972). All colour absorptions were measured by spectrophotometrical methods. Temperature, pH and dissolved O_2 levels were also measured every week. O_2 levels were measured from three sites using a standard laboratory automatic analyser (see Figure 1). pH was measured on a digital pH meter.

On day 0, 200 juvenile *Penaeus merguiensis* (total 100-150 g wet wt and C.L. 5-10 mm) were added to each system. These animals provided a conditioning load only and mortalities were not recorded. Dead animals were replaced to maintain a constant animal load on the bacterial filter. The prawns were fed once daily to excess with commercial dried prawn food pellets. Leftover food and faecal matter were removed before each feeding period.

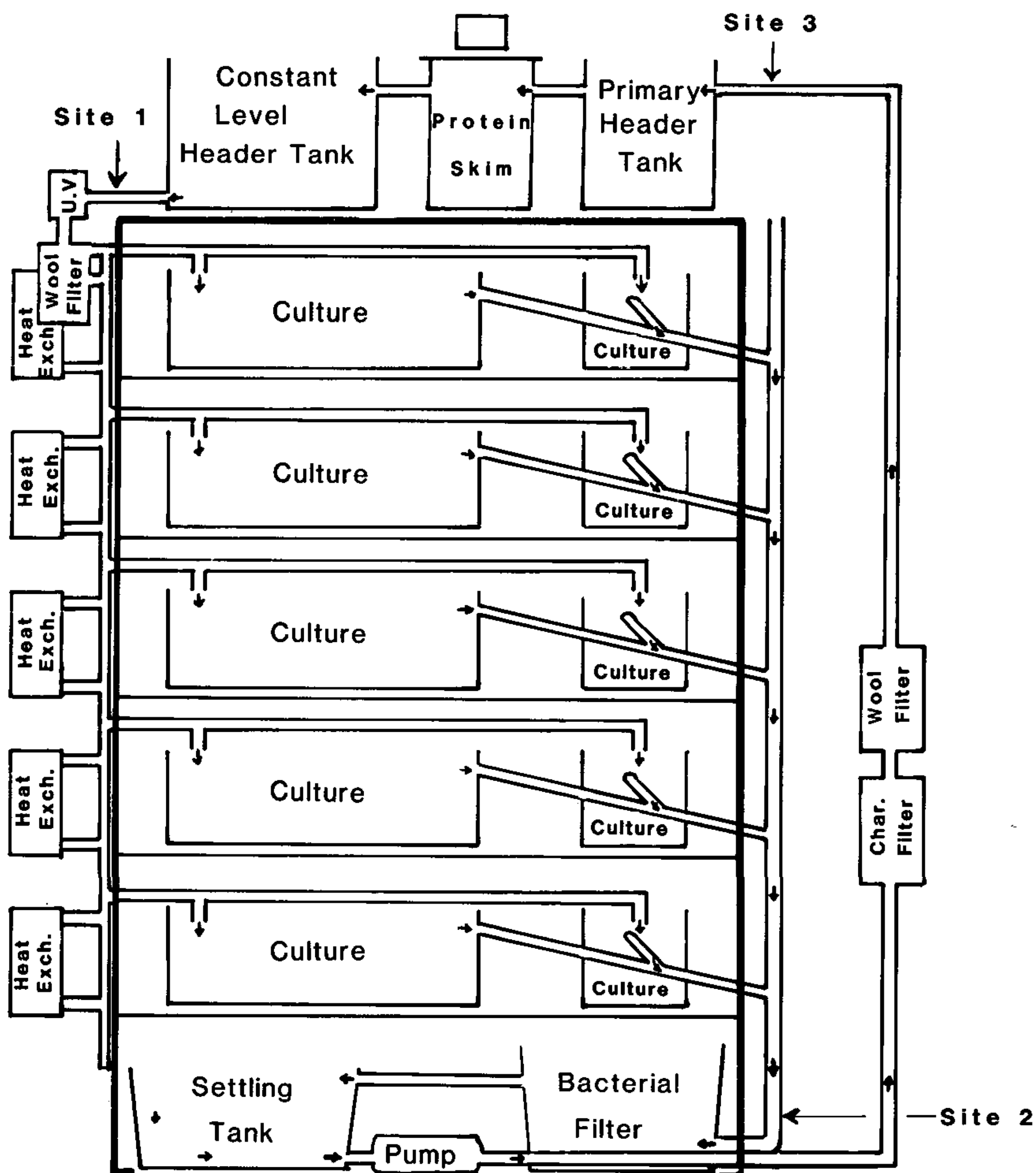


Figure 1. One of the closed recirculating seawater systems used in the experiment. Sites 1, 2 and 3 indicate sampling points for dissolved oxygen

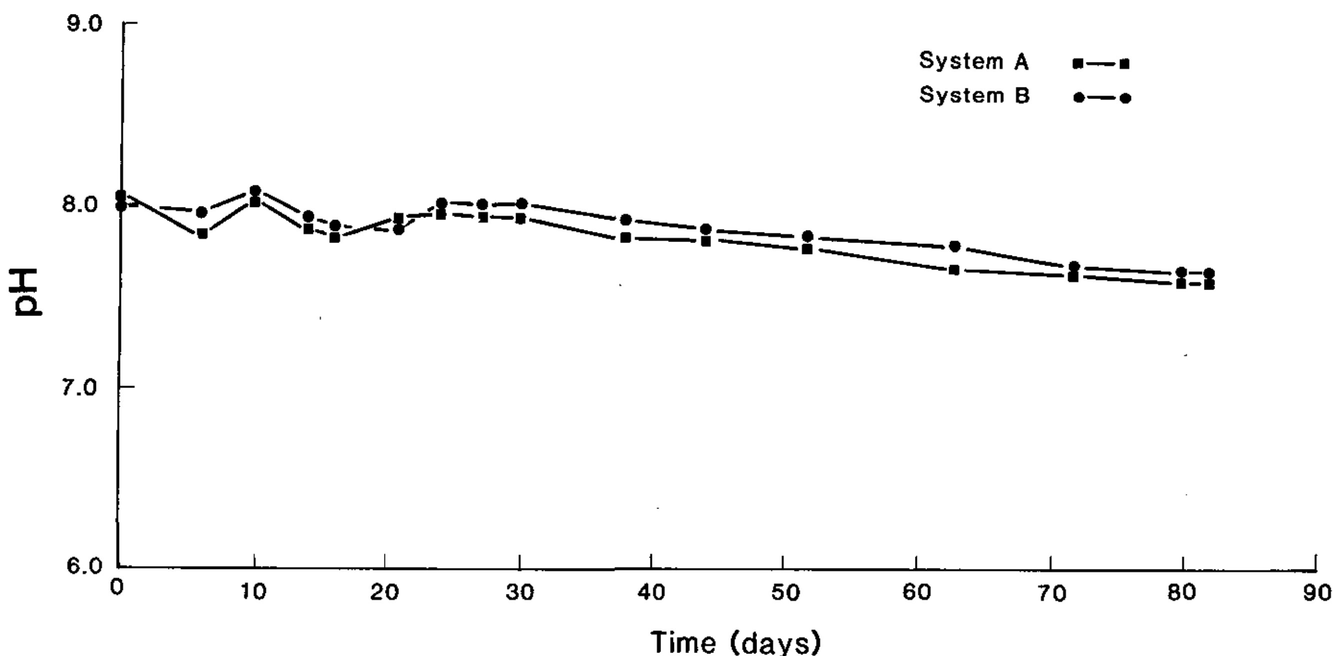


Figure 2. Changes in pH during the conditioning of closed seawater systems

Results

Temperature varied slightly from 26°C (± 1.5°C). A slight variation in pH levels was seen in both replicates (Figure 2). The decrease from 8.0 to 7.69 was due to an acid build-up from the nitrification processes.

Dissolved oxygen saturation percentages at three sites are shown in Table 1. The highest was 100% at the culture tank influent water. The lowest recording was 94% at site 3, before the header tank in system A.

Total ammonia-nitrogen rose rapidly over the first 13 days to reach 4.0 and 4.5 mg l^{-1} (systems A, B respectively) then fell dramatically as nitrifying bacteria populations stabilised (Figure 3). Total ammonia levels then remained at or below 0.1 mg l^{-1} for the remainder of the experiment. From the observed pH range of

Table 1. Percentage saturation of dissolved O_2 at three sites in replicate systems. (SD = standard deviation).

Sites	System A		System B	
	O_2 (%)	SD	*O_2 (%)*	SD
1. U.V. light	98	0.171	100	0.083
2. Bacterial filter	97	0.131	98	0.131
3. Primary header tank	96	0.099	97	0.138

8.1 to 7.69, the level of toxic NH_3-N was calculated to be between 0.005 and 0.01 mg l^{-1}.

As ammonia oxidising populations of bacteria became established, nitrite levels rose steadily (Figure 4) until day 42, (8.1 mg l^{-1}) in system A, and day 48, (11.8 mg l^{-1}), in system B. A 24 h pump

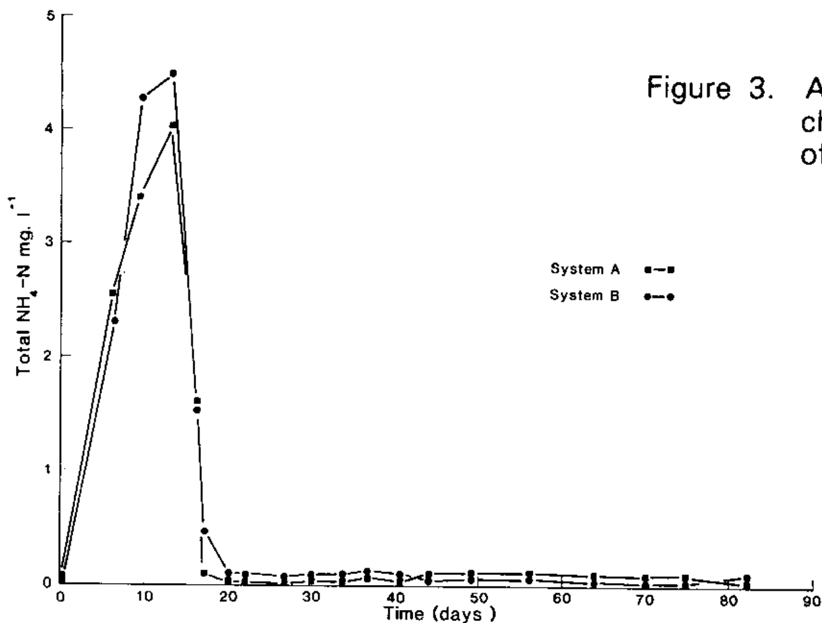


Figure 3. Ammonia-nitrogen concentration changes during the conditioning of closed seawater systems

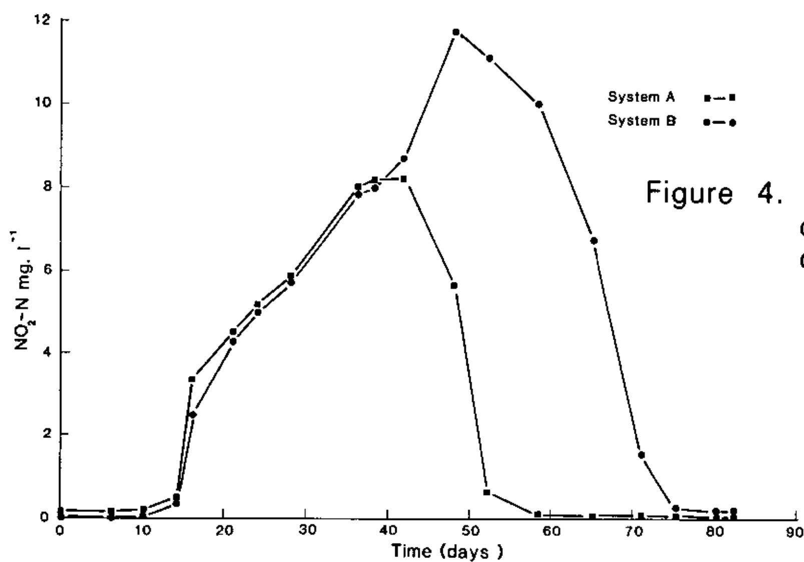


Figure 4. Nitrite-nitrogen concentration changes during the conditioning of closed seawater systems

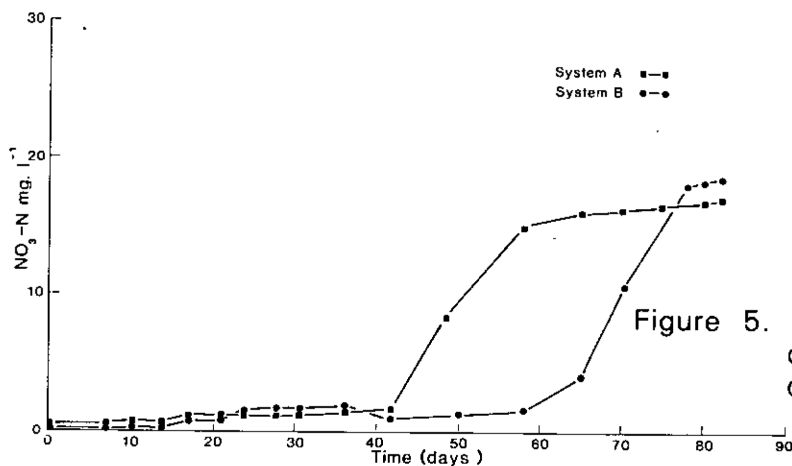


Figure 5. Nitrate-nitrogen concentration changes during the conditioning of closed seawater systems

failure on day 43 in system B was the most likely cause of the discrepancy between systems. System B lagged behind A for the rest of the experiment. Nitrite fell below 0.5 mg l^{-1} by day 58 and day 75 in systems A and B respectively.

Nitrate levels (Figure 5) rose slowly from the start of the experiment, indicating some activity of nitrite oxidising bacteria. From day 42 and day 58 in systems A and B respectively, the *Nitrobacter spp.* population became firmly established and levels climbed steadily to 15-18 mg l^{-1} before levelling out after 58 days in system A and 78 days in system B. Nitrate thereafter increased slowly at a rate of approximately 0.5 mg week^{-1} until the conclusion of the experiment at 82 days. Final levels of 16.8 and 18.6 mg nitrate-nitrogen in systems A and B respectively were well within the safe limits of 50 mg l^{-1}.

Discussion

The system described is still in experimental stages. Contact time between water and activated charcoal is limited to when the pump is forcing water through the pellets. Relocation to a position where gravity feed slows the passage of water would ensure more efficient adsorption.

Estimates of major cation concentrations were omitted because Gerhardt (1978) found that no significant changes occurred in Na^+, K^+, Ca^{++} and Mg^{++} levels in similar closed systems over a period of 70 days. He also noted no significant changes in trace element concentrations. Wickins (1976) found decreases in inorganic carbon levels due to nitrification processes. Levels of inorganic carbon less than 10-12 mg l^{-1} may be lethal to prawns. Gerhardt (1978), on the other hand, found that concentrations of inorganic carbon never fell in a 65-day period to a level where they could be lethal to prawns.

Suitable culture water was available after 58 days in system A and 75 days in system B. NH_3-N and NO_2-N levels in both systems were below the maximum safe limits of 0.1 and 1.0 mg l^{-1} after the conditioning period. Increasing nitrate levels of 0.5 mg l^{-1}.week^{-1} presented no problems to short term experiments of up to 20 weeks. Water replacement of 10% each week is recommended for longer experiments.

Acknowledgements

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TASMANIAN LABORATORY
Castray Esplanade, Hobart, Tas
G.P.O. Box 1538, Hobart, Tas 7001

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233 Middle Street, Cleveland, Qld
P.O. Box 120, Cleveland, Qld 4163

WESTERN AUSTRALIAN LABORATORY
Leach Street, Marmion, WA
P.O. Box 20, North Beach, WA 6020

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