Macroalgae as biofilters of dissolved inorganic nutrients in an integrated mariculture tank system in Zanzibar

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ABSTRACT

A laboratory experiment was conducted to determine the capacity of the macroalgae *Eucheuma denticulatum*, *Gracilaria crassa* and *Ulva reticulata* to remove dissolved inorganic nutrients from finfish effluents in serially arranged tank systems. The aim was to determine the capacity of macroalgae which could be used in an integrated maricultre pond system at Makoba, Zanzibar. Seawater from the reservoir entered the finfish tanks first, then drained through plastic pipes into the shellfish units and finally into the macroalgae units before being discharged back into the sea. The water residence time in each tank was 4 days. The performance of each of the different units was assessed in terms of total ammonia-N, phosphate-P and nitrate-N produced by finfish, and then, the removal of these nutrients by shellfish and macroalgae biofilters. The levels of dissolved inorganic nutrients were highest in the finfish units and lowest in the macroalgae units. Increase in stocking density of macroalgae from 1g/l to 2g/l was related to their increase in daily growth rates, specific growth rates and inorganic nutrient uptake rates. However optimum stocking densities were not reached. Of the three inorganic nutrients, ammonia-N and phosphate-P was taken up by the macroalgae much more than nitrate-N. Ammonia-N removal efficiency in the system reached a maximum of about 63% at 2g/l of *Ulva reticulata* whereas, phosphate-P and nitrate-N maximum removal capacity were 58 and 54% respectively. Among the three species of macroalgae, *Ulva reticulata* seems to be the most efficient biofilter. It is concluded that locally available macroalgae are good bioregulators of water quality, thus can be applied in small scale mariculture activities and can also generate income.

INTRODUCTION

Mariculture possesses great potential for protein production worldwide. However, the development of mariculture in Africa has experienced several failures including low

human capital which prevent people from going into fish farming due to low output per work day (Christensen, 1995). Land-based mariculture development has also caused environmental problems including salinisation of terrestrial environments, and pollution downstream originating from fish effluents when discharged without prior treatment. Fish grown in mariculture systems retain only 20 to 30% of the food supplied as body flesh (Porter et al., 1987; Hall et al., 1992). The remaining 70 to 80% is excreted in two major forms: (1) as dissolved nutrients, and (2) as particulate organic matter. Microbial degradation of leftover food, fish faeces and fish excretion has been reported to cause several pollution problems within the culture media and outside, such as reduction of oxygen content and algal blooms (Bell et al., 1989).

Previous studies have used phytoplankton to reduce excess dissolved inorganic nutrients from fish effluents (Gordin et al., 1981; Krom and Neori, 1989). However, the phytoplankton are subject to uncontrollable blooms that are likely to cause changes in water quality, particularly in terms of ammonia and dissolved oxygen (Krom et al., 1985).

Therefore the development of sustainable integrated mariculture is vital if we are to feed an ever-growing population with minimum damage to the environment. Ryther et al., 1975 suggested the use of seaweeds as biofilters for the first time. Following their work, several studies have reported that, submerged macroalgae such as *Ulva lactuca* can efficiently remove dissolved nutrients from fish effluents (Neori et al.,1991; Cohen and Neori, 1991; Vandermeulen and Gordin, 1990). Such seaweeds (macroalgae), cannot only remove dissolved inorganic nutrients, but they also produce a biomass which could be used in several ways (Kissil et al., 1992; Arieli et al., 1993; Neori et al., 1996; Haglund and Pedersen, 1993). In this study we proposed to investigate the performance of the macroalgae *Eucheuma denticulatum* (Rhodophyta), *Gracilaria crassa* (Rhodopyta) and *Ulva reticulata* (Chlorophyta) in removing ammonia-N, phosphate-P and nitrate-N in an integrated mariculture tank system. This was attempted through a culture of rabbitfish (*Siganus sutor*) in tanks connected to shellfish (*Pinctada magartifera*) and the macroalgae.

MATERIALS AND METHODS

The experiment was conducted in the green house at Mazizini from 5 December 1998 to 2 April 1999. Mazizini is situated on the western coast of Zanzibar, south of Zanzibar town (Figure 1).

Experimental systems

Two set ups were investigated. In the first *Ulva reticulata* and *Gracilaria crassa* were studied in a system comprising six 400 litre plastic tanks with a surface area of $1m^2$ each and one 500-litre tank as a seawater reservoir. The six tanks were connected in two series of three tanks each. Each series had (1) a finfish tank (FF), (2) a shellfish tank (SF) and (3) a seaweed tank (SW) containing either *Gracilaria crassa* (GC) or *Ulva*

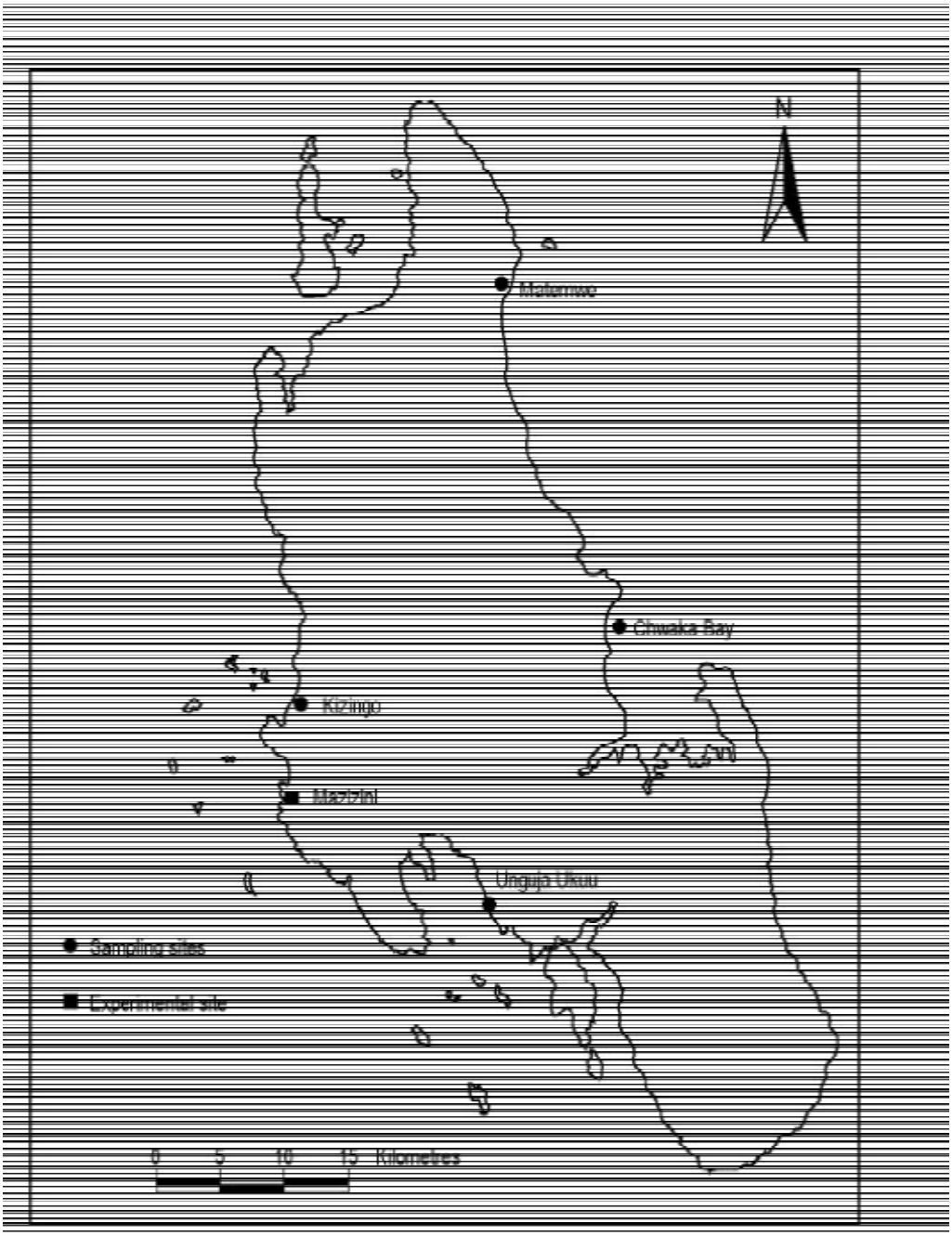


Figure 1. A map of Zanzibar showing areas of sample collection and site where laboratory experiments were conducted

reticulata (UR). The tanks were arranged as follows; Reservoir ▬ Finfish ▬ Shellfish ▬ Macroalgae. The first series consisted of FF^1, SF^1 and GC, while the second series consisted of FF^2, SF^2 and UR. In the first and second series conducted between 5 December 1998 to 4 February 1999, the GC and UR stocking densities were 1g/l (i.e. 1g fresh weight of macroalgae per litre of effluent). From 8 February 1999 to 16 March 1999 the stocking densities were 2g/l.

In the second set up *Eucheuma denticulatum* (ED) was studied in the third series from 1 February 1999 to 2 April 1999. In this series, four tanks, two of about 800 litres and two of 400 litres were used. The two 800 litre tanks were used for FF^3 and SF^3 and the two 400-litre tanks were stocked with 1g/l and 2g/l of ED respectively. The effluent from FF^3 was discharged into SF^3 systems, and from the latter, half of the effluents were discharged into a tank with an ED density of 1g/ l and the remaining half was directed into a tank with an ED density of 2g/ l. The superscripts 1, 2 and 3 above FF and SF refer to the first, second and third series of experiments. As in series 1 and 2, the reservoir (R) supplied seawater to FF^3.

A control experiment was carried out to determine nutrient loss by physical and/ or microbial processes. The effluents with known concentrations of inorganic nutrients were subjected to three aerated plastic buckets without macroalgae. The amount of nutrient lost was determined from samples collected concurrently from experimental setups in series 1, 2 and 3. The water retention time in each series was four days. Aeration of the experimental tanks was achieved using air pumps.

Experimental materials

Macroalgae

The macroalgae *Ulva reticulata*, *Gracilaria crassa* and *Eucheuma denticulatum* were collected from Kizingo beach, Chwaka Bay and Matemwe respectively. They were transported to the laboratory in 20-litre plastic containers filled with seawater.

Finfish

Fingerlings of *Siganus sutor* were collected along the shore near the Institute of Marine Sciences (IMS), Zanzibar. The collection was done using small beach seine net during low tide. The collected fingerlings were kept in aerated tanks at the IMS for two days before being transferred into 40-litre plastic buckets. Tanks FF^1 and FF^2 were stocked with 20 fingerlings with mean body weight of 5g and FF^3 was stocked with 40 fingerlings. The finfish were fed twice a day at 0830 and 1830 hours, with 5% of their body weight. Their diet was made from a combination of *Ulva* sp. and sardines (1:1 w/w).

Shellfish

Individuals of the bivalve *Pinctada margaritifera* were collected from Unguja Ukuu situated on the west coast of Unguja Island. The animals were brought into the laboratory in 40-litre plastic buckets full of seawater. Tanks SF^1 and SF^2 were stocked with 2kg of shellfish regardless of the differences in shellfish body weight. 4kg of the shellfish were

stocked SF[3]. It was difficult to properly clean the shellfish tanks as this could disturb the shellfish attachment. As a result, opportunistic photosynthetically active flora particularly cyanobacteria (*Lyngbya* sp.) were found growing on the insides of the tanks.

Data collection and sampling

Temperature, light, salinity and pH were measured 3 times during the day, using a thermometer, light meter, hand-held refractometer and a pH meter respectively. The means of the three readings for each parameter were calculated.

Water samples for dissolved inorganic nutrient analysis were taken after every four days. The samples were filtered through a 25mm glass-fibre filter. 10 ml of water samples for ammonia-N and phosphate-P were taken in triplicate and duplicate respectively and stored in 20ml acid-washed vials. About 100ml were sampled for nitrate-N analysis. All samples except those for nitrate-N were analysed after a maximum of four days. Analysis was done as described in Parson's et al. (1984).

Survival of finfish as percentage of the initial stock, body weight and length were determined monthly. The survival was determined using the formula below:

$$\text{Survival (\%)} = (N_f/N_i) * 100 \quad (1)$$

where N_i = number of fingerlings at stocking time, N_f = number of fingerlings during weighing time. Weighing balance and measuring board were used to estimate fish weight and length respectively. Half of the finfish were measured and their mean weight and length were recorded. Ectoparasites observed on the body of live and dead fish were collected and preserved in 5% formalin for subsequent identification. The finfish tanks were then restocked. 5% potassium permanganate solution was used to clean the tanks so as to eliminate ectoparasites.

Survival of shellfish as percentage of the initial stock (total weight) were determined using the following formula:

$$\text{Survival (\%)} = ((W_f/W_i) * 100) \quad (2)$$

where W_i = initial weight during stocking time and W_f = final total weight at time of weighing.

Macroalgae wet weight was determined weekly using a weighing balance and returned to the tanks. Daily growth rate (DGR) was calculated using the formula below (Lignell et al., 1987):

$$DGR = [(W_t/W_0)^{1/t}-1]*100 \quad (3)$$

where W_0 and W_t are initial biomass and final biomass at day t, respectively.

The dissolved inorganic nutrient uptake rates were determined as a loss of that nutrient in the culture medium using the formula below modified from Krom et al. (1985):

$$V = (S_0 - S_1) \quad (4)$$

where V = uptake rates (μg-at.l/day), S_0 = inflow nutrient concentration and S_1 = ouflow nutrient concentrations.

RESULTS

Physical and chemical factors

The temperature ranged between 25°C in the morning to 27°C in the afternoon. A maximum mean irradiance of 32μ photon m^2/s was recorded at the surface and 18μmol photon m^2/s was recorded at the bottom of the containers.

The salinity ranged between 35.6 and 36.2‰ in the reservoir and between 38.0 and 38.7‰ in macroalgae systems. pH values of 8.5 to 8.6 were measured in *Eucheuma denticulatum*. In *Ulva reticulata* the pH varied from 8.2 to 8.3 and pH values of 8.3 to 8.4 were recorded in *Gracilaria crassa* systems.

Shellfish and finfish growth and survival

The mean body weight of finfish (FF^1, FF^2 and FF^3) ranged from 10.8–18.4g and their length ranged from 7.9–12.6cm. The weight and length of shellfish were not determined. The survival of shellfish and finfish were about 65 and 70% respectively.

Dissolved inorganic nutrient concentrations

Figures 2a–2c, 3a–3c and 4a–4c show the mean concentrations of ammonia-N, phosphate-P and nitrate-N. The concentrations in the finfish effluents gradually decreased as the effluents flowed to the macroalgae tanks via the shellfish tanks at all stocking densities of macroalgae. In all units, the concentration of ammonia-N and phosphate-P were higher in the finfish tanks. The lowest concentrations were recorded in the macroalgae biofilter tanks. The minimum mean concentrations recorded in the macroalgae were 26.2 (36.5%) μg-at.N/l for ammonia-N, 17.2 (41.7%) μg-at.P/l for phosphate-P and 0.34 (44.4%) μg-at.$N0_3$-N/l of nitrate-N.

Reduction and uptake rates of nutrients by shellfish and macroalgae

The percentage of ammonia-N reduction reached a maximum of 63% at 2g/l of *Ulva reticulata*. For phosphate-P, the maximum reduction was 58% at 2g/l of *Ulva reticulata*. Maximum reduction of nitrate-N was 56% at 2g/l of *Gracilaria crassa*. The reduction of dissolved inorganic nutrients in the control experiments were 0.5, 0.3 and 0.2% for ammonia-N, phosphate-P and nitrate-N respectively. The uptake rates of dissolved ammonia-N, phosphate-P and nitrate-N by macroalgae were found to increase with increase in stocking densities. Higher uptake rates of 7.4μg-at.N/l/day for ammonia-N was recorded in *Ulva reticulata* at 2g/l followed by 7.0μg-at.N/l/day recorded in *E. denticulatum* at 2g/l . Phosphate-P taken at 2g/l of *U. reticulta* reached a rare value of up to 4.12μg-at.N/l/day. The highest nitrate-N uptake rates of 0.11μg-at.$N0_3$-N/l/day

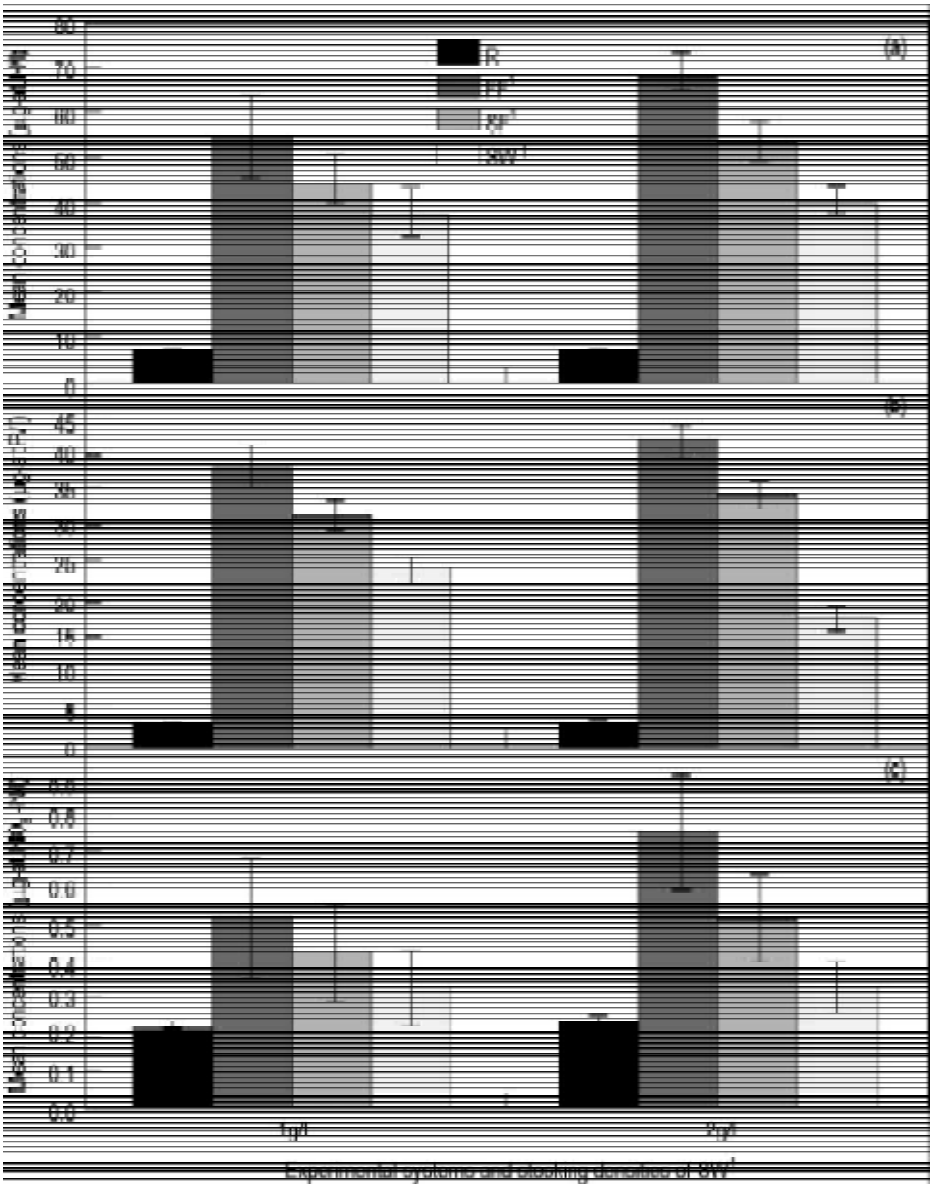


Figure 2a. Mean concentration of ammonia-N from 5/12/98–16/3/99

Figure 2b. Mean concentration of phosphate-P from 5/12/98–12/3/99

Figure 2c. Mean concentration of nitrate-N from 5/12/98–12/3/99
R = reservoir, FF = finfish, SF = shellfish, SW = *G. crassa* and 1 = series number

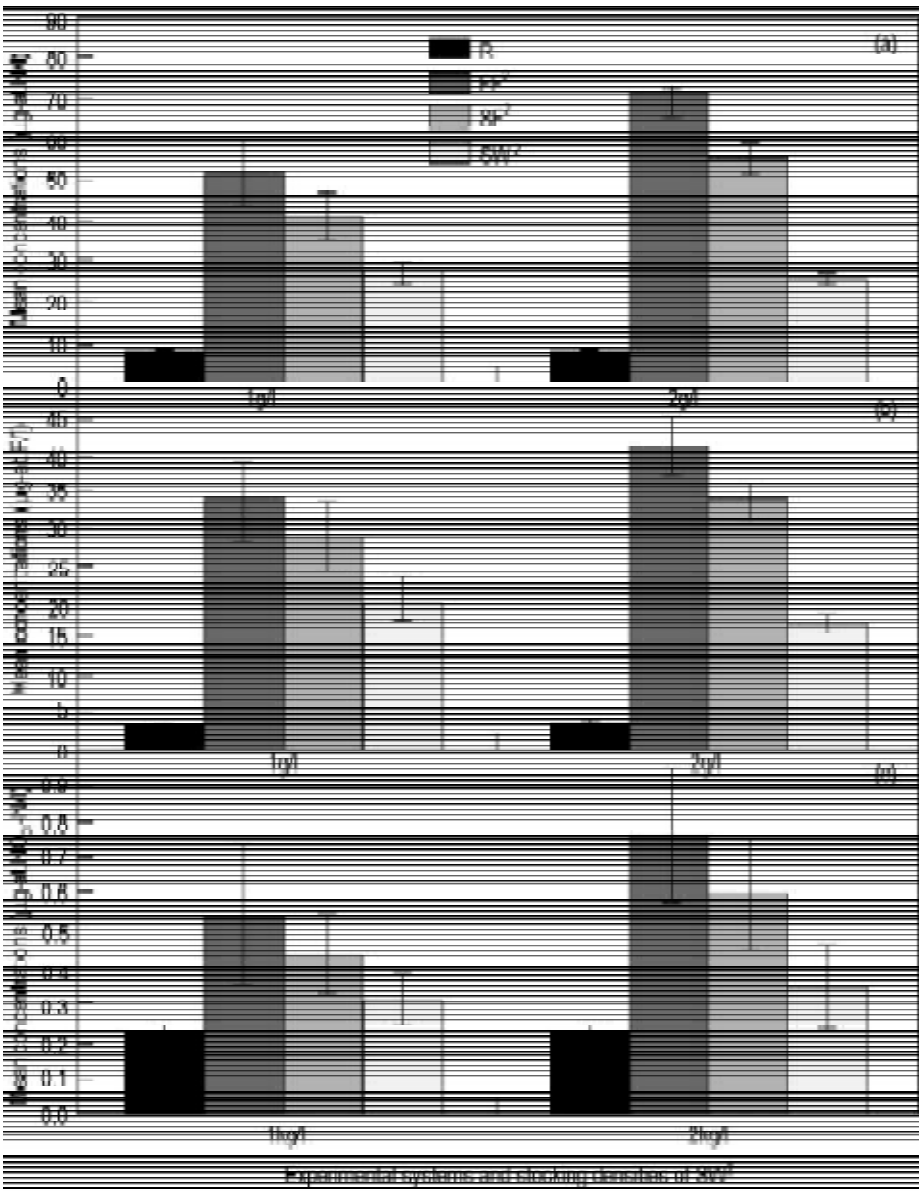


Figure 3a. Mean concentration of ammonia-N from 12/8/98–16/3/99

Figure 3b. Mean concentration of phosphate-P from 8/12/98–12/3/99

Figure 3c. Mean concentration of nitrate-N from 5/12/98–12/3/99
R = reservoir, FF = finfish, SF = shellfish, SW = *U. reticulata* and 2 = series number

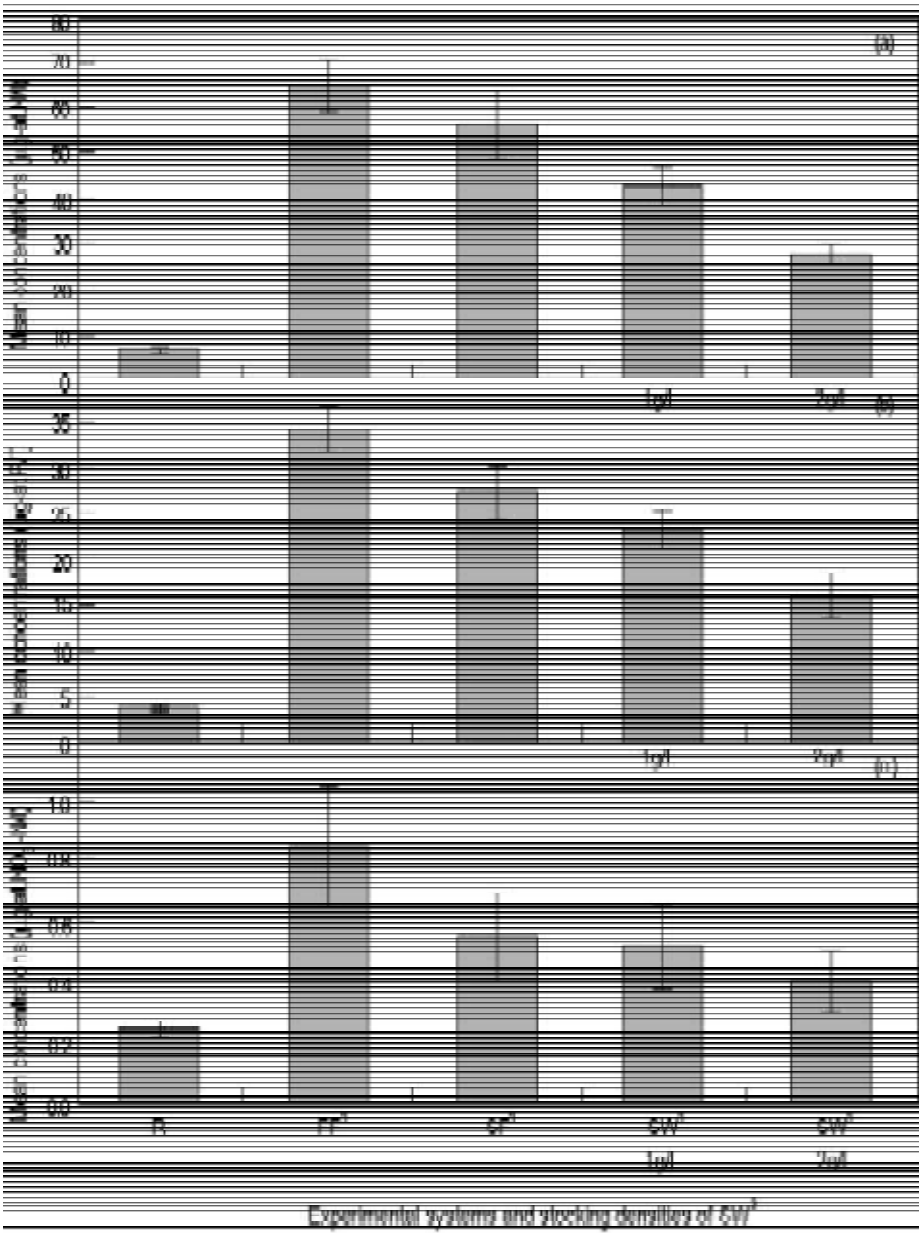


Figure 4a. Mean concentration of ammonia-N from 1/2/99–2/4/99

Figure 4b. Mean concentration of phosphate-P from 1/2/99–2/4/99

Figure 4c. Mean concentration of nitrate-N from 1/2/99 –2/4/99
R = reservoir, FF = finfish, SF=shellfish, SW= *E. denticulatum* and 3 = series number

was recorded in *E. denticulatum* at 2g/l. The contribution of shellfish systems in both uptake rates and percentage reduction was probably due to the presence of cyanobacteria rather than to the shellfish themselves.

Growth of macroalgae

Table 1 shows the mean daily growth rate of *E. denticulatum*, *Ulva reticulata* and *G. crassa*. The mean daily growth rates (DGR) and dissolved inorganic nutrient uptake were found to be linked to the stocking densities. The maximum mean DGR was recorded in *U. reticulata* at a stocking density of 2g/l followed by *E. denticulatum* also at 2g/l, whereas, the minimum mean DGR was recorded in *G. crassa* at 2g/l. On some days there was a loss of wet weight of macroalgae, possibly due to fragmentation, bleaching and grazing by amphipods.

Table 1. Daily growth rates (DGR) of *Ulva reticulata* (SW^2), *Eucheuma denticulatum* (SW^3) and *G. crassa* (SW^1) at 1g/l and 2g/l stocking densities

	DGR	
Macroalgae species	1g/l	2g/l
Ulva reticulata (SW^2)	5.52	8.27
Eucheuma denticulatum (SW^3)	5.32	5.96
Gracilaria crassa (SW^1)	3.44	3.52

DISCUSSION

The concentrations of dissolved inorganic nutrients recorded in the reservoir were very low compared to those recorded in the experimental tanks, and were thus neglected. The main source of dissolved inorganic nutrients into the system was from the food for the cultured fish. Food given to cultured fish can give rise to inorganic nutrients through fish metabolites and leftovers which could be degraded by microbial activities (Krom et al., 1989; Neori et al., 1989).

The low concentrations of inorganic nutrients observed in the macroalgae systems was possibly due to uptake by macroalgae. The intermediate concentrations in the shellfish tanks were probably due to uptake by microphytes especially cynobacteria. It was difficult to properly clean the tanks with shellfish as this could disturb the shellfish attachment. As a result, opportunistic photosynthetically active flora such as *Lyngbya* sp. grew on the sides within the tanks.

The highest growth rates were recorded in *Ulva reticulata* followed by *Eucheuma denticulatum* and *Gracilaria crassa* at the same stocking densities. This may be attributed to high surface area of *Ulva reticulata*. The differences in the ratio of surface area to volume (S:V), may lead to differences in nutrient uptake rates among macroalgae (Rosenberg and Ramus, 1984; Cohen and Neori, 1991). Although the macroalgae were

grown at a low irradiance their high ability for photoacclimation (Vandermeulen, 1989) might have contributed to the high growth rate and nutrient uptake rates. It is also possible that light limitation had negative effects on growth rates and nutrient uptake rates.

The low percentage reduction of ammonia-N, phosphate-P and nitrate-N observed in the control experiments compared to those recorded in tanks with macroalgae show that the macroalgae biofilters are responsible for inorganic nutrient reduction in the systems.

The finfish mortality recorded during this study was probably due to infection by ectoparasites found on the body of live and dead fish. The mortality of shellfish was probably due to culturing conditions as they were completely immersed in the effluents most of the time except during effluent exchange. Such condition might have interfered with the normal rhythms of their physiological processes. In the marine environment, the shellfish are found in the intertidal areas where they are exposed during the low tide and submerged during the high tides almost twice per day. The survival of shellfish probably can be improved by allowing them to receive a splash of seawater as in the natural environment.

CONCLUSIONS

The macroalgae used in this study can efficiently remove dissolved inorganic nutrients from fish effluents, and their biofiltration capacities increased with increase in stocking density of macroalgae. Among the three species of macroalgae, *Ulva reticulata* showed the highest nutrient uptake rate and high growth rates. Thus, the results from this study can be applied in developing a small-scale wastewater treatment pond system.

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