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Ulva reticulata and *Gracilaria crassa*: Macroalgae That Can Biofilter Effluent from Tidal Fishponds in Tanzania

Flower E. Msuya[1,2] and Amir Neori[2]

[1]*Department of Plant Sciences, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel;* [2]*The National Centre for Mariculture, Israel Oceanographic and Limnological Research, P.O. Box 1212, Eilat 88112, Israel*

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Abstract**—**Macroalgae (seaweed) can be cultured effectively for the production of useful algal biomass and removal of nutrients from fishpond effluents. A land-based, tide/gravity-driven flow-through, fish-macroalgae integrated system was studied at Makoba Bay, Zanzibar, Tanzania, during May–October, 2000. Rectangular cages made of 1-inch mesh netting were constructed in channels that received the outflows of the fishponds. Four species of macroalgae were planted in the cages and compared for their usefulness as biofilters. *Gracilaria crassa* and *Ulva reticulata* grew at average rates of 1.5 and 1.2 %, respectively. Both species removed nitrogen as seaweed protein at rates of up to 0.4 g $N/m^2/d$. The algal biomass produced was of good quality with protein dry weight contents of 13% for *G. crassa* and 26 % for *U. reticulata*. The biofilters also raised the pH values of the fishpond effluents and oxygenated the water. In contrast to *Ulva* and *Gracilaria*, species of *Eucheuma* and *Chaetomorpha* performed poorly in the fishpond effluents.

INTRODUCTION

Besides their other use as food, fertilisers and in the production of phycocolloids, macroalgae find application in the removal of nutrients from effluent waters of sewage, industry and mariculture fishponds.

Semi-intensive and intensive mariculture involves feeding fish by fertilisation of the ponds, either with live or formulated feeds. Fed aquaculture creates much pollution, as fish excrete 60–70 % of the nitrogen they ingest in dissolved forms (Porter et al., 1987), which can cause eutrophication of the marine coastal environment leading to harmful algal blooms. To avoid such effects, mariculture effluent water should be recycled or treated before it is released back to the sea.

The use of macroalgae as 'biofilters' of effluent water from fishponds and cages has been a recent venture. In Chile, Troell et al. (1997) studied the integration of cage culture of salmon and the red macroalga *Gracilaria chilensis* on ropes. The authors extrapolated their results to show that each hectare of alga cultivated close to finfish cages had the potential to remove at least 5 % of fish-excreted inorganic nitrogen. The integration of macroalgae with shellfish/crustaceans culture was also studied by Qian et al. (1986) and Ni et al. (unpublished) in China. The treatment of land-based fishpond effluents using macroalgal biofilters has been studied mostly in Israel (Vandermeulen & Gordin, 1990; Neori & Shpigel, 1999; Neori et al., 2000), where the authors obtained significant ammonia-nitrogen removals with re-aeration and restoration of the pH of the water. Trials on the culturing and use of macroalgae to filter nutrients from fishponds in Tanzania, as reported by Mmochi et al. (1999)

Corresponding author: FM. Permanent address: Institute of Marine Sciences, University of Dar es Salaam, P.O. Box 668, Zanzibar, Tanzania. E-mail: flowerem@hotmail.com

and Mwandya (2001), were not successful. Another attempt to use macroalgae to treat wastewater in Tanzania (although not from fishponds) was by Haglund and Lindstrom (1995) who used sewage water to culture macroalgae under laboratory conditions. The use of macroalgae biofilters for the removal of nutrients in land-based mariculture has been described as a cost-effective method of removing nutrients *in situ* (Vandermeulen & Gordin, 1990).

The Institute of Marine Sciences (IMS), Zanzibar, Tanzania, in collaboration with the National Centre for Mariculture (NCM), Eilat, Israel, have been conducting a study on the use of macroalgae to remove nutrients from an integrated finfish-shellfish-macroalgae system at Makoba, Zanzibar, for the past three years. During this period, it has been difficult to grow macroalgae in the integrated system due to environmental problems. Trial experiments by Mmochi et al. (1999) on macroalgae led to decomposition and rotting followed by death of the algae, the main cause of which was thought to be the substratum. This consisted of a 'mangrove substratum' formed by mangrove trees that were buried at the site as a result of a hurricane in the 18th century. The resultant substratum has very fine sediment that is re-suspended whenever water is allowed into the ponds, and this settles on the algal fronds, hindering growth. The sediment also contains some microorganisms that may also settle on the algae. Apart from the twice daily water exchange, the macroalgae were planted in almost stagnant water. The aim of this study was to use the water flow regime used successfully in Eilat to culture macroalgae in fishpond effluent water at Makoba. To our knowledge, except for the ongoing trials with abalone and *Gracilaria* in South Africa (Alan Critchley, pers. commun.), this is the first study of its kind in Africa.

MATERIALS AND METHODS

Study site

Experiments were conducted at Makoba Bay, on the northwest coast of Zanzibar, Tanzania (Fig. 1). Water trapped in a reservoir (40,000 m^2) during each high tide was later released through channels into six fishponds of about 260 m^2 each (Fig. 2). Since the refilling of the reservoir depended on the spring tides, it was only achieved twice per month when the tidal height was large enough to enter the reservoir (at least 3.7 m above chart datum).

Fig. 1. Map of Unguja Island, Zanzibar, showing the study sites

Experimental design

Macroalgae cages were installed in the fishpond outflow channels (Fig. 2). Water was allowed to flow continuously through the cultivated macroalgae cages and then to the sea. All experimental procedures, with respect to stocking, sampling and harvesting, were similar in all experiments unless otherwise indicated.

The green macroalgae, *Ulva reticulata* and *Chaetomorpha crassa*, and the red *Gracilaria crassa* were collected from wild stocks at Chwaka Bay, whereas commercially farmed *Eucheuma denticulatum* was purchased from seaweed farmers at Uroa and Matemwe (Fig. 1).

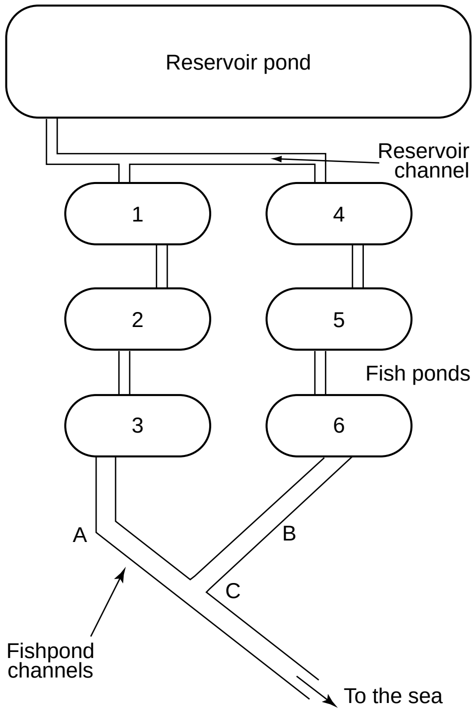

Fig. 2. Sketch of the Makoba integrated system showing the layout of ponds and channels

The experimental material was sorted in the laboratory to separate and clean the desired species from other unwanted algae, seagrasses and debris. The experimental material was then kept overnight in 300-litre plastic containers filled with seawater, at room temperature, and aerated until the next morning when it was transported to the experimental site. *Eucheuma denticulatum* was, however, kept on the floor in the laboratory and not in water as it is known to be affected by long-term storage in stagnant water and especially in containers (Msuya, 1996). Samples were taken for chemical analyses of the stocked biomass, in which protein, carbohydrate, ash, phosphorus and fibre contents were assessed.

Two experiments were conducted: one on growth and a second on the nutrient removal capacity of the macroalgae. The experiments for each algal species required six weeks, and were conducted from May to October, 2000. In the first experiment, all four species were cultured in the same period, whereas in the second experiment each species was cultured six weeks after the previous one.

Growth rates of the macroalgae

Four species of algae, *Chaetomorpha crassa*, *Ulva reticulata*, *Eucheuma denticulatum* and *Gracilaria crassa* were planted in cages at the channels draining the fishponds (positions 'A' and 'B' in Fig. 2), and in channels leading to the fishponds; i.e., between the reservoir and fishponds as controls. Two-square-metre chamber cages (referred here as 'units') were constructed using 1-inch netting material and thin nylon ropes commonly known as 'tie tie' (for seaweed cultivation). *Ulva reticulata*, *Chaetomorpha crassa* and *Gracilaria crassa* were planted into these units, at a stocking density of 3 kg/m^2. *Eucheuma denticulatum* was planted using 20-mm nylon ropes placed 20 cm apart. Eleven pieces of *Eucheuma* (about 100 g each) were tied to each rope using 'tie tie', as described by Msuya et al. (1996), about 10 cm apart. Some *E. denticulatum* units were installed at the intersection of two series of fishponds (point 'C' in Fig. 2), where effluent waters were thought to have the highest concentrations of nutrients.

This experiment was designed to establish which of the four species would survive under such conditions before their abilities to remove nutrients were evaluated. The units and ropes were cleaned and 'weeded' twice a week to remove other epiphytic algae which grow with the targeted species and to remove any accumulated debris.

Nutrient biofiltration abilities of the macroalgae

Six (2 m^2) macroalgae units (three units in two replicates) were placed in a series at the outflow channels of the fishponds (Fig. 2). Two control macroalgae units also placed in series were placed within the channel from reservoir to the fishponds.

Sampling and analytical procedures

Algal yields

Macroalgae were harvested once a week, squeezed (for *Ulva*) or shaken (for the rest of the species), to remove excess water and their fresh weights measured on a commercial scale. This was done by the same person and in the same way to ensure consistency of the measurements. Growth rates were calculated from biomass data according to Lignell et al. (1987) and yields were calculated per unit area and time.

Nutrients

Weekly water samples were taken in the morning at 08:30 and in the afternoon at 14:00 hours and filtered in the field (glass microfibre filters, 25 mm) using syringes. The samples were analysed for dissolved inorganic nutrients (NH_4^--N, NO_3^--N and PO_4^--P) as in Parsons et al. (1984).

Protein content of the macroalgae

Samples of the macroalgae, 150 g (fresh weight) each, were taken from each unit to the laboratory, where they were sorted and rinsed with fresh water. The rinsed samples were oven-dried at 60 ºC for 24 hrs and then at 105 ºC to constant weight. Dry samples were packed in sealed plastic bags and taken to the NCM in Eilat, for Kjeldahl organic nitrogen analysis.

Basic parameters

Oxygen concentration and temperature were measured with an oxygen meter (OxyGuard Handy Mk III), water velocity using a mini current meter (Model SD-6000), the pH of the water with a pH meter (HI 8424) and salinity with a hand refractometer (SPER 300011).

RESULTS

In both fishpond outflow channels, water velocity averaged 8 cm/s. Ammonia-N concentration in the water entering from the tidal water reservoir into the control seaweed units averaged 7 µM. In the water exiting these control units ammonia-N concentration averaged 4 µM, which rose to 7.7 µM in the fishpond outlets. However, variability of these concentrations and of the concentrations in the water exiting the treatment seaweed units was high and inconsistent, disallowing a rigorous use of these data for nutrient removal calculations. Such calculations were, however, possible with the Yield and Protein Content data.

Chemical composition of the stocked macroalgae

The culturing of the algae at Makoba fishponds generally resulted in improved quality of the algae. Their protein and carbohydrate contents increased and their ash content dropped (Table 1). In *Ulva reticulata*, protein content increased, while the other components either remained the same or dropped slightly.

In *Gracilaria*, contents of protein, carbohydrate and fibre rose, while ash content dropped. Even in *Chaetomorpha* and *Eucheuma*, which did poorly as biofilters, there were significant changes in composition as a result of the culture in the tidal waters, including rises in protein and carbohydrate contents and drops in ash content.

Chemical composition of the cultured algae

Protein content, which is considered the major indicator of the quality of the macroalgae biofilters (Neori et al., 2000), gave higher values in the treatments than in the respective controls (Table 2).

Table 1. Composition of macroalgae collected from wild stocks at Chwaka Bay and Matemwe, Zanzibar, Tanzania

Species	Protein	Carbohydrate	Ash	Phosphorus	Fibre
	Ingredient (% in dw) on stocking				
Ulva reticulata	18.9 ± 4.0	23.1 ± 5.4	22.2 ± 2.0	0.1 ± 0.0	37.7 ± 3.6
Gracilaria crassa	11.4 ± 2.3	28.2 ± 3.1	37.7 ± 3.6	0.1 ± 0.0	22.7 ± 2.2
Chaetomorpha crassa	10.1 ± 1.0	13.4 ± 4.3	39.7 ± 6.9	0.1 ± 0.0	35.7 ± 4.2
Eucheuma denticulatum	2.0	15.2	56.9	0.1	25.9
	Ingredient (% in dw) at the end of the experiments				
Ulva reticulata	25.7 ± 1.7	21.1 ± 2.0	18.3 ± 0.6	0.1 ± 0.0	38.5 ± 2.9
Gracilaria crassa	13.2 ± 0.7	33.1 ± 4.4	15.0 ± 0.9	0.04 ± 0.0	38.7 ± 2.9
Chaetomorpha crassa	13.1 ± 1.1	15.6 ± 6.7	35.3 ± 9.3	0.1 ± 0.1	36.0 ± 2.8
+*Eucheuma denticulatum*	7.6 ± 0.3	23.5 ± 4.4	46.6 ± 6.9	0.04 ± 0.0	22.3 ± 3.8

+Material from control units.

Protein content in the *Ulva* fishpond effluents rose by 5 % in 6 weeks, while it dropped slightly in the control *Ulva*. In *Gracilaria* cultured in the fishpond effluents, protein content rose by 4 % in 6 weeks, while in the control units a rise of 2 % was measured. Protein content of macroalgae in the units placed in series at the fishpond effluents was slightly but repeatedly higher in the first unit compared to those from the second and third units (data not shown).

Growth rate and yield

Three of the four species of algae survived in the outflow channels of the fishponds. The red alga *Eucheuma denticulatum* did not survive. The alga started by lightening in colour during the first week. White lesions were then observed at the tips, a typical sign of stress—peroxide formation; the plants finally rotted and died. Epiphytes were found growing on the test algae, decreasing their growth even further. Cross-contamination of the units was observed between *Ulva reticulata* and *Gracilaria crassa*, probably a result of very small pieces of material which remained even after careful sorting (in nature the three species grow together in the intertidal areas), or fouling. The pieces of *E. denticulatum* that were cultured at the intersection of the two series of ponds (C in Fig. 2) died after 10 days. Those pieces of *E. denticulatum* planted in the fishpond effluent channels, just before their intersection, survived until the fourth week.

Gracilaria had the best daily growth rate of the remaining three algal species, followed by *Ulva* and *Chaetomorpha* (Table 2). All four species survived the experimental period in the control units.

Table 2. Growth, yield, protein content and N removal rate by macroalgae at Makoba, Zanzibar, May–October 2000

Macroalgae species	Growth rate (% per day)		Yield, fresh ($g/m^2/d$)		Protein content (% of dw)			Nitrogen removal rates ($g N/m^2/d$)+	
	Treatment	Control	Treatment	Control	Initial	After 6 weeks	Control	Treat-ment	Cont-rol
Ulva reticulata	1.2 ± 2.7	0.5 ± 0.4	72 ± 53	13 ±14	20.7 ± 0.7	25.7 ± 1.7	20.1 ± 0.4	0.43	0.05
Gracilaria crassa	1.5 ± 0.4	1.0 ± 0.5	105 ± 60	45 ±28	9.4 ± 0.4	13.2 ± 0.7	11.8 ± 0.4	0.18	0.05
Chaetomorpha crassa	< 0	<0	26 ±124	23 ±14	10.1 ± 0.3	13.1 ± 1.1	10.4 ± 3.4	0.07	0.05
Eucheuma denticulatum	< 0	3.0 ± 0.6	++	18 ± 8	2.0	++	7.6 ± 0.3	++	0.025

+Calculated from yield and protein content; ++No material was left.

The fresh yield of *Ulva* in the fishpond outflow channels was 0.5 kg in the first 2 weeks (Fig. 3), declined slightly and then rose again to above 1 kg during the final 2 weeks. In contrast, the yield in the control units was low (Fig. 3). *Gracilaria* growth was steady, and was higher in the fishpond outflow channels than in the control channels (Fig. 4). *Chaetomorpha* on the other hand, did not produce an easily defineable pattern (Fig. 5). *Eucheuma* grew during the first two weeks by 1 kg, but lost weight by the fourth week (Fig. 6).

Biofiltration ability

Water column nutrient concentrations at Makoba were 7.7 ± 4.6 (ammonia-N), 1.9 ± 1 (nitrate-N) and 0.7 ± 0.4 (orthophosphate). However, as mentioned above, these measurements gave inconsistent results probably because of organic matter breakdown processes in the sediment at the bottoms of the different mariculture facilities. Therefore, the nutrient removal rates presented here are based on algal yield and its nitrogen content. The algae at Makoba removed significant amounts of nitrogen. During the six-week experiments, *Ulva* removed much more nitrogen than either *Gracilaria* or *Chaetomorpha* (Table 2). Areal removal (removal per unit area) rates ranged from 0.43 g N/m^2/d by *Ulva* down to 0.07 g N/m^2/d by *Chaetomorpha*.

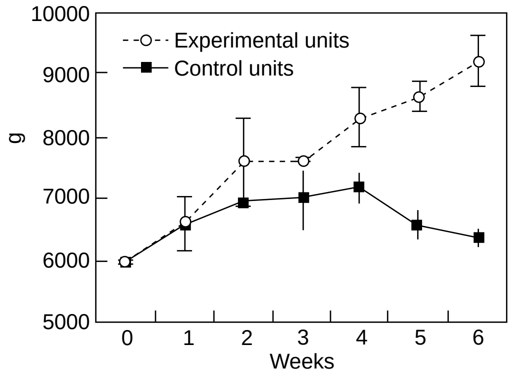

Fig. 4. Cumulative harvest of *Gracilaria crassa* (wet wt ± SD), Makoba, Zanzibar, May 2000

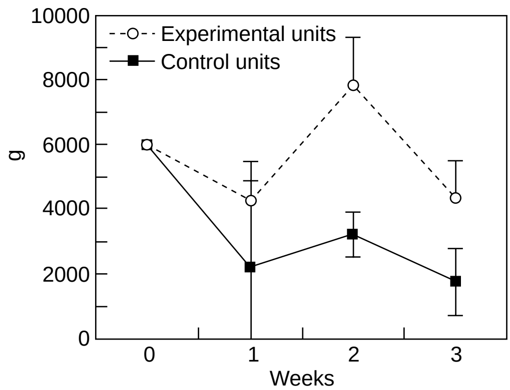

Fig. 5. Cumulative harvest of *Chaetomorpha crassa* (wet wt ± SD), Makoba, Zanzibar, May 2000

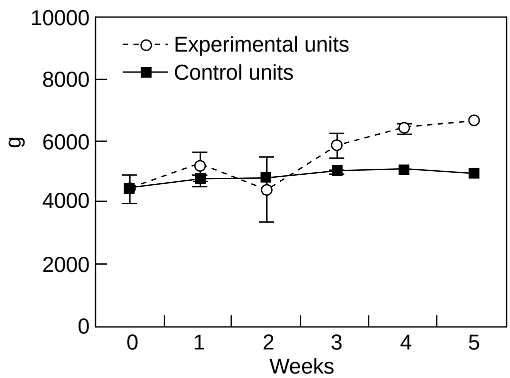

Fig. 3. Cumulative harvest of *Ulva reticulata* (wet wt ± SD), Makoba Zanzibar, May 2000

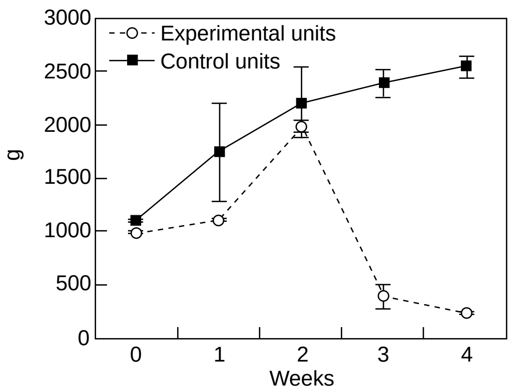

Fig. 6. Cumulative harvest of *Eucheuma denticulatum* (wet wt ± SD), Makoba, May 2000

Effect of seaweed on water quality

The macroalgae raised the pH of the fishpond outflow water, from 7.7 ± 0.16 at the outflow of the fishponds to 8.16 ± 0.15 at the outflows of the last macroalgae units (Fig. 7), with significant differences between fish and macroalgae (P < 0.001). In the control units, constant average pH values of 8.13 ± 0.12 were measured. The macroalgae also aerated the water, with significant statistical differences between fishponds and macroalgae units (P < 0.001) in oxygen content. From an average value of 4.6 mg/l at the outflows of the fishponds, the oxygen concentration at the outflow of the last macroalgae units rose to 7.2 mg/l (Fig. 8). Minimum dissolved oxygen concentrations obtained were 3.1 and 5.3 mg/l for fishpond and macroalgae outflows respectively whereas the maximum concentrations were 6.4 and 8.7 mg/l respectively. In the control channels, oxygen concentrations hovered around 6.8 mg/l throughout the study period. Temperatures in the fish and macroalgae outflow waters were similar (P > 0.05) at an average of 29 ºC (Fig. 9) with minimum and maximum values at 27.3 and 29.6 ºC for fish and 27.1 and 29.5 ºC for macroalgae ponds respectively.

Salinity of the water varied from 23–40 ‰ (Fig. 10), with higher fluctuations during June when it was raining. Towards the end of July and in August, salinity was stable, at 35 ‰.

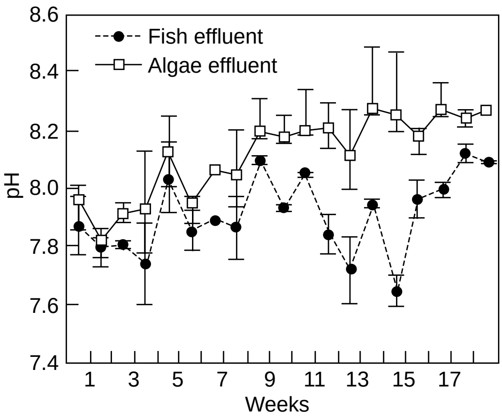

Fig. 7. pH values (± SD) at the fish-macroalgae integrated system, Makoba, Zanzibar, May–October 2000

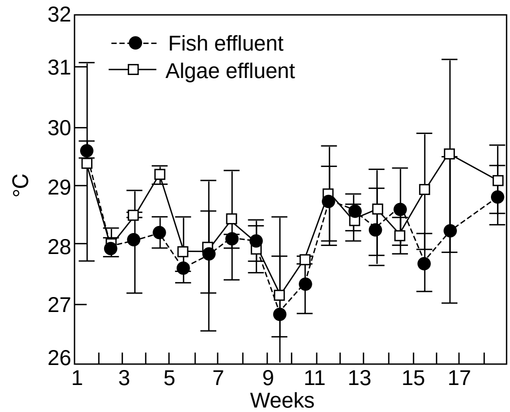

Fig. 9. Temperature values (± SD) at the fish-macroalgae integrated system, Makoba, Zanzibar, May–October 2000

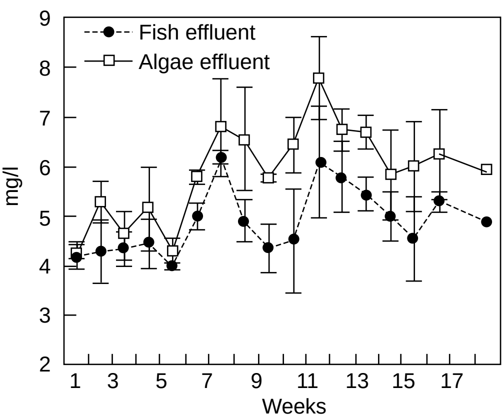

Fig. 8. Dissolved oxygen concentration (± SD) at the fish-macroalgae integrated system, Makoba, Zanzibar, May–October 2000

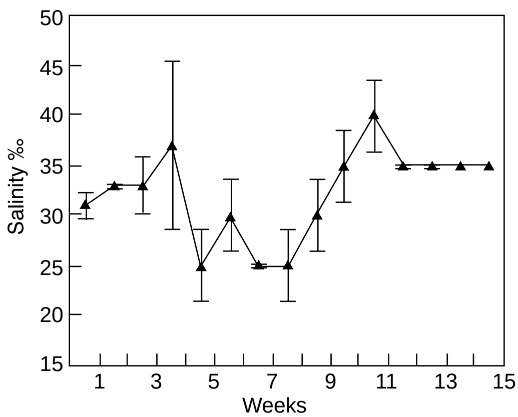

Fig. 10. Salinity values (± SD) at the fish-macroalgae integrated system, Makoba, Zanzibar, May–August 2000

DISCUSSION

Chemical composition

Cultured *Ulva* and *Gracilaria* can be rich in protein and so can be a good food source for shellfish, such as abalone (Neori et al., 2000) or as an ingredient in fish feed as used at the Makoba integrated system (Mmochi et al., 1999). The macroalgae *Chaetomorpha* is also rich in protein but *Eucheuma* has low protein content. *Eucheuma* is a well-known source of the phycocolloid carrageenan. When cultured in the tidal reservoir water, the chemical composition of *Eucheuma* improved greatly, with higher levels of protein and carbohydrates (polysaccharides) and lower levels of ash. This observation gives hope that by improving the culturing technology, *Eucheuma* culture in tidal mariculture ponds can still become a viable industry for a high-quality product. There is no known commercial use of *Chaetomorpha*, but its biomass could be possibly converted into biogas.

The increase in protein content in the macroalgae thalli when cultured in fishpond effluents show that the macroalgae removed more nitrogen than they needed and therefore stored it in their thalli, a useful feature if the biomass is used to culture marine herbivores.

Growth rate and yield

The relatively lower growth rate of *Ulva* compared to *Gracilaria* could have been a result of an observation that during the experiments the former alga was always buoyant, rising to the surface of the water where it was exposed and became bleached or desiccated. This was mostly observed during the afternoon whereas during the morning the alga was submerged. *Chaetomorpha* and *Gracilaria* did not show the same buoyancy.

In the case of *Eucheuma*, these results indicate that whatever was killing it came from the fishponds. Possible causes are low pH, low salinity and relatively higher nutrient fluxes considering that nutrient concentrations in the sea where the algae are farmed are very low (ammonia-N concentrations have been below 0.1 μM). Similar results were obtained in Hawaii where it was reported that stress signs on the algae could be a result of intolerance to salinity or mineral contents in the water, observations that were also reported from the Philippines and Malaysia (Doty, 1986). In China, however, Qian et al. (1996) integrated the culture of *Kappaphycus* (formerly placed in the genus *Eucheuma*) and oyster and obtained good growth rates for both the macroalga and the oyster.

From our results with *Eucheuma* that were planted at the intersection of the two pond series (i.e. the algae that died after 10 days), it is possible that the algae obtained relatively higher nutrient concentrations coming from both pond series, thus speeding up their death.

Yields and growth rates of the macroalgae obtained in this study are indicative of the possibility of culturing macroalgae at Makoba. Ammonia-N concentrations were higher than those reported earlier at Makoba (Mwandya, 2001 and Msuya, 2001). At this point we would like to correct the data reported by the latter report. The nutrient concentrations should be multiplied by the respective atomic weights of each nutrient. The higher nutrient concentrations in the present study may be due to the fact that there were no sedimentation or shellfish ponds placed between the fishponds and the seaweed (as was the case during the study by Mwandya, 2001) that would have reduced nutrient concentrations before the water drained into the seaweed. The study by Mwandya also reported that the integrated system at Makoba, did not produce ammonia-N concentrations that exceeded 1 μM.

The present study was conducted during the rainy season, which could have affected the growth of the macroalgae because of high dilution of seawater at the channels and ponds. Salinity at the outflow of fishponds was below 30 ‰ most of the time between May and July (Fig. 10), and became even lower during the downpours (e.g. on 9th June, salinity was 23 ‰ in the afternoon). Despite all these factors, the macroalgae were growing satisfactorily as also shown by their good photosynthesis rates (data not shown).

Biofiltration ability

Macroalgae can be used to remove a significant amount of nitrogen in this kind of low-tech integrated system. The results also show that with high water velocity, high removal rates can be obtained even with relatively lower nutrient concentrations. In this study, the macroalgae biofilters were able to restore the pH of the water and aerate the water in the integrated system. For a system like the one at Makoba where the performance of the macroalgae biofilters is limited by water availability, these removal rates are significant. Results of *Gracilaria* in this study showed that it performed less efficiently than *Ulva* in N removal. These results are similar to those obtained by Neori et al. (2000) who reported that the efficiency of *Gracilaria conferta* in removing nutrients from fishpond effluents was lower than that of *Ulva lactuca*.

The present study is useful in Tanzania where the development of mariculture of finfish, shellfish, crustaceans and seaweed, is currently taking place. Seaweed farming is one of the most important industries in the country, and especially on the Zanzibar Islands. The activity employed more than 20,000 people in 1993/1994, and contributed 14.7 and 27.3 % of Zanzibar's exports during the two years respectively (Msuya et al., 1996). If seaweed farming can be integrated with fish farming as a means of reducing the mariculture's ecological impact, the economic activities of the communities will become more diversified and sustainable. The results of this study will be useful to the (generally underdeveloped) marine fish farming industry in Tanzania and Africa in general, as a low-tech, low-cost method of cleaning water from mariculture operations.

CONCLUSIONS

The macroalgae *Ulva reticulata*, *Gracilaria crassa* and *Chaetomorpha crassa* can be cultured and used to remove nutrients from mariculture fishpond effluents by simple facilities and using only water tidal flow. Of the species tested, the macroalga *U. reticulata* and *G. crassa* were superior biofilters in this type of integrated system.

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