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Performance of the seaweeds *Gracilaria salicornia* and *Caulerpa lentillifera* as biofilters in a hatchery scale recirculating aquaculture system for juvenile spotted babylons (*Babylonia areolata*)

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Abstract This study was conducted to determine the feasibility of using seaweeds as biofilters in a hatchery scale recirculating aquaculture system for juvenile spotted babylons (*Babylonia areolata*). Two seaweeds *Gracilaria salicornia* and *Caulerpa lentillifera* were used with three initial biomass levels of each species (280, 560 and 840 g wet weight m^{-3}). Spotted babylon with an average initial shell length of 1.32 ± 0.01 cm and body weight of 0.37 ± 0.01 g was used with a stocking density of 300 snails m^{-2}. The experiment was carried out in triplicate over a period of 120 days. Results showed that seawater quality (water temperature, conductivity, salinity, pH, dissolved oxygen and total suspended solids) gradually changed with no significant differences between treatments throughout the experimental period, except alkalinity, total ammonia nitrogen, nitrite–nitrogen, nitrate–nitrogen and phosphate. Growth rates in weight and shell length of the spotted babylon cultured in all seaweed treatments used were not significantly different between seaweeds and density treatments, but significant differences in final survival rate of the spotted babylon were found between seaweed and density treatments. Growth rate and biomass gain of *C. lentillifera* were significantly higher than those of *G. salicornia* for all stocking density treatments. This study shows that *Gracilaria salicornia* and *Caulerpa lentillifera* can be used as biofilters for regulating water quality in a recirculating culture system for spotted babylons.

Keywords Biofilter · Recirculating culture system · Water quality · *Gracilaria salicornia* · *Caulerpa lentillifera* · *Babylonia areolata*

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Introduction

At present, commercial aquaculture of juvenile spotted babylons, *Babylonia areolata*, in Thailand, is undertaken in flow-through seawater systems. This type of culture requires about 100% water exchange per day to maintain water quality. Furthermore, this type of culture has encountered some problems due to its tendency to release waste effluents containing elevated levels of nitrogen or phosphorus—reactive compounds that may be considered as water pollutants. One of the main drawbacks of spotted babylon culture lies in the wastes derived from food and their metabolic products. The main products are uneaten food, faeces and excreted dissolved inorganic nutrients which are transported in the water at various concentrations (Chaitanawisuti et al. 2005). Many valuable marine species can be successfully grown and have high growth and survival rates in recirculating aquaculture systems (RAS) because of high-quality culture water. Water reuse methods require maintaining good water quality with a system that is easily operated at low cost. RAS have attracted much attention because they consume less water than flow-through systems and also reduce water exchange rates. The RAS are biologically and technically feasible and will produce extra income for the producers. They have been used extensively for rearing and maintaining adult and juvenile marine species. Aquaculture water treatment systems use bacteria to convert ammonium and nitrite into nitrate under aerobic conditions, while nitrate removal can be accomplished using sophisticated denitrification systems under anaerobic conditions (Chuntapa and Powtongsook 2003). Pagand et al. (2000) reported that the use of recirculating water systems is one approach used to limit the impact of aquaculture on the aquatic environment. Although the total quantity of nutrients released is similar in open and recirculating systems, the small volumes of concentrated effluents that are produced should be easier to deal with, although proper technical and economic solutions have yet to be implemented. The use of seaweeds for treatment of marine aquaculture wastes has been proposed by a number of authors since marine seaweeds have high capacities for absorption and metabolism of *N* and *P*—compounds excreted by marine animals. In addition to improving water quality, seaweeds can provide an additional source of income as a valuable by-product in the process. The special interest is the use of seaweeds in recycling some dissolved nutrients, in particular nitrogen in the form of ammonia (Buschmann 1996; Neori et al. 1996; Chow et al. 2001; Hernandez et al. 2002; Martinez-Aragon et al. 2002; Msuya et al. 2006; Kovitvadhi et al. 2008). We developed a simple recirculating system for the production of juvenile spotted babylons that are available for commercial use. The aim of the present study was to assess the capability of two seaweeds (*Gracilaria salicornia* and *Caulerpa lentillifera*) as potential biofilters of dissolved wastes in a hatchery—scale recirculating aquaculture system for juvenile spotted babylons (*Babylonia areolata*).

Materials and methods

Recirculating system design and components

Each experimental unit was designed as an independent closed—recirculating system consisting of a rearing tank, a sedimentation tank and a biological filter tank. Cylindrical plastic tanks of 300 l capacity were used for each tank of the recirculating unit. The bottom area of the rearing tank was 0.78 m^2 and was covered with a 5 cm layer of coarse sand (0.5–1.0 mean grain size) to serve as a substrate. The sedimentation tank contained oyster shell fragments and the biological filter tank contained one of the marine seaweeds as a biofilter.

All experimental units were located in an indoor hatchery, but the roof over all biofilter tanks was transparent and permitted about 80% of sunlight to pass thus enabling seaweed photosynthesis. Seawater was pumped from an earthen pond, filtered mechanically, and circulated through the experimental unit. The water depth in all tanks was 30 cm. Water flowed from the biofilter tank through the rearing tank and was maintained in a small submersible pump at a constant flow rate of 530 l h^{-1} (14.3 vol d^{-1} water flow) before it was returned to the rearing tank. No seawater was exchanged throughout the experimental period of 120 days. The tanks were moderately aerated by air diffusers placed at the bottom of each tank. Water temperature was maintained at room temperature ±1.5°C. Salinity was monitored daily, as necessary, to keep the variation within ±2.0 ppt by addition of fresh water to correct for any increased salinity due to water evaporation. Photoperiod was a natural 12 l:12 D.

Experimental animals and seaweeds

Juvenile spotted babylons, *B. areolata*, used in growth and survival experiments were produced at a private hatchery in Rayong province, located on the eastern coast of the Gulf of Thailand. Individuals from the same cohort were sorted by size to prevent possible growth retardation of small spotted babylons when cultured with larger ones. Their initial shell length and whole body weight averaged 1.33 ± 0.01 cm (1.31–1.34 cm) and 0.37 ± 0.001 g (0.35–0.38 g), $n = 30$, respectively. Mean shell lengths and body weights used in each treatment were not statistically different, and hence, treatments could be compared statistically. Juveniles were held in the experimental rearing tanks at a stocking density of 300 individual m^{-2} or 192 snails per tank.

Two seaweeds *Gracilaria salicornia* and *Caulerpa lentillifera* were used as biofilters. The first was collected from intertidal pools along the shore of Samui Island, Surattani province in southern Thailand, and the latter was collected from broodstock-rearing ponds of an intensive shrimp farm. Healthy thalli were carefully rinsed to remove mud or associated animals or other attached seaweeds. Seaweeds were placed in a plastic basket of 25.0 × 35.0 × 25.0 cm which contained numerous pores of 1.5 cm^2 (4 holes cm^{-2}) at each side and were suspended 30 cm above the bottom of biofilter tanks. Seaweeds in all tanks were not harvested throughout the experiments. Visible epiphytes and invertebrate naturally occurring in the biofilter tanks were carefully removed on a weekly basis.

Experimental design

This study was conducted at the Spotted Babylon Aquaculture Research and Training Unit, Aquatic Resources Research Institute, Chulalongkorn University, Petchaburi province, Thailand. The laboratory experiment was designed to test the combined effects of two seaweeds (*Gracilaria salicornia* and *Caulerpa lentillifera*) and three stocking densities of (280, 560 and 840 g m^{-3}) on growth performance of juvenile *B. areolata* and seaweeds as well as water quality. The experiment was a 2 × 3 factorial design with three replications. An additional tank without algae was used as a control. The experiment was terminated after a 120 days during May to August, 2009.

Rearing method

The snails were fed ad libitum with fresh trash fish once daily at 10:00 h. The amount of food consumed was recorded daily, and uneaten food was removed immediately after the animals stopped feeding and air dried for a period of 10 min before weighing. Size grading of snails in all treatments was not done throughout the grow-out period. No chemical and antibiotic agents were used throughout the entire experimental period. To determine growth performance, 50% of snails were sampled randomly from each treatment at 15 days intervals, and shell length and whole body weight were determined. Shell length was measured with calipers to the nearest millimetre from the maximum anterior to posterior distance of a shell, and the whole weight was measured after air drying for a period of 10 min before weighing and then returned to the tank. The number of dead individuals was recorded at 15-day intervals. Seaweeds from each tank were also weighed to determine the increase in biomass, and specific growth rates were calculated at 15-day intervals. Seaweeds were placed in a plastic basket to drain off excess water. Visible epiphytes were carefully removed. Average shell length increments, body weight gains and growth rates were calculated after the method of Chaitanawisuti and Kritsanapuntu 1999). Body weight gains ($BW_f - BW_i$), and monthly growth rates for body weight ($BW_f - BW_i$)/T) were calculated, in which BW_f = mean final body weight, BW_i = initial body weight, and T = time in months. The specific growth rate (SGR, % day^{-1}) = 100 × [ln (final weight, g) − ln (initial weight, g)/ (culture period, days). Mortality, expressed as the percentage of the initial stocking density, was calculated from the difference between the number of snails stocked and harvested.

Water analysis

The following seawater quality parameters in each rearing tank were analysed weekly: water temperature (Hg thermometer), salinity (portable multiparameter metre model YSI#63), conductivity (portable multiparameter metre model YSI#63), pH (pH metre), total alkalinity (phenolphthalein methyl orange indicator), dissolved oxygen (DO metre model YSI#52), total ammonia–nitrogen (phenate method), nitrite–nitrogen (colorimetry method), nitrate–nitrogen (cadmium reduction) and orthophosphate (ascorbic acid method) (APHA et al. 1998).

Statistical analysis

Data on growth performance and water quality were analysed using the SPSS statistical software package (version 10). Analysis of variance (ANOVA) was used to test the interaction of seaweeds and stocking density at $\acute{\alpha} = 0.05$, and differences between means were compared using Tukey's test at $\acute{\alpha} = 0.05$.

Results

Water quality

Seawater quality in the recirculating aquaculture system for juvenile spotted babylons (*Babylonia areolata*) using two seaweeds at different densities as biofilters over 120 days is shown in Fig. 1. Water temperature, salinity, dissolved oxygen and pH did not differ significantly ($P > 0.05$) between the two seaweed biofilters and density treatments, with ranges of 27.24–27.38°C, 30.9–31.1, 6.11–6.20 mg/l and 7.75–7.79, respectively. Significant differences in total ammonia nitrogen, nitrite, nitrate, phosphate and total alkalinity were found between seaweeds and density treatments ($P < 0.05$), with ranges of 0.0624–0.1041 mg/l, 0.0458–0.0619, 7.7005–12.5973, 0.4501–0.6074, and 72.28–78.69 mg/l, respectively.

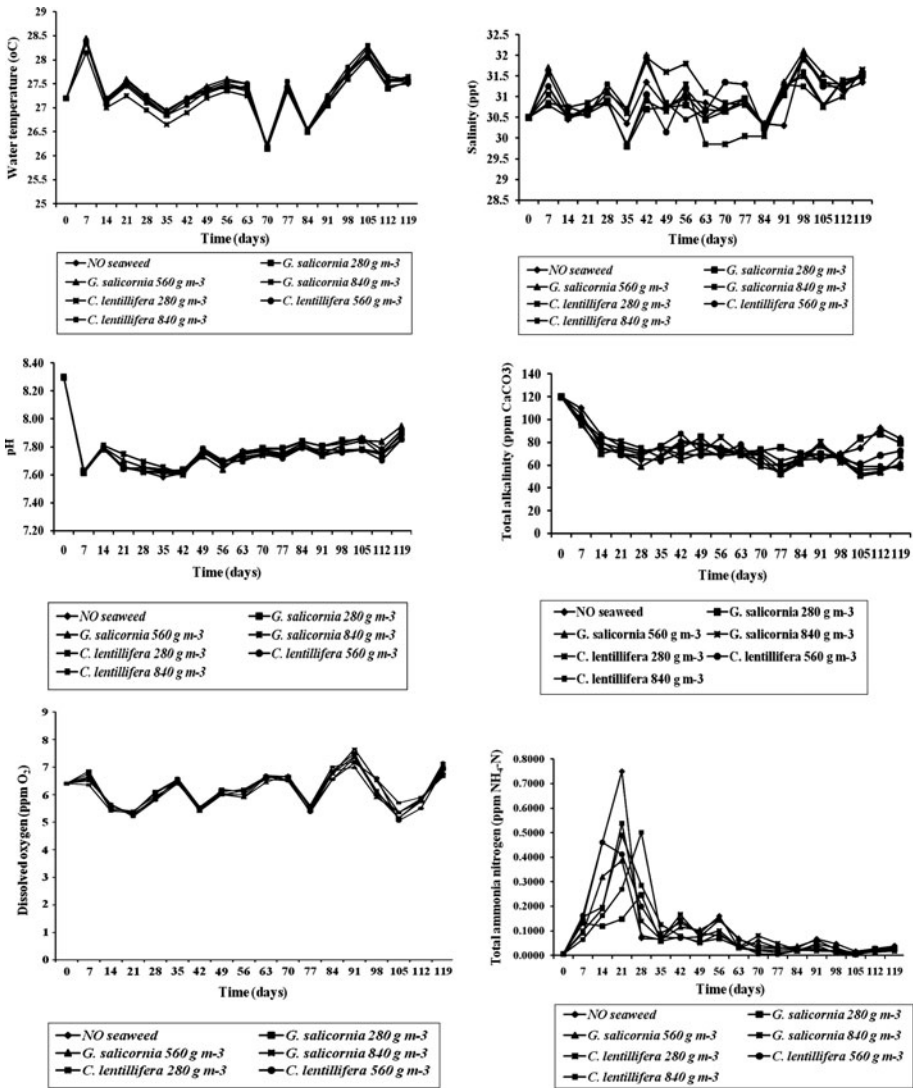
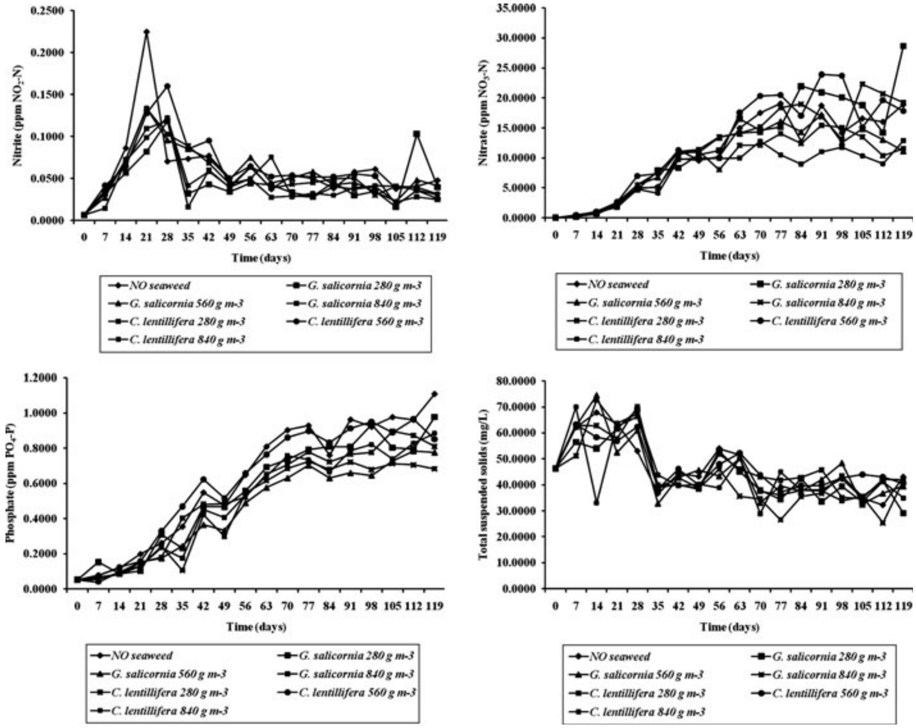


Fig. 1 Seawater quality in a recirculating aquaculture system for juvenile spotted babylons (*Babylonia areolata*) using two seaweeds at different densities as biofilters for 120 days

Growth and survival of spotted babylon

Growth in shell length and body weight of juvenile spotted babylons (*Babylonia areolata*) in a recirculating aquaculture system using two seaweeds at different densities as biofilters over 120 days is shown in Figs. 2 and 3. Two-way ANOVA showed no significant differences in growth rate in shell length and body weight of spotted babylons between two seaweed biofilters and density treatments ($P > 0.05$) varying from 1.00 to 1.17 g mo^{-1}. Overall average growth rates of spotted babylons at all density treatments were 1.11, 1.13 and 0.90 g mo^{-1} for all systems using *Gracilaria salicornia* and *Caulerpa lentillifera*) as



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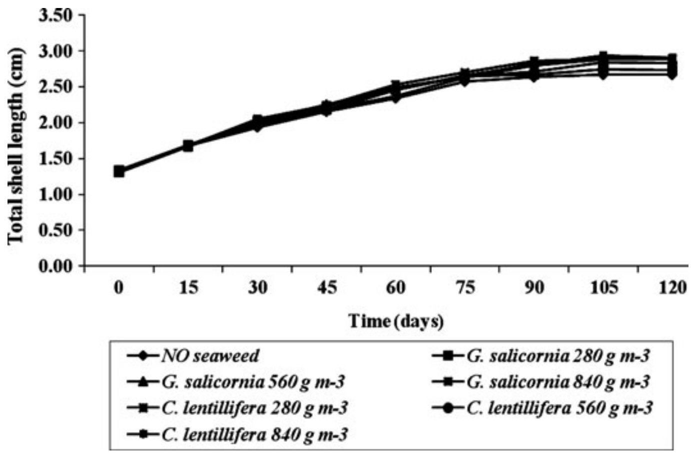

Fig. 2 Growth in shell length of juvenile spotted babylons (*Babylonia areolata*) in a recirculating aquaculture system using two seaweeds at different densities as biofilters for 120 days

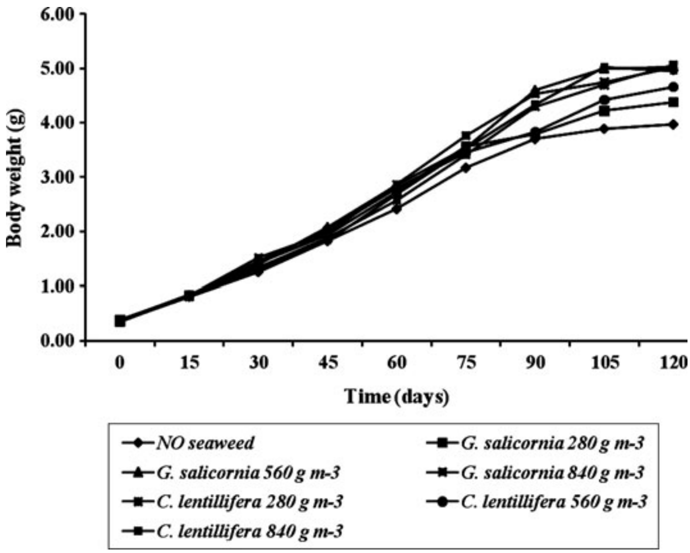

Fig. 3 Growth in weight of juvenile spotted babylons (*Babylonia areolata*) in a recirculating aquaculture system using two seaweeds at different densities as biofilters for 120 days

biofilters and the control (no seaweed). Significant differences in final survival rate of spotted babylon were found between seaweeds and density treatments ($P < 0.05$), ranging from 86.98 to 92.97% compared with those of the control (84.64%). No significant difference in the yield of spotted babylon was found between seaweeds and density treatments ($P > 0.05$) ranging from 689.5 to 826.2 g but it was significantly higher than that of control (577.3 g) (Table 1).

Growth and biomass of seaweeds

The growth of seaweeds (*Gracilaria salicornia* and *Caulerpa lentillifera*) used as biofilters in a recirculating system for rearing juveniles spotted babylon (*Babylonia areolata*) for 120 days is shown in Fig. 4. Two-way ANOVA showed that there were significant differences in specific growth rates (SGR) between the two seaweeds and density treatments with an interaction effect ($P < 0.05$). SGRs of *C. lentillifera* (1.70–2.52% d^{-1}) were significantly higher than those of *G. salicornia* (0.44–0.61% d^{-1}). For both seaweeds, the highest SGR was found at a density of 280 g m^{-3} and SGRs at densities of 560 g m^{-3}, and 840 g m^{-3} was not significantly different. In addition, the overall average specific growth rate for all density treatments of *C. lentillifera* (2.07% d^{-1}) was about 4 times higher than that of *G. salicornia* (0.49% d^{-1}). Significant differences in biomass gains were found between seaweed treatments ($P < 0.05$) but there was a significant difference in density treatments without an interaction effect ($P > 0.05$). The biomass gain of *C. lentillifera* (4,498.5–5,237.2 g) was significantly higher than that of *G. salicornia* (270.4–526.4 g) for all stocking density treatments ($P < 0.05$). The average biomass gain for all density treatments of *C. lentillifera* (4,920.8 g) was about 12 times higher than that of *G. salicornia* (380.1 g) (Table 2).

Table 1 Growth of juvenile spotted babylons (*Babylonia areolata*) reared in a recirculating aquaculture system using two seaweeds at different densities as biofilter

Parameters	No seaweed	*Gracilaria salicornia*			*Caulerpa lentillifera*			Significance
		390 g/m^2	780 g/m^2	1,170 g/m^2	390 g/m^2	780 g/m^2	1,170 g/m^2	
Initial length (cm)	1.33 ± 0.01	1.33 ± 0.01	1.31 ± 0.02	1.33 ± 0.01	1.34 ± 0.01	1.33 ± 0.00	1.32 ± 0.00	
Initial body weight (g)	0.36 ± 0.01	0.38 ± 0.01	0.35 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.37 ± 0.00	0.37 ± 0.00	
Final shell length (cm)	2.67 ± 0.10	2.73 ± 0.07	2.88 ± 0.14	2.89 ± 0.16	2.90 ± 0.15	2.83 ± 0.10	2.91 ± 0.09	
Final body weight (g)	3.98 ± 0.46	4.38 ± 0.45	5.03 ± 0.65	5.02 ± 0.65	5.07 ± 0.64	4.66 ± 0.40	4.96 ± 0.35	
Length increment (cm)	1.36 ± 0.11	1.40 ± 0.08	1.58 ± 0.12	1.57 ± 0.16	1.56 ± 0.14	1.50 ± 0.10	1.59 ± 0.09	
Body weight gain (g)	3.62 ± 0.46	4.00 ± 0.47	4.68 ± 0.64	4.66 ± 0.66	4.70 ± 0.63	4.29 ± 0.40	4.59 ± 0.35	
Growth rate (cm mo^{-1})	0.34 ± 0.03	0.35 ± 0.02	0.39 ± 0.03	0.39 ± 0.04	0.39 ± 0.04	0.38 ± 0.02	0.40 ± 0.02	
Growth rate (g mo^{-1})	0.90 ± 0.12	1.00 ± 0.12	1.17 ± 0.16	1.16 ± 0.16	1.17 ± 0.16	1.07 ± 0.10	1.15 ± 0.09	
Feed conversion ratio	1.68 ± 0.21	1.41 ± 0.16	1.62 ± 0.27	1.52 ± 0.21	1.61 ± 0.34	1.76 ± 0.17	1.64 ± 0.11	
Final survival rate (%)	84.64 ± 1.10	90.63 ± 0.74^{bc}	86.98 ± 0.74^{b}	92.97 ± 0.37^{c}	88.29 ± 2.95^{a}	88.54 ± 0.74^{b}	90.36 ± 1.84^{bc}	
Yield (g)	577.3 ± 83.11	689.5 ± 87.7	773.3 ± 113.0	826.2 ± 121.0	727.4 ± 70.3	721.4 ± 73.9	788.9 ± 43.8	

Values are means ± standard deviation. Means with different superscript in the same row are significantly different ($P < 0.05$)

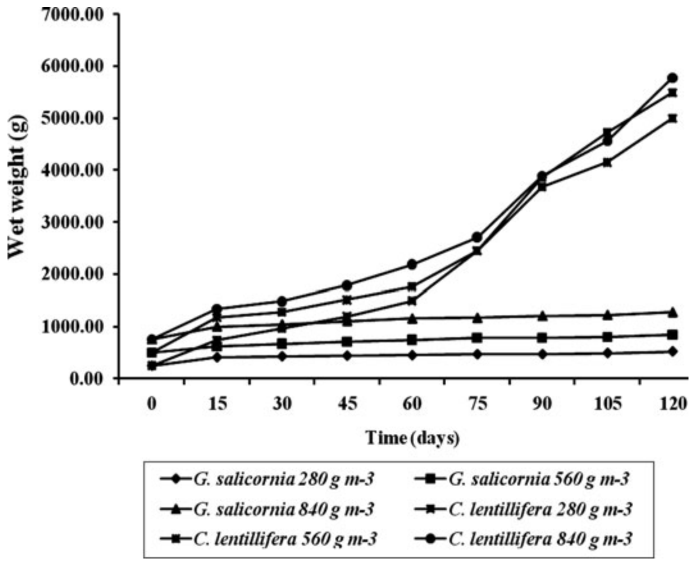

Fig. 4 Growth of seaweeds (*Gracilaria salicornia* and *Caulerpa lentillifera*) as biofilters in a recirculating system for rearing juvenile spotted babylons (*Babylonia areolata*) for 120 days

Discussion

The main goal of this study was to examine the effect of biological filter treatments in recirculating aquaculture systems (RAS) using two seaweeds (*Gracilaria salicornia* and *Caulerpa lentillifera*) at three initial stocking densities. Growth and mortality of juvenile spotted babylons (*Babylonia areolata*) and seaweeds as well as water quality were compared in seven rearing treatments over a long-term culture period of 120 days. Results were favourable for complete reuse of the treated waste water in the recirculating systems, and the system is seemed feasible. Growth of spotted babylons did not decrease in any of the experimental rearing systems over the 4-months, and it also had a positive effect on survival rate. All seawater quality parameters in all rearing systems used were below the safety criteria for growth and survival of the spotted babylon, except alkalinity. In addition, the seaweed (*Caulerpa lentillifera*) showed a better growth rate and yield than that of *Gracilaria salicornia* at all stocking densities. This study shows that *Gracilaria salicornia* and *Caulerpa lentillifera* can be used as biofilters for regulating water quality in a recirculating culture system for spotted babylons. Paul and de Nys (2008) reported that seaweed from the genus *Caulerpa* will not be easily integrated into settlement ponds in tropical aquaculture. However, because some species of *Caulerpa* grew well in tank-based systems (*C. racemosa* grew at >7% day^{-1}) and others are capable of good uptake (*C. serrulata* and *C. taxifolia* almost doubled internal nitrogen in nutrient—rich water), *Caulerpa* species have an application in the bioremediation of intensive tank-based aquaculture and perhaps treated pond aquaculture effluent. Martinez-Aragon et al. (2002) indicated that the seaweeds *Ulva rotundata* and *Enteromorpa intestinalis* and *Gracilaria gracilis* efficiently removed dissolved phosphate in waste water from fish culture tanks. Removal efficiency was highest in *U. rotundata* (99.6%) and lowest in *G. gracilis* (62.2%). The maximum uptake rate of phosphate occurred in *U. rotundata*, slightly greater than that for *E. intestinalis*, while *G. gracilis* showed the lowest uptake rate. Deviller et al. (2004)

Table 2 Growth and biomass gain of seaweeds (*Gracilaria salicornia* and *Caulerpa lentillifera*) as biofilters in a recirculating aquaculture system for rearing juvenile spotted babylons (*Babylonia areolata*) for 120 days

Parameters	*Gracilaria salicornia*			*Caulerpa lentillifera*		
	390 g/m^2	780 g/m^2	1,170 g/m^2	390 g/m^2	780 g/m^2	1,170 g/m^2
Initial wet weight (g)	250.0 ± 0.00	500.0 ± 0.00	750.0 ± 0.00	250.0 ± 0.00	500.0 ± 0.00	750.0 ± 0.00
Final wet weight (g)	520.4 ± 63.9	843.5 ± 20.2	1,276.4 ± 61.7	5,487.6 ± 67.2	4,998.5 ± 31.3	5,776.9 ± 81.0
Biomass gain (g)	270.4 ± 63.9[a]	343.5 ± 32.2[a]	526.4 ± 61.7[a]	5,237.3 ± 59.8[b]	4,498.5 ± 31.3[b]	5,026.9 ± 81.0[b]
Specific growth rate (%/day)	0.61 ± 0.10[a]	0.44 ± 0.12[a]	0.44 ± 0.04[a]	2.58 ± 0.09[c]	1.92 ± 0.02[b]	1.70 ± 0.12[b]

Values are means ± standard deviation. Means with different superscript are significantly different ($P < 0.05$)

showed that reuse of the high-rate algae pond (HRAP) treated water did not decrease sea bass (*Dicentrarchus labrax*) growth in a recirculating system over a 1-year period. It also had a positive effect on fish survival, and the nitrification in the biological filter was not reduced.

This study indicated that total ammonia–nitrogen, nitrite–nitrogen, nitrate–nitrogen and orthophosphate throughout the experiment were in the range of accepted criteria for effluents from aquaculture in Thailand. Many studies have shown that ammonium, nitrite and phosphate can be biofiltered in recirculating systems, establishing seawater quality which is well within the safety conditions for grow-out of various aquatic animals. Deviller et al. (2004) suggested that in order to improve nitrate and phosphate removal rates, periodic harvesting of algae is necessary and a higher water inflow in a high-rate algae pond (HRAP) should partly make up for inorganic carbon depletion in high nitrate and phosphate effluents. Jones et al. (2001) also suggested that the nutrient removal efficiency by macroalgae may be improved with increased light, particularly for nitrate uptake, increased water flow to reduce the boundary layer, and higher stocking densities. In addition, Hernandez et al. (2002) indicated that *U. rotundata* and *E. intestinalis* and *G. gracilis* efficiently removed ammonium dissolved in the waste water from fish culture tanks. *U. rotundata* and *E. intestinalis* showed the highest rated removal of ammonium (97.7%), whereas *G. gracilis* removed 93.2% of the nutrient during incubation. In addition, the minimum uptake rate of ammonium occurred in *G. gracilis*. Wang et al. (2007) showed that growth rate of *Ulva pertusa* was 3.3% day^{-1} in an indoor recirculating system for production of juvenile sea cucumbers (*Apostichopus japonicas*). *U. pertusa* was efficient in removing toxic ammonia and in maintaining the water quality within acceptable levels for sea cucumber culture. *U. pertusa* removed 68% of the total ammonia nitrogen and 26% of the orthophosphate from the sea cucumber culture effluent; the macroalgae biofilter removed ammonia at an average rate of 0.459 g N m^{-2} day^{-1}. *Gracilaria chilensis* culture was highly efficient at a biofilter for the soluble nutrients but had little effect on particulate emission. The best growth of *G. chilensis* occurred in the ammonium—rich effluent from fish culture (Chow et al. 2001). In addition, results indicated the possibility of extending this aquaculture system with few modifications to large-scale grow-out operations for this species.

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