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Feasible use of rock oyster (*Crassostrea commercialis*) and seaweeds (*Gracilaria salicornia* and *Caulerpa lentillife*) as biofilter in a laboratory - scale closed recirculating system for juveniles spotted babylon (*Babylonia areolata*)

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ABSTRACT:

This study was conducted to assess the feasible use of rock oyster (*Crassostrea commercialis*) in biofiltration and two seaweeds (*Gracilaria salicornia* and *Caulerpa lentillife*) as nutrient absorbant in a laboratory - scale recirculating system for growing of juveniles spotted babylon (*Babylonia areolata*). The experiment was carried out in triplicates over a period of 90 days. The experiment was a complete randomized design with three growth trials: Treatments 1: without oyster and seaweed biofilter used as a control; Treatment 2: Oyster biofiltration (1,500 g per tank) and seaweed (*G. salicornia*) absorption (250 g per tank); and Treatment 3: Oyster biofiltration (1,500 g per tank) and seaweed (*C. lentillife*) absorption (250 g per tank). No significant differences (P>0.05) in final shell length, final body weight, body weight gains, shell length increment and growth rate among all treatments. Growth rate in shell length and body weight of spotted babylon ranged from 0.33 – 0.34 cm mo^{-1} and 0.62 – 0.67 g mo^{-1}, respectively. Significant differences (P<0.05) in final survival rate of spotted babylon were found among treatments, ranging from 86.72 to 86.98 % compared with those of the control (84.27%). There were no significant differences (P>0.05) in water temperature, salinity, pH, dissolved oxygen, ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen and phosphate-phosphorus among the growth trials but not for alkalinity. This study can conclude that *G. salicorni*and *C. lentillifera* can be used as nutrient biofilter for regulating of water quality in a closed recirculating system for growing juveniles spotted babylon but not suitable on using oyster.

Keywords:
Seaweed, biofilter, recirculating culture, water quality, *Gracilaria salicornia*, *Caulerpa lentillifera*, *Babylonia areolata*.

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INTRODUCTION:

Commercial growing of juveniles spotted babylon, *Babylonia areolata*, in Thailand are cultivated under the flow-through seawater system. This type of culture needs about 100% water exchange per day to maintain water quality and also had some criticism regarding its environmental impact due to its tendency to release waste effluents containing elevated levels of nitrogen or phosphorus – rich compounds which may be considered as water pollutants. One of the main drawbacks of spotted babylon culture lies in the wastes derived from feed 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, Krisanapuntu and Natsukari 2005). Many valuable marine species can be successfully grown and have high growth and survival rates in recirculating aquaculture system (RAS) due to the high-quality culture water. Water reuse methods require maintaining good water quality with a system that easily operated at a low cost. RAS have attractive much attention because they consume less water than flow-through systems and also reduce water exchange rates. Therefore, RAS is biologically and technically feasible and secondly that it will produce worthwhile income to the producers. It has been used extensively for rearing and maintaining adult and juvenile marine species. 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 economical solutions have to be implemented. The use of seaweeds for the 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 – rich compound excreted by marine animals. In addition to improving the water quality, seaweeds can provide an additional source of income from a valuable by-product in the process. Jones, Dennison & Preston (2001) suggested that nutrient removal efficiency of macroalgae may be improved with increased light, particularly for nitrate uptake, increased water flow to reduce the boundary layer, and higher stocking densities. The special interest is the use of seaweeds for recycling some dissolved nutrients, in particularly nitrogen in the term of ammonia (Buschmann 1996, Neori et al. 1996, Chow et al. 2001, Hernandez et al. 2002, Matinez-Aragon et al. 2002, Msuya et al. 2006). Thus, we have developed a simpler recirculating system for the production of juvenile spotted babylon available use for commercial applications. This study was conducted to assess the capable use of rock oyster (*Crassostrea commercialis*) as biofiltration and two seaweeds (*Gracilaria salicornia* and *Caulerpa lentillife*) as nutrient absorption in a laboratory - scale closed recirculating system for growing of juveniles spotted babylon (*Babylonia areolata*)

MATERIALS AND METHODS

This study was conducted at the Spotted Babylon Aquaculture Research Unit, Aquatic Resources Research Institute, Chulalongkorn University, Petchaburi province, Thailand. The laboratory experiment was designed to test the use of rock oyster (*C. commercialis*) and seaweeds (*G. salicornia* and *C. lentillife*) as biofilter in a laboratory - scale closed recirculating system for growing juveniles spotted babylon (*B. areolata*). The experiment was a complete randomized design with three replications as following: Treatment 1: Without oyster and seaweed biofilter used as a control; Treatment 2: Oyster biofiltration (1,500 g per tank) and seaweed (*G. salicornia*) absorption (250 g per tank); and Treatment 3: Oyster biofiltration (1,500 g per tank) and seaweed (*C. lentillife*) absorption (250 g per tank). Each experimental unit was designed as an independent closed - recirculating system consisting of a rearing tank, an oyster filtration tank and a seaweed absorption 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 seaweed absorption tank contained one of the marine seaweeds as a biofilter. All experimental units were located in an indoor hatchery, but the roof over all seaweed absorption 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} before it was returned to the rearing tank. No seawater was exchanged throughout the experimental period of 90 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 ±1.0 ppt by addition of fresh water to correct for any increased salinity due to water evaporation. Photoperiod was a natural 12 l:12 day:night.

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 1.23 ± 0.005 cm and 0.37 ± 0.01 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. The rock oyster *Crassostrea commercialis* with average body weight of 4.0 - 10.0 g were collected from the oyster farm at Ang-sila, Cholburi province. Two seaweeds *Gracilaria salicornia* and *Caulerpa lentillifera* were used as biofilters. The first was collected from intertidal pools along the coastal water of Samui Island, Surattani province, and the latter was collected from broodstock-rearing ponds of an intensive shrimp farm. Oysters and seaweeds were placed in a plastic basket of 25.0 x 35.0 x 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. Oyster and seaweeds in all tanks were not harvested throughout the experiments.

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. The experiment was terminated after a 90 days during May to June, 2009. Average shell length increments, body weight gains and growth rates were calculated after the method of Chaitanawisuti and Kritsanapuntu 1999). Body weight gains (BWf - BWi), and monthly growth rates for body weight (BWf - BWi)/T) were calculated, in which BWf = mean final body weight, BWi = initial body weight, and T = time in months. The specific growth rate (SGR, % day-1) = 100 9 [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. 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).

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

RESULTS

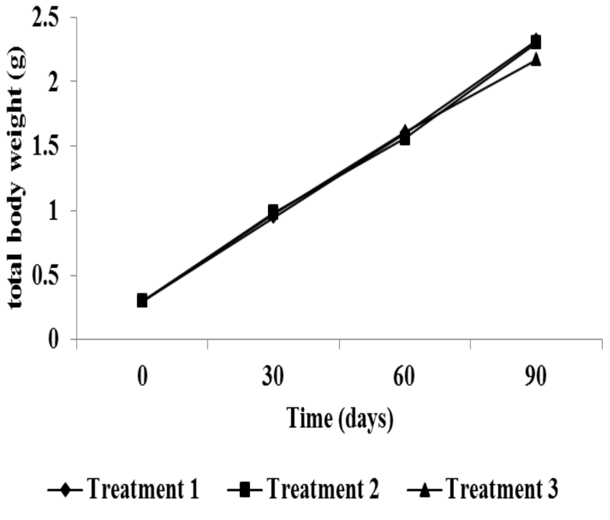
Growth in shell length and body weight of juvenile spotted babylon (*Babylonia areolata*) in a laboratory - scale closed recirculating system using oyster and two seaweeds as biofilters over 90 days is shown in Figure 1. One-way ANOVA showed no significant differences ($P>0.05$). in final shell length, final body weight, body weight gains, shell length increment and growth rate among all treatments (Table 1). Shell length increments of spotted Babylon ranged from 0.98 to 1.00 cm and 1.88 to 2.03 g for those of body weight gains. Growth rate in shell length and body weight of spotted babylon ranged from 0.33 – 0.34 cm mo^{-1} and 0.62 – 0.67 g mo^{-1}, respectively. Significant differences ($P<0.05$). in final survival rate of spotted babylon were found among rearing treatments ($P<0.05$), ranging from 86.72 to 86.98 % compared with those of the control (84.27%).

Growth of seaweeds (*G. salicornia* and *C. lentillifera*) were used as biofilters in a laboratory scale, closed recirculating system for juveniles spotted babylon (*B. areolata*) for 90 days is shown in Figure 2. There were significant differences ($P<0.05$) in absolute growth rates between the two seaweed biofilter treatments. Weight gain of *C. lentillifera* (716.0 g) was significantly higher than those of *G. salicornia* (353.0 g mo^{-1}). Growth rate of *C. lentillifera* (238.6 g mo^{-1}) was significantly higher than those of *G. salicornia* (117.6 g mo^{-1}). However, growth of rock oyster (*C. commercialis*) is very low with less than 0.05 g mo^{-1} for all treatments but final survival was high (100%) for Treatment 1 and 2.

Water quality parameters in a closed recirculating system using rock oyster (*C. commercialis*) and seaweeds (*G. salicornia* and *C. lentillifera*) as biofilters over 90 days is shown in Table 2. There were no significant differences ($P>0.05$) in water temperature (24.2 – 24.6^{0}C), salinity (30.5 – 30.7 psu), pH (8.35 – 8.42), dissolved oxygen (6.5 – 6.7 mg/l), ammonia - nitrogen (0.0766 – 0.0922 mg/l), nitrite -nitrogen (0.1555 – 0.1728 mg/l), nitrate - nitrogen (0.8584 – 0.9073 mg/l) and phosphate - phosphorus (0.4863 – 0.5185 mg/l) among the growth trials but not for total alkalinity (77.0 – 81.0 mg/l) ($P<0.05$).

DISCUSSION

This study showed that no significant differences in final shell length, final body weight, body weight gains, shell length increment and growth rate among all treatments. Growth rate in shell length and body weight of spotted babylon. Significant differences in final survival rate of spotted babylon were found among treatments, ranging from 86.72 to 86.98 % compared with those of the control (84.27%). There were no significant differences in water temperature, salinity, pH, dissolved oxygen, ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen and phosphate-phosphorus among the growth trials but not for alkalinity. This study can conclude that *G. salicorni* and *C. lentillifera* can be used as nutrient biofilter for regulating of water quality in a closed recirculating system for growing juveniles spotted babylon but not suitable use of oyster. As compared to the study of Chaitanawisuti, Kritsanapuntu and Natsukari



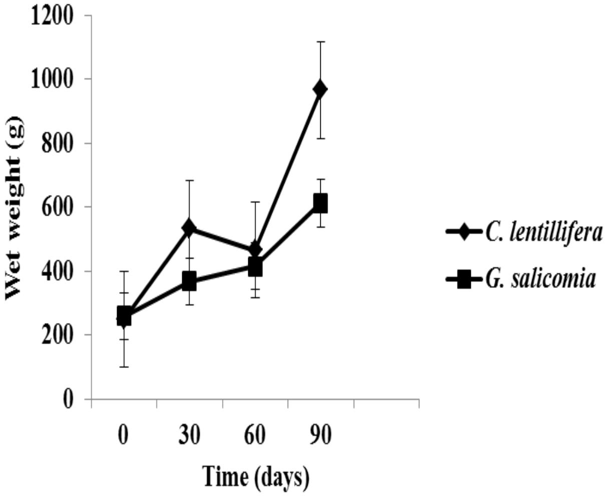


Table 1. Growth of juveniles spotted babylon (*B. areolata*) in a laboratory - scale closed recirculating system using oyster and two seaweeds as biofilters for 90 days

Parameters	Treatment 1	Treatment	Treatment
	1.23±0.02	1.23±0.01	1.23±0.001
	0.30±0.02	0.29±0.01	0.30±0.004
	2.23±0.04	2.23±0.16	2.21±0.10
	2.32±0.11	2.30±0.57	2.17±0.27
	1.00±0.03	1.00±0.15	0.98±0.10
	2.03±0.11	2.01±0.58	1.88±0.27
	0.33±0.01	0.34±0.05	0.33±0.03
	0.67±0.04	0.67±0.19	0.62±0.09
	84.27^{b}	86.72^{a}	86.98^{a}

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

(2005), growth rate of *B. areolata* in this study (0.62 to 0.67 g mo^{-1}) is the same as those cultured in the recirculating system used oyster shells as biofilter (0.62 to 0.68 g mo^{-1}) but lower than those cultured in flow-through system (1.05 to 1.25 g mo^{-1}) and This study agreed with the study of Chow et al. (2001) who indicated that *Gracilaria chilensis* culture was highly efficient at biofiltration of the soluble nutrients but had little effect on particulate emission. The best growth of *G. chilensis* occurred in the ammonium – rich effluent from the fish culture. Jones, Dennison & Preston (2001) also recommended that nutrient removal efficiency of macroalgae may be improved with increased light, particularly for nitrate uptake, increased water flow to reduce the boundary layer, and higher stocking densities. Paul & Nys (2008) reported that seaweed from the genus *Caulerpa* culture will not be easily integrated into settlement ponds in tropical aquaculture. However, because some species of *Caulerpa* grew well in tank-based system (*C. racemosa* grew at > 7% day^{-1}) and others are capable of luxury uptake (*C. serrulata* and *C. taxifolia* almost doubled internal nitrogen in nutrient – rich water), *Caulerpa* species have application in bioremediation of intensive tank-based aquaculture and perhaps treated pond aquaculture effluent. As compared to other seaweeds commonly used for water treatment in mariculture purposes, Msuya et al. (2006) reported that seaweed, *Ulva reticulate*, grew at an average of 4.0% day^{-1} at the fishpond outflow with a biomass yield averaging 46 g m^{-2} day^{-1}, compared with averaging 2.5% and 2.7 g m^{-2} day^{-1}at the fishpond inflow. Martinez-Aragon et al. (2002) indicated that seaweeds (*Ulva rotundata* and *Enteromorpa intestinalis* and *Gracilaria gracilis* removed efficiently the phosphate dissolved in the waste water from the fish culture tanks. Removal efficiency was highest in *U. rotundata* (99.6%) and lowest in *G. gracilis* (62.2%). In addition, 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. Hernandez et al. (2002) also indicated that *U. rotundata* and *E. intestinalis* and

Table 2. Seawater quality in a laboratory - scale closed recirculating system for juveniles spotted babylon (*B. areolata*) using oyster and two seaweeds as biofilters for 90 days

Parameters	Treatment 1	Treatment	Treatment
Water temperature (C)	24.2±2.4	24.3±2.4	24.6±2.3
Salinity psu)	30.6±1.3	30.7±1.7	30.5±1.2
pH	8.42±0.11	8.37±0.12	8.35±0.15
Alkalinity (mg/L)	81±9 ^{a}	74±8^{c}	77±9^{b}
Dissolved oxygen (mg/L)	6.5±0.7	6.7±0.6	6.5±0.5
Ammonia - nitrogen (mg-N/L)	0.0766±0.0590	0.0900±0.0538	0.0922±0.0604
– -	0.1728±0.0404	0.1638±0.0809	0.1555±0.0802
– -	0.9073±0.3112	0.8584±0.3036	0.8827±0.3230
– -	0.4863±0.2109	0.5185±0.2516	0.4922±0.2643

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

G. gracilis removed efficiently the ammonium dissolved in the waste water from the fish culture tanks. *U. rotundata* and E. intestinalis showed the highest ammonium disappearance (97.7%), whereas *G. gracilis* removed 93.2% of the nutrient during the 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 a indoor recirculating system for production of juvenile sea cucumber (*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}. This study can conclude that *G. salicorni*and *C. lentillifera* can be used as nutrient biofilter for regulating of water quality in a closed recirculating system for growing juveniles spotted babylon but not suitable use of oyster. However, the stocking density and production of *G. salicorni*and *C. lentillif* should be considered for optimal use in the recirculating system for the spotted Babylon.

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