**Gracilariopsis longissima** as biofilter for an Integrated Multi-Trophic aquaculture (IMTA) system with *Sciaenops ocellatus*: Bioremediation efficiency and production in a recirculating system

Qing He\(^1,2\), Yuanzittuo\(^1,2\), Jianheng Zhang\(^1,2\), Zhaoyang Chai\(^1,2\), Hailong Wu\(^1,2\), Shanshan Wen\(^1,2\) & Peimin He\(^1,2\)

\(^1\)College of Fisheries and life Science, Shanghai Ocean University, Shanghai 201 306, China

\(^2\)Marine Scientific Research Institute, Shanghai Ocean University, Shanghai 201 306, China

\(^[\text{E-mail address: pmhe@shou.edu.cn]}\)

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A recirculating integrated system producing *Sciaenops ocellatus* and *Gracilariopsis longissima* (a red seaweed) was evaluated with respect to nutrient removal efficiency and production. *G. longissima* was found to be efficient in removing toxic ammonia and maintaining the water quality within an acceptable level for *S. ocellatus* culture. Specific growth rate (SGR) of *S. ocellatus* ranged from 0.064 ± 0.006% d\(^{-1}\) to 0.099% ± 0.010% d\(^{-1}\). Survival rates of *S. ocellatus* were 100% in the Integrated Multi-Trophic Aquaculture (IMTA) systems. *G. longissima* had average SGRs of 3.03 ± 0.11% d\(^{-1}\), 2.48 ± 0.04% d\(^{-1}\), 1.86 ± 0.26% d\(^{-1}\) and 1.12 ± 0.16% d\(^{-1}\) under initial densities of 1 g L\(^{-1}\), 3 g L\(^{-1}\), 6 g L\(^{-1}\) and 9 g L\(^{-1}\), respectively. Daily average nitrogen and phosphorus uptake rates of *G. longissima* were negatively correlated to cultivation densities in the recirculating system. Biofiltration capacity of *G. longissima* was confirmed by significantly reduced concentrations of ammonia, nitrate, nitrite and phosphate in the integrated system with *S. ocellatus*. Results indicated that *G. longissima* is suitable as a good candidate for IMTA systems.

**Keywords:** Biofilter, Macroalgae, Recirculating aquaculture system, Specific growth rate

**Introduction**

Intensive mariculture activities of fish, shrimp, shellfish or the other economic aquatic animals have a great impact on organic matter and nutrients loading in coastal areas, affecting the sediments beneath culture installations, producing variations in the nutrient composition of the water column\(^1\). These environmental modifications not only affect the production of the aquatic animals but also have great influences on the other biological components of the ecosystem such as bacteria, benthic fauna, bird, macroalgae, phytoplankton, zooplankton etc\(^2\). Increasing concerns on negative environmental impacts of aquaculture aroused a renewed interest to employ integrated techniques in the early 1990s\(^3\). Since then Integrated Multi-Trophic Aquaculture (IMTA) which provides nutrient bioremediation capability, mutual benefits to co-cultured organisms and economic diversification had been widely accepted and achieved rapid developments\(^3,4\).

According to water flow, intensive mariculture systems can be classified as flow-through or recirculating. In the former, the water is used once only and then discharged into the receiving water with or without treatment\(^5\). Unlike flow-through systems, Recirculating Aquaculture Systems (RASs) are closed or partially closed systems and require water treatment to enable the water to be re-used. RAS saves the energy, reduces nutrient release and water consumption, avoids drawing in toxic microalgae which have the potential to wipe-out the entire stock\(^6,7\). Current research and development in RAS are largely devoted to bacteria-based systems\(^8\), while reports of macroalgae-based water re-use systems are limited\(^6,9,10\).

Gracilariales species (mainly *Gracilaria* but also *Gracilariopsis*) is one of the world’s most cultivated seaweeds because of its values in agar production, food and environmental protection\(^9,11,12\). Species of genus *Gracilaria* and *Gracilariopsis* studied in IMTA systems for the bioremediation include *Gracilaria vermiculophylla*\(^13,14\), *Gracilaria lichenoides*\(^15\), *Gracilaria lemaneiformis*\(^16,17\), *Gracilaria birdiae*\(^18\), *Gracilaria chilensis*\(^19,20\) and *Gracilariopsis longissima*\(^12\). *G. longissima* was identified as *Gracilaria verrucosa* which has been reported as an efficient biofilter significantly
removing inorganic nitrogen and inorganic phosphate under the closed sea\textsuperscript{31} and semi-closed bay conditions\textsuperscript{32}. \textit{G. longissima} as the current accepted name (www.algaebase.org) is used in this study. Up to now, only a few papers on the bioremediation efficiency of \textit{G. longissima} in closed recirculation integrated systems with fish were available. Thus, in the present study, we ran a simple recirculating system to evaluate the bioremediation efficiency of \textit{G. longissima} in an IMTA system with \textit{S. ocellatus} and to explore the feasibility of co-culturing \textit{G. longissima} with \textit{S. ocellatus} without water exchange.

**Materials and Methods**

**Algal and animal material: pre-culture conditions**

Fronds of \textit{G. longissima} were collected manually from Xiangshan Harbor and transported to the laboratory under cool conditions in sufficient seawater to remove epiphytes and visible encrusting organisms. Algal fronds were then kept in a plastic container illuminated with white light fluorescent lamps at 60 \textmu mol photons m\textsuperscript{-2} s\textsuperscript{-1}, under a 12:12 light: dark photoperiod. Temperature was maintained as 25°C throughout the study. \textit{S. ocellatus} were bought from a local market in Zhejiang Province, China. Fish were fed with headless river shrimps everyday 8:00 a.m. Water containing algal fronds and fish was continuously aerated.

**Experimental design**

The recirculating system included a series of glass tanks. Four 20 cm \times 36 cm \times 25 cm glass tanks and three 27 cm \times 36 cm \times 25 cm glass tanks were used for \textit{G. longissima} culture. An 80 cm \times 39 cm \times 25 cm glass tank was used for \textit{S. ocellatus} culture. Seawater was recirculated among these tanks all the time so there was practically no difference in nutrient concentrations among different tanks. The experiment included two treatments. Each treatment had a fish monoculture system served as a control. Fig. 1 included two treatments. Each treatment had a concentration among different tanks. The experiment was performed for a period of 30 days during 15 May to 15 June 2011. \textit{G. longissima} was incubated for 4 days in the recirculating system for acclimatization before the \textit{S. ocellatus} was introduced. Water samples for nutrient concentrations measurement were collected 9:00 a.m. daily during this period (4 days). Then the \textit{S. ocellatus} was introduced into the recirculating system. Fish were fed with headless river shrimps everyday 8:00 a.m. On the first day when the \textit{S. ocellatus} was introduced into the recirculating system, water samples were collected every four hour (5 times in total) to testify the effect of \textit{S. ocellatus} on the water quality at the early stage of the experiment. After that water samples were collected 9:00 a.m. daily for the following 26 days. On the 20\textsuperscript{th} day, the fish density of treatment 1 and 2 was raised to 27 g L\textsuperscript{-1} and 45g L\textsuperscript{-1}, respectively. Meanwhile the same fish densities were settled in control groups. All glass tanks were illuminated with white light fluorescent lamps at 60 \textmu mol photons m\textsuperscript{-2} s\textsuperscript{-1}, under a 12:12 light: dark photoperiod. Temperature was maintained as 25°C and salinity was maintained at 20. During the whole experimental period, there was no water exchanged in all tanks except for the renewed supply to compensate evaporation loss. Epiphytes on the inner wall of the glass container were cleaned every day. Fish survival was carefully checked every day.

**Analytical procedures**

Water samples for ammonium-nitrogen (NH\textsubscript{4}-N), nitrate-nitrogen (NO\textsubscript{3}-N), nitrite-nitrogen (NO\textsubscript{2}-N) and inorganic phosphate (PO\textsubscript{4}-P) measurements were filtered through cellulose membrane filters (Millipore HAWP 0.45 \mu m) and determined according to protocols of JOGFS\textsuperscript{23}. Total nitrogen and phosphorus contents in \textit{G. longissima} fronds were determined after peroxymonosulphuric acid digestion\textsuperscript{24} in triplicates for each sample. Specifically, samples containing 100 mg (dry matter) were digested with 4 mL concentrated sulfuric acid at 440°C and treated with 17 mL of 30% hydrogen peroxide. Total nitrogen and phosphorus contents in samples were determined spectrophotometrically after chemical reactions\textsuperscript{25}. To determine the ratio of dry weight to fresh weight, centrifuged fresh seaweed samples of 10 g were oven-dried (48 h; at 60°C) and weighed after cooling down in a Silica-desiccator\textsuperscript{26}. Fish and seaweed in each tank were weighed to calculate specific growth rate (SGR, % d\textsuperscript{-1}) determined as:

\[
SGR = \frac{\ln W_t - \ln W_o}{t} \times 100
\]

where \(W_0\) and \(W_t\) refer to the initial wet weight and wet weight at time \(t\), respectively, and \(t\) is the time interval (d).
Daily nutrient production rate ($NPR$, g kg$^{-1}$ d$^{-1}$) was calculated according to nutrient measurements of control groups:

$$NPR = \frac{\left(N_t - N_0\right) \cdot V}{W \cdot t}$$

where $N_0$ refers to initial nitrogen or phosphorus concentrations (g L$^{-1}$), $N_t$ represents final nitrogen or phosphorus concentrations (g L$^{-1}$), $V$ represents the volume of the glass tank (L), $W$ represents the fresh weight of $S. ocellatus$ (kg) and $t$ refers to the time interval (d).

Nutrient removal efficiency ($NRE$, %) was calculated according to nutrient concentrations in recirculating systems:

$$NRE = \frac{M_0 - M_t}{M_0} \times 100$$

where $M_0$ and $M_t$ refer to the initial nutrient concentration and the nutrient concentration at time $t$, respectively, and $t$ is the time interval (d).

**Statistical analysis**

All statistical analyses were performed using SPSS software (V.17.0). One-way Analysis of Variance (ANOVA) was used to compare the SGR of $S. ocellatus$, $G. longissima$ and nutrient concentrations. Differences were considered significant when $P<0.05$.

**Results**

Nutrient removal efficiency of $G. longissima$ before the $S. ocellatus$ was introduced into recirculating systems

Nutrient removal efficiencies of two treatments were evaluated before the $S. ocellatus$ was introduced into recirculating systems. After four days cultivation, the concentration of NH$_4$-N, NO$_3$-N, NO$_2$-N and PO$_4$-P was decreased by 80.2%, 78.3%, 65.6% and 62.6% in treatment 1, respectively. The NH$_4$-N, NO$_3$-N, NO$_2$-N and PO$_4$-P was removed 96.8%, 86.6%, 86.5% and 77.1% in treatment 2, respectively. Nutrient removal efficiency of treatment 2 was significantly higher than that of treatment 1 ($P<0.05$) (Fig. 2).

Nutrient removal efficiency of $G. longissima$ after the $S. ocellatus$ was introduced into recirculating systems

$S. ocellatus$ had great effects on the water quality of the recirculating system. Fig. 3 showed
variations of NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N and PO$_4^3-$-P concentrations on the first day when the $S$. ocellatus was introduced into recirculating systems. NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N and PO$_4^3-$-P concentrations all increased with time in both treatment 1 and 2. Sixteen hours later, the concentration of NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N and PO$_4^3-$-P increased to 0.09 mg L$^{-1}$, 2.74 mg L$^{-1}$, 0.40 mg L$^{-1}$, 0.08 mg L$^{-1}$ in treatment 1 and 0.20 mg L$^{-1}$, 3.00 mg L$^{-1}$, 0.43 mg L$^{-1}$, 0.16 mg L$^{-1}$ in treatment 2, respectively. The NH$_4^+$-N concentration in treatment 2 was significantly higher than that in treatment 1 ($P<0.05$).

Fig. 4 showed variations of NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N and PO$_4^3-$-P concentrations in treatment 1 and 2.
from the 2nd to the 20th day after the S. ocellatus was introduced into recirculating systems. NH$_4$-N concentrations increased rapidly to the maximum level then began to decrease significantly in both treatments. From the 10th day to the 20th day, NH$_4$-N in both treatments maintained at low concentration levels. NO$_3$-N concentrations in treatment 1 and 2 decreased significantly and then maintained at low levels. NO$_2$-N and PO$_4$-P concentrations in treatment 1 and 2 increased with time then decreased slowly. Though the initial fish density of treatment 2 was twice as much as that of treatment 1, NH$_4$-N concentrations in two treatments showed no significantly difference from the 2nd to the 20th day (P>0.05).

On the 20th day, the fish density was raised from 18 g L$^{-1}$ to 27 g L$^{-1}$ in treatment 1 and 36 g L$^{-1}$ to 45 g L$^{-1}$ in treatment 2, respectively. Fig. 5 showed influences of the increase of fish density on water quality in recirculating systems. Concentrations of NH$_4$-N, NO$_3$-N, NO$_2$-N and PO$_4$-P in both treatments increased with the increase of fish density and then began to decrease significantly with time. NH$_4$-N, NO$_3$-N and NO$_2$-N concentrations in two treatments showed no significantly difference from the 21st to the 27th day (P>0.05). PO$_4$-P concentration of treatment 2 was significantly higher than that of treatment 1 (P<0.05).

**Growth of G. longissima and S. ocellatus**

The average SGR of G. longissima was 3.03 ± 0.11% d$^{-1}$, 2.48 ± 0.04% d$^{-1}$, 1.86 ± 0.26% d$^{-1}$ and 1.12 ± 0.16% d$^{-1}$ during the whole experimental period under the initial density of 1 g L$^{-1}$, 3 g L$^{-1}$, 6 g L$^{-1}$ and 9 g L$^{-1}$, respectively. SGR of G. longissima in the 1 g L$^{-1}$ condition was significantly higher than that in other conditions (P<0.05) (Fig. 6). S. ocellatus had the average SGR of 0.099 ± 0.010% d$^{-1}$, 0.080 ± 0.004% d$^{-1}$, 0.073 ± 0.008% d$^{-1}$ and 0.064 ± 0.006% d$^{-1}$ during the whole experimental period under the initial density of 18 g L$^{-1}$, 27 g L$^{-1}$, 36 g L$^{-1}$ and 45 g L$^{-1}$, respectively. SGR of S. ocellatus in the 18 g L$^{-1}$ condition was significantly higher than that in the 36 g L$^{-1}$ condition (P<0.05) during the first 20 days after the S. ocellatus was introduced to the integrated system. The SGR of S. ocellatus in the 27 g L$^{-1}$ condition was significantly higher than that in the 45 g L$^{-1}$ condition (P<0.05) during the last 7 days (Fig. 6).

**Daily average nutrient uptake rates of G. longissima and daily nutrient (nitrogen and phosphorus) production rates in the recirculating system**

The initial tissue nitrogen and phosphorus content of G. longissima were 3.9% and 0.35% dry weight, respectively. At the end of the experiment, the tissue nitrogen and phosphorus of G. longissima...
increased to 4.2% and 0.37% dry weight, respectively. Daily average nitrogen and phosphorus uptake rates of *G. longissima* were calculated based on its SGR and the tissue nitrogen and phosphorus content. Fig. 7 showed daily average nitrogen and phosphorus uptake rates of *G. longissima* were decreased with the increasing algal density. Based on results, *G. longissima* could assimilate 0.44 g nitrogen, 0.038 g phosphorus and 0.53 g nitrogen, 0.048 g phosphorus every day in treatment 1 and treatment 2, respectively.

NPRs in recirculating systems were calculated according to nutrient concentrations in control groups. The NO$_2$-N, NO$_3$-N, NH$_4$-N and PO$_4$-P production rate was 0.025 g kg$^{-1}$ d$^{-1}$, 0.18 g kg$^{-1}$ d$^{-1}$, 0.056 g kg$^{-1}$ d$^{-1}$, 0.032 g kg$^{-1}$ d$^{-1}$ under the initial fish density of 18 g L$^{-1}$ and 0.02 g kg$^{-1}$ d$^{-1}$, 0.13 g kg$^{-1}$ d$^{-1}$, 0.049 g kg$^{-1}$ d$^{-1}$, 0.023 g kg$^{-1}$ d$^{-1}$ under the initial fish density of 36 g L$^{-1}$, respectively (Fig. 7). Ca. 0.364 g N and 0.045 g P in treatment 1 and 0.557 g N and 0.064 g P in treatment 2 were produced in the recirculating system every day. According to the daily nitrogen and phosphorus uptake rate of *G. longissima*, we concluded that *G. longissima* could assimilate most of the nitrogen and phosphorus produced in the recirculating system.

**Ecological and economical cost-benefit analysis**

During the whole experimental period, ca. 16.57 g nitrogen and 1.43 g phosphorus were removed from the recirculating system in treatment 1. The net weight gain of algae and fish was 358.4 g and 42 g
which accounted for 72.9% and 2% of the initial weight, respectively. In treatment 2 ca. 20.37 g nitrogen and 1.74 g phosphorus were removed from the recirculating system. The net weight gain of algae and fish was 422.7 g and 57 g which accounted for 51.7% and 1.6% of the initial weight, respectively. Survival rates of \textit{S. ocellatus} were 100% in both treatment 1 and 2. In control groups nitrogen and phosphorus concentrations increased from the beginning of the experiment and no fish survived at the end of the experiment. Although treatment 2 supported more algae and fish than treatment 1, the ratio of total cost to benefit of treatment 2 was higher than that of treatment 1 (Table 1).

**Discussion**

Maintaining an acceptable water quality is extremely important for the RAS\(^{27}\). Accumulation of inorganic nitrogen and phosphorus, particularly NH\(_4\)-N may have significant toxic effects on economic aquatic animals\(^{6}\). The results of present study showed that \textit{G. longissima} had high nutrient removal potential and could maintain good water quality in the recirculating system. Seaweed removed most of the NH\(_4\)-N excreted by the fish and oxygenated the water. Survival rates of \textit{S. ocellatus} were 100% in both treatments while no fish survived in the monoculture system at the end of the experiment. Reducing the investment and bringing extra income, seaweed biofilters constitute a logical alternative to phytoplankton and bacterial biofilters for the maintenance of water quality in land-based mariculture\(^{27}\).

The principle of seaweed utilization as biofilter is based on their nutrient removal potential\(^{20}\). Nutrient removal efficiencies of \textit{G. longissima} in treatment 1 and 2 were evaluated before the \textit{S. ocellatus} was introduced into recirculating systems. The reduction efficiency of \textit{G. longissima} in the present study was higher than that of the same species cultivated in an enclosure sea\(^{21}\) and a semi-closed bay\(^{22}\). However, \textit{G. longissima} was less efficient when compared with \textit{Gracilaria edulis} which achieved 87% of its total NH\(_4\)-N uptake within the first hour in a laboratory scale study\(^{28}\). Maximal NO\(_3\)-N and NO\(_2\)-N removal efficiency of \textit{G. longissima} in this study was 86.6% and 86.5%, respectively, which was relatively lower than that of the red macroalga \textit{Kappaphycus alvarezii} which achieved NO\(_3\)-N and NO\(_2\)-N removal efficiency of 66.0% and 83.3%, respectively, within 24 hours in a co-culture system with \textit{Pinctada martensi}\(^{29}\). With respect to PO\(_4\)-P removal, the maximum reduction efficiency within the first four days was 77.1% in this study. The similar result was obtained by Mao \textit{et al.} for \textit{G. lemaneiformis} in a polyculture system with scallop \textit{Chlamys farreri}\(^{30}\). The maximal nutrient removal efficiency obtained in this study was within the range of values reported in literatures, which confirmed that \textit{G. longissima} was one of the available species for an IMTA system.

In the present study, nitrogen and phosphorus uptake rates decreased with the increasing algal density (Fig. 7). Similar result was obtained by Mao \textit{et al.} when studied the ammonium uptake rate of \textit{G. lemaneiformis}\(^{30}\). Abreu \textit{et al.} also reported that nutrient removal efficiency of \textit{G. vermiculophylla} was negatively related to the cultivation density\(^{13}\). The yield and nitrogen uptake efficiency of \textit{Gracilaria bursa} decreased when stocking density

<p>| Table 1—Costs and benefits of the two treatments |
|-------------------------------|-------------------|---------------|---------------|-----------------|-----------------|</p>
<table>
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<th>Treatments</th>
<th>Fish (¥)</th>
<th>Algae (¥)</th>
<th>Fish (¥)</th>
<th>Algae (¥)</th>
<th>Costs:benefits</th>
</tr>
</thead>
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<td>1</td>
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<td>6.1</td>
<td>98.5</td>
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<tr>
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<td>10.4</td>
<td>163.6</td>
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</table>
increased from 5 to 7 kg m$^{-2}$\textsuperscript{31}. Removal efficiency varied according to the system and depended on the water volume and circulation system, initial biomass, light source and photon flux density, temperature, salinity and the physiological state of the seaweed\textsuperscript{10}. Nutrients removal efficiency of treatment 2 was higher than that of treatment 1 in the first four days indicated that treatment 2 had better combination of biomass yields and nutrient uptakes than treatment 1.

In both treatments, concentrations of NO$_3$-N began to decrease soon after the S. ocellatus was introduced into the recirculating system. On the other hand, concentrations of NO$_2$-N, NH$_4$-N and PO$_4$-P didn’t show a sign to decrease until 6 or more days later (Fig. 4). NH$_4$-N is often the preferred nitrogen source for seaweeds\textsuperscript{32}. This had already been observed for other Gracilaria species\textsuperscript{28,33}. Abreu et al. reported the ability of G. vermiculophylla to remove both ammonia and nitrate from the culture media, but with a higher affinity for the NH$_4$-N source in laboratory experiments\textsuperscript{13}. Our study showed that G. longissima had high NO$_3$-N uptake rate when both NH$_4$-N and NO$_3$-N were present in the recirculating system. Marinho-Soriano et al. also demonstrated that G. birdiae showed high efficiency in NO$_3$-N removal with no apparent inhibition due to the higher NH$_4$-N concentration\textsuperscript{18}.

Another criterion for choosing a proper biofilter for IMTA is the growth rate of the seaweed. A higher growth rate means a higher biomass yield, i.e. a better economic benefit. Our results indicated that cultivation density negatively affected the growth rate of G. longissima which was in accordance with previous results reported by Zhou et al.\textsuperscript{16} and Abreu et al.\textsuperscript{13}. Generally speaking, culturing seaweeds in IMTA system could promote higher productivity levels and with less variability than natural seaweed beds due to higher and more constant nutrients availability\textsuperscript{34}. However, the growth rate of G. longissima in our experiment was lower than that of the same species cultivated in a semi-closed\textsuperscript{22,35} and an enclosed sea area\textsuperscript{21}. The NH$_4$-N, NO$_2$-N, NO$_3$-N and PO$_4$-P concentrations in our recirculating systems were higher than those in field studies\textsuperscript{21,22}. Besides, the tissue N of G. longissima in this study was higher than the mean critical N value (the tissue N concentration needed to sustain maximum growth) reported for different macroalgae\textsuperscript{36}, indicating that the growth of G. longissima was not limited by nitrogen resource. Low growth rate of G. longissima in our experiment could attribute to light-limited by self-shading.

An efficient algal-based integrated mariculture system maintains optimal standing stocks of all cultured organisms, considering the respective requirement of each for water and nutrients and the respective rate of excretion and uptake of the important solutes by each of them\textsuperscript{4}. In the present study, though treatment 2 had higher nutrient removal efficiencies and supported more biomass than treatment 1, the latter had higher ratio of benefit to cost. To optimize an IMTA system more attention should be given not only on the nutrient removal efficiency, biomass yields but also on the food production efficiency. More studies need to done on co-cultivation proportion and co-operation relationship among the extractive species and fed species.

High nutrient removal efficiency of G. longissima ensured the recirculating system in good conditions. Amount of water consumed was small in our recirculating systems and there was zero discharge of wastewater with high nutrient concentrations. Besides it could reduce operating costs because it was unnecessary to heat large amounts of cold seawater\textsuperscript{6}. However, the RAS is not very common in many areas and currently accounts for a small fraction of worldwide aquaculture production because of its revenue and system instability. Uncontrollable fluctuations in nutrient concentrations, populations and their performance are consequences of dynamic properties of a recirculating system\textsuperscript{27,38}.

**Conclusion**

Ecological and economical cost-benefit analysis showed that G. longissima could be an efficient component of a RAS with environmental as well as potentially economic benefits for the fish farm. Results of present investigation suggested a practical solution to major managements and environmental problems of intensive land-based mariculture. Although macroalgae-based RAS may not be economically viable for some species and locations, it is the method of choice under certain conditions.

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