

Effects of salinity, light intensity and sediment on growth, pigments, agar production and reproduction in *Gracilaria tenuistipitata* from Songkhla Lagoon in Thailand

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SUMMARY

The effects of salinity, light intensity and sediment on *Gracilaria tenuistipitata* C.F. Chang & B.M. Xia on growth, pigments, agar production, and net photosynthesis rate were examined in the laboratory under varying conditions of salinity (0, 25 and 33 psu), light intensity (150, 400, 700 and 1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and sediment (0, 0.67 and 2.28 mg L^{-1}). These conditions simulated field conditions, to gain some understanding of the best conditions for cultivation of *G. tenuistipitata*. The highest growth rate was at 25 psu, 700 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ with no sediments, that provided a 6.7% increase in weight gain. The highest agar production (24.8 ± 3.0 %DW) was at 25 psu, 150–400 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ and no sediment. The highest pigment contents were phycoerythrin (0.8 ± 0.5 $\text{mg g}^{-1}\text{FW}$) and phycocyanin (0.34 ± 0.05 $\text{mg g}^{-1}\text{FW}$) produced in low light conditions, at 150 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. The highest photosynthesis rate was 161.3 ± 32.7 $\text{mg O}_2\text{ g}^{-1}\text{DW h}^{-1}$ in 25 psu, 400 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ without sediment in the short period of cultivation, (3 days) and 60.3 ± 6.7 $\text{mg O}_2\text{ g}^{-1}\text{DW h}^{-1}$ in 25 psu, 700 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ without sediment in the long period of cultivation (20 days). The results indicated that salinity was the most crucial factor affecting *G. tenuistipitata* growth and production. This would help to promote the cultivation of *Gracilaria* cultivation back into the lagoon using these now determined baseline conditions. Extrapolation of the results from the laboratory study to field conditions indicated that it was possible to obtain two crops of *Gracilaria* a year in the lagoon, with good yields of agar, from mid-January to the end of April (dry season), and from mid-July to the end of September (first rainy season) when provided sediment was restricted.

Key words: agar production, *Gracilaria tenuistipitata*, growth, light intensity, salinity, sediment, Thailand.

INTRODUCTION

Gracilaria spp. show the highest commercial value among seaweeds at present because they are the most

important raw materials for producing agar and they are relatively easy to farm. *Gracilaria* species contribute 50% of the world's agar, which has a world market value of US\$ 82.2 million (McHugh, 2003) In Thailand, although wet and dry *Gracilaria* production has reached 80 tons and 20 tons per year, respectively, the commercial requirement is for more than 2400 tons (Tachanaravong and Daroonchoo, 1988). Songkhla Lagoon, which is mesotrophic and the largest natural lagoon in Thailand, is an important source of *Gracilaria*, especially the harvests from both wild and planted beds at Koh Yor. However, in recent times the yields of *Gracilaria* have declined because of developments in and near the lagoon, which have dramatically changed the salinity and turbidity (Angsupanich & Rakkheaw 1997; Angsupanich & Kuwabara 1999; Panapitukkul *et al.* 2005).

Salinity is an important factor for photosynthesis, respiration, and growth of *Gracilaria* spp (Israel *et al.* 1999; Li-hong *et al.* 2002). Lower salinities often inhibit growth of seaweeds, affect branching patterns and promote changes in their chemical composition (Ekman *et al.* 1991; Choi *et al.* 2006). Light intensity is also important for photosynthesis, and ultimately for all their biological processes. The quality and quantity of light depends on the depth, the density of particles in the water (turbidity) and day length (Lobban *et al.* 1985). The ability of seaweeds to absorb light energy varies, depending on their quantity of pigment and density of their photosynthetic units (Darley 1982). Adaptation is always important for the algae populations at all depths. Red algae adapt well to variations in light. The ratio of phycoerythrin increases with increased depth or low light intensities, and is a good example of chromatic adaptation (Beer & Levy 1983; Carnicas *et al.* 1999).

An increase of sediment load has been recognized as a major threat to marine biodiversity on a global scale.

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Changes in sedimentation caused by deforestation, agriculture, coastal developments, construction, mining, drilling and dredging have been especially problematic for aquaculture. Sediments influence the macroalgal community and their associated animals (Airoldi 2003). Generally, organisms that rely upon sexual reproduction are more vulnerable than those using vegetative means, probably due to the lack of substrate stability and the likelihood of smothering of new recruits (Chapman & Fletcher 2002; Airoldi 2003). On the other hand, organisms with sediment trapping morphologies, opportunistic species, and those with good physical adaptations because sediments could tend to dominate in sediment-affected environments (Prathep *et al.* 2003).

The aims of this study were to assess the effects of salinity, light intensity and sediment on the growth, biomass, pigment content, rate of photosynthesis, respiration rate, and the agar content, of *Gracilaria tenuistipitata* C.F. Chang & B.M. Xia. In addition, it was hoped to provide sufficient information that would allow us to encourage *G. tenuistipitata* cultivation in the Songkhla Lagoon, where most cultivation for the production of *Gracilaria* occurs. The baseline information obtained will be extrapolated to establish the ideal conditions for periods for cultivation in the wild or to simulate such conditions in cultivation ponds.

MATERIALS AND METHODS

Study site

The studies were carried out at Koh Yor (94°29'12", 5°58'30"), a 15 km² island in the lower part of Songkhla Lagoon, Songkhla Province, Southern Thailand. The site has an abundance of *Gracilaria* spp.; and previously was the main cultivation area for *Gracilaria* in Thailand. There are two dominant seasons. The dry season is dominated by the Southwest monsoon (February to April) and the rainy season, which can be separated into two parts the first being May to September, dominated by the Southwest monsoon, and the second October to January which is dominated by the Northeast monsoon).

Salinity in the lagoon is a function of the balance of river water and seawater from tidal flux. Thus, salinity increases during periods of high tides. However, coastal developments have changed the balance of freshwater and seawater and caused a change of salinity within the lagoon. There are two species of *Gracilaria* in this area: *G. tenuistipitata* C.F. Chang & B.M. Xia and *G. percurrens* (Abbott) Abbott. Another dominant species of this closely related group was *Hydropuntia (Gracilaria) fisheri* (B.M. Xia & I.A. Abbott) M.J. Wynne (Lewmanomont & Ogawa 1995). They occur on both the soft bottom and the hard substrates in the shallow bay from depths of 0–5 m. The *Gracilaria* population is mostly laid loose on

the soft bottom substrate, but they can be found on fishing gear or plastic bags, that provide some hard substrates.

Field study

Salinity, light intensity, sediments and sedimentation, temperature, transparency and algal abundance were recorded monthly around the coast of Koh Yor from 2006 to 2007, at four stations, where *Gracilaria* was observed to be present both seasonally and spatially regardless of water quality within the lagoon. Salinity, light intensity, temperature and transparency were measured by five replications using a refracto-salinometer, a light meter (LI-250A, LI-COR, Lincoln, NE, USA), a thermometer and a secchi disk, respectively. The water samples were measured at around 3 m depth, 10 cm above the bottom where *Gracilaria* is normally found at the highest tide. Suspended sediment traps, five per site set 50 cm above the bottom collected sediment and water samples for 24 h after which time they were brought back to the laboratory. These data were calculated to represent the average value of environmental conditions of Koh Yor, Songkhla lake, which would provide baseline information for the laboratory study to simulate field conditions. Nitrogen and phosphorous data were provided by the National Institute of Coastal Aquaculture, Songkhla province from January to December (2006 to 2007); the average concentrations throughout the year were 0.096 and 0.281 mg L⁻¹ respectively.

Sediment from the traps was washed and dried at 30°C for 6 days; 10% H₂O₂ (v/v) was added and left for 24 h to remove organic matter. Then the H₂O₂ was removed by evaporation by boiling and the sediments rinsed with distilled water. Grain sizes were investigated using a particle size analyzer (LS 13 320, Beckman Coulter, Miami, FL, USA) and dried again at 60°C for 24 h (Buakaew 2005) and weighed. Total sediment is presented, but the grain size is not shown; however, the majority of the sediment was fine silt (11–16 µm). These sediments were kept and used in the experiment in the laboratory study.

Gracilaria tenuistipitata was collected from March to November 2006–2007. The plants were transported to the laboratory in an aerated container and washed with seawater to remove sand, mud and epiphytes, and acclimated in aerated filtered seawater tanks for 7 days under daylight, ambient temperature, and salinity of 28 psu.

Laboratory study

The experiments were established to test the effects of light, salinity and sediment under both short-term (3 days) and long-term (20 days) conditions. Approximately 3 g wet weight of *G. tenuistipitata* were incubated in 750 mL sterilized seawater at 25 ± 5°C, for 5

days to acclimatize the algae. The experiment was divided into a combination of three levels of salinity, three light intensities and three sediment levels, and five replications for each condition, with continuous aeration, there was a total of 360 bottles. The sterilized seawater (31 psu salinity), or diluted with deionized water to 25% or 0% in enriched nutrients were prepared in 750 mL flasks; pH values ranged 7.0–7.2. Exposure to light at 1000, 700, 400 and 150 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Philips, TL-D SUPER 80 58W/865 SLV/25 fluorescent lamps) (12:12 h light : dark), such as was measured in the field during a preliminary study, were provided. The amount of sediment used in each experiment was calculated so that it represented the rainy season at 2.28 mg L^{-1} , the dry season at 0.67 mg L^{-1} and the control at 0 mg, as established by the field study. Light intensity in each condition was measured in the enriched medium with and without sediment using an underwater light meter (Li-Cor, LI-250A) to ensure that the algae were cultivated in each light condition. In the high sediment treatment, fans were provided to help keep the temperature since light sources were close to the experimental flasks.

Algae were cultivated for 3 days (short term) and 20 days (long term). The enriched medium was renewed every 3 days by using NaNO_3 0.799 mg L^{-1} and Na_3PO_4 1.486 mg L^{-1} . Aeration was provided by bubbling with an air pump at a rate of 3.4 min L^{-1} (Daivo pump, NS 8200, Japan) to keep the sediment suspended.

Morphology and reproduction

The change of color and occurrence of the reproductive cells of *G. tenuistipitata* were observed and recorded in each condition.

Growth rate

After 20 days the algae were harvested and their wet weight determined. Biomass was calculated by the following where %WG is the percentage of weight gain, A is the weight after culture and B is the weight before culture.

$$\%WG = (A - B)/B \times 100$$

Pigment analysis

Chlorophyll *a* (90% acetone) and phycobilins (0.05 M phosphate buffer pH = 6.8) were extracted by grinding 0.3 g (for chlorophyll *a*) and 0.5 g (for phycobilins) wet weight thalli. The extracts were centrifuged at 2000 rpm for 20 min and their absorption determined for chlorophyll *a* plus phycobilins according to Jeffrey and Humphrey (1975) and for phycobilins according to Kursar *et al.* (1983) respectively.

Photosynthesis and respiration

The effect of light intensity on the photosynthetic rate in *G. tenuistipitata* was measured using 0.1 g of thallus. Samples were placed in closed chambers connected to a dissolved oxygen meter (MI 605, Martini Instruments, Rocky Mount, NC, USA) that measured $\text{mg O}_2 \text{ L}^{-1}$ (ppm). Oxygen evolution was first monitored in the dark for 15 min, then with increasing irradiance: 150 to 1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, for 5 min each at 25°C. Halogen lamps were used as a light source. The net photosynthetic rate was calculated according to the following equation (Falkowski & Raven 1997):

$$P = \frac{(P_{max} \times I)}{(I_k + I)} - R$$

where *P* is the net photosynthetic rate ($\text{mg O}_2 \text{ g}^{-1}$ dry weight (DW) h^{-1}), *I* is irradiance, P_{max} is the maximum photosynthetic capacity, I_k is the half-saturation irradiance level and *R* is the respiration rate.

Agar extraction

Agar was extracted according to the methods of Marinho-Soriano (1999). After long term cultivation, 2 g of thallus in each container were dried for 24 h at 60°C. The dried samples were added to 30 mL of distilled water, adjusted to pH 6.5 in an Erlenmeyer flask, and heated for 1 h at 110°C in an autoclave (Hiclave HVE-50, Hirayama, Indianapolis, IN, USA). The extracts were filtered through a hot glass fiber filter (Whatman GF/C, Maidstone, UK). The extracts were allowed to gel at room temperature and then placed in a freezer (15°C) overnight. The frozen gel was thawed, washed with distilled water and dried for 24 h at 60°C. The agar yield (%) was calculated as a percentage of the initial dry matter.

Statistical analyses

Statistical analyses were carried out using SPSS for Windows, Version 13.0 (SPSS, Chicago, IL, USA). One-way ANOVA was used to determine differences in subjects (year and month). A three-way ANOVA and Stepwise multiple regression were used to analyze the effects of salinity, light intensity and sediment on biomass, pigment content, agar yields, net photosynthesis and respiration of *G. tenuistipitata*.

RESULTS

Field study/environmental parameters

There were significant differences in air temperature, water temperature, salinity, transparency, light intensity and sediments over the months from January 2006 to

Environmental parameters

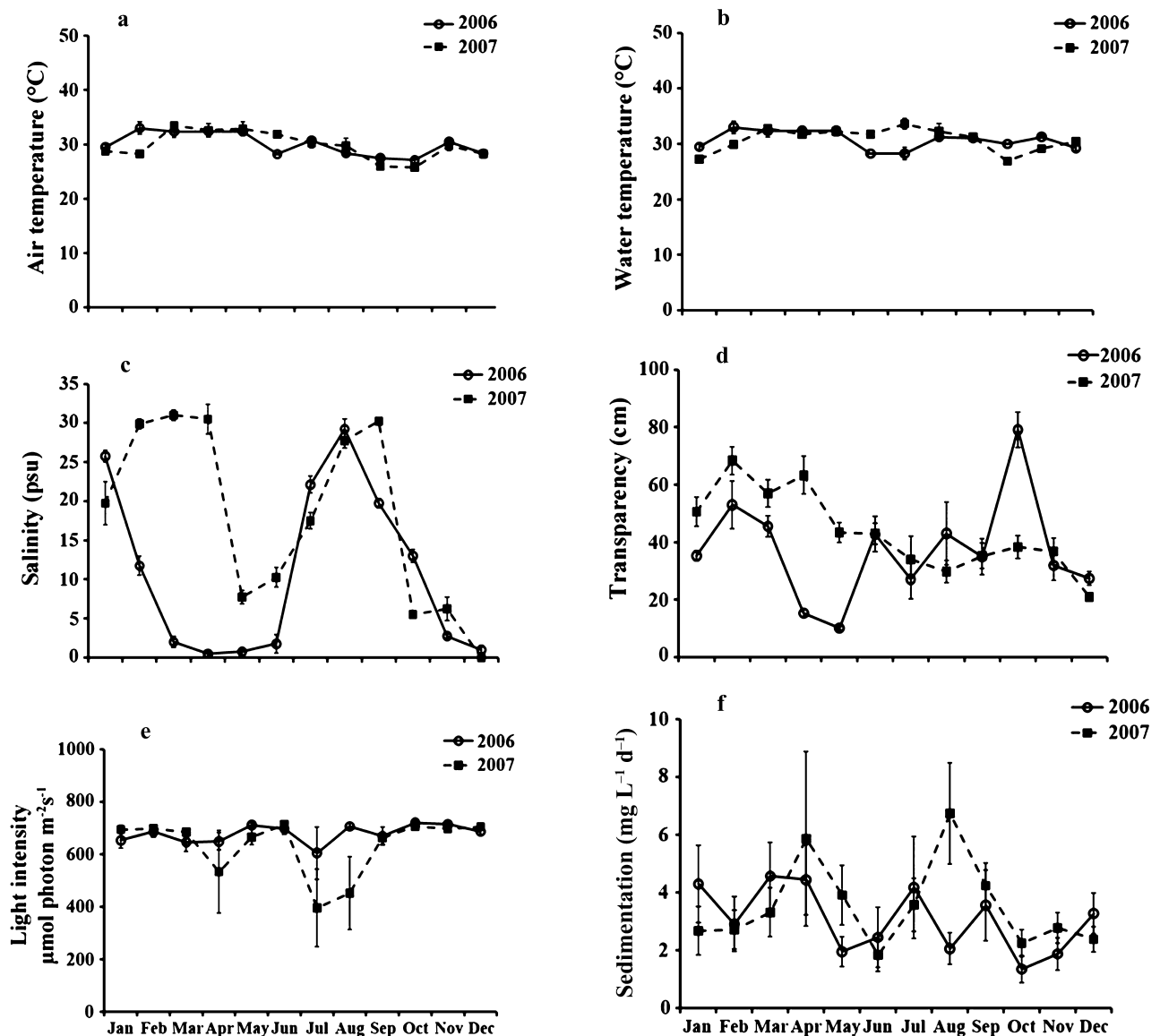


Fig. 1. The environmental parameters were investigated monthly around the lagoon coast from four stations around Koh Yor during 2006 to 2007. (a) Air temperature; (b) water temperature; (c) salinity; (d) transparency; (e) light intensity and (f) sediments ($n = 20$, mean \pm SE).

December 2007 (Fig. 1). The average air temperature and water temperature at noon was $29.9 \pm 2.6^\circ\text{C}$ and $30.8 \pm 2.1^\circ\text{C}$, respectively. Temperatures ranged from a low of 25°C from June to January (rainy season) and to a high of 36°C from February to May (dry season). The average salinity was 14.5 ± 11.6 psu. The high was 33 psu from January to April (dry season) and the low of 0 psu from May to July and October to December (rainy season) except in December 2005 to January 2006 (before the dry season) due to an unexpected heavy rainfall that decreased salinity.

There was a significant difference in transparency between seasons. This is a function of the density

of suspended particles. The high transparency was 62.9 ± 3.4 cm from February to April (dry season) and the low transparency was 32.2 ± 3.2 cm from August to December (rainy season) because of a high wave motion and increasing sediment load from run-off during the rainy season. There was a big flood in the rainy season in 2005, which caused a very low transparency of only 8.5 cm in May 2006.

The average underwater light intensity at noon was 656.0 ± 17.0 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$. The lowest was 394.6 ± 148.0 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ in July 2007 and the highest was 720.1 ± 6.3 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ in October 2006.

The average sediment was $3.3 \pm 0.1 \text{ mg L}^{-1} \text{ d}^{-1}$. The lowest was $0.05 \text{ mg L}^{-1} \text{ d}^{-1}$ in October 2006 (rainy season) and the highest was $7.2 \text{ mg L}^{-1} \text{ d}^{-1}$ in August 2007 (dry season). There were significant differences in the amounts of sediment and grain size during different months. There was an average of $3.1 \pm 0.1 \text{ mg L}^{-1} \text{ year}^{-1}$ sediment load of primarily fine silt ($11\text{--}16 \mu\text{m}$) in the lagoon.

Laboratory study

Morphology

After 20 days, the algae growing under low light intensity ($150\text{--}400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), 25 psu with no sediments became green at 0 psu and were bleached at high light intensity ($1000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Thalli covered by sediment had a darker color than those under sediment free conditions. Also they became fragile with 0 psu when combined with a high light intensity. Thalli were sturdier under 25 and 30 psu with no sedimentation conditions. No reproductive fronds were observed.

Biomass

There were significant differences in algal biomass associated with salinity, light intensity and interactions of the three factors ($P < 0.05$), whereas the sediment produced no significant differences ($P = 0.68$). The highest biomass was $137 \pm 3.6\%$ in the sediment-free conditions, at 25 psu and $700 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$, while the weight decreased, $-15.8 \pm 2.3\%$, with no sediment, 0 psu and $700 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ conditions. The biomass was lowest when cultured in fresh water (0 psu) combined with high light intensity ($400\text{--}1000 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$) under sediment-free conditions (Fig. 2). Stepwise multiple regression analysis revealed that the biomass shows a positive correlation with salinity and light intensity (Table 1).

Agar

There were significant differences in agar production associated with the interactions between salinity, light intensity and the amount of sediment ($P < 0.05$). The highest yield was $24.8 \pm 3.0 \%$ DW when cultured with no sediment, 0 psu and under $1000 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ conditions. The lowest was $4.0 \pm 0.6 \%$ DW when cultured with a high sediment, 25 psu, and $150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ condition (Fig. 2). Moreover, agar production was highest when cultured with no sediment combined with low salinity (0 psu). Stepwise multiple regression analysis revealed that the agar production shows a positive correlation with light intensity but negative correlations with salinity and sediment (Table 1).

Pigment content

Chlorophyll *a* levels differed significantly with varying conditions of salinity, light intensity and sediment as seen in the interactions of the three factors ($P < 0.05$). It increased to $0.2 \pm 0.02 \text{ mg g}^{-1}$ fresh weight (FW) with no sediment, 0 psu and $700 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ condition and then decreased to $0.02 \pm 0.002 \text{ mg g}^{-1}$ FW in low sediment, 0 psu and $150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ conditions. Chlorophyll *a* was highest when cultured under low light ($150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$) and at low salinity (Fig. 3a). The thalli also looked darker. Chlorophyll *a* showed negative correlations with salinity and light intensity (Table 1).

There was a significant difference in the phycoerythrin content associated with sediments and interactions among the three factors ($P < 0.05$), whereas differences in salinity and light intensity produced no significant differences ($P = 0.80$ and $P = 0.09$, respectively). It increased to $0.8 \pm 0.04 \text{ mg g}^{-1}$ FW in a high sediment, 31 psu and $150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ conditions and then decreased to $0.02 \pm 0.003 \text{ mg g}^{-1}$ FW in a low sediment, 0 psu and $150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ conditions (Fig. 3b). The phycoerythrin content was higher than the chlorophyll *a* and phycocyanin content when cultured in low light ($150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$). Stepwise multiple regression analysis showed that there was no correlation between the phycoerythrin content and other factors (Table 1).

There was a significant difference in the phycocyanin contents associated with light intensity, sediments and their interactions ($P < 0.05$), whereas salinity had no significant effect. The highest phycocyanin was $0.34 \pm 0.05 \text{ mg g}^{-1}$ FW when cultured in high sediment, 31 psu and $150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ condition. The lowest phycocyanin was $0.014 \pm 0.002 \text{ mg g}^{-1}$ FW when cultured in a low sediment, 0 psu and $150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ condition (Fig. 3c). Also, the phycocyanin content increased when cultured at a low light intensity ($150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$) combined with a low salinity and high amounts of sediment. The phycocyanin content showed a negative correlation with the light intensity but a positive correlation with sediment (Table 1).

Net photosynthesis

There was no significant difference in the net photosynthesis rate by thalli associated with sediment, light intensities and their interactions ($P = 0.07$, $P = 0.11$ and $P = 0.09$), whereas differences in salinity and interactions of the three factors showed significant differences over a short culture period ($P < 0.05$ and $P < 0.01$, respectively); and there was no significant differences of any factors when cultured over long periods ($P = 0.44$).

In the short period cultivation, the highest net photosynthesis was $161.3 \pm 32.6 \text{ mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ when

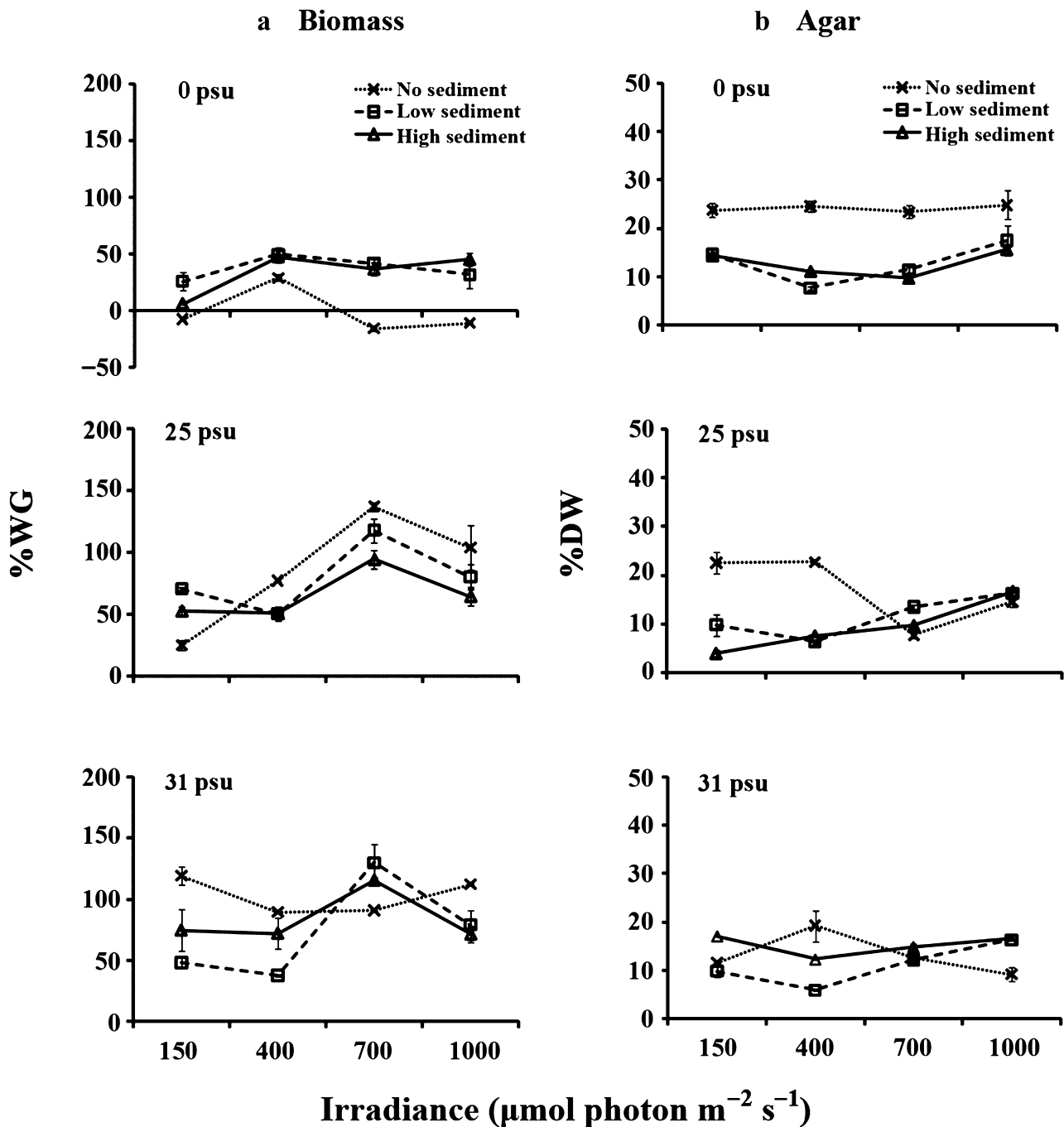


Fig. 2. Effects of salinity, light intensity and sediment on the biomass (% WG, weight gain) and agar production (% DW, dry weight) of *Gracilaria tenuistipitata* after 20 days. (a) Biomass; (b) agar production ($n = 5$, mean \pm SE).

cultured with no sediment, 25 psu and 400 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ condition and the lowest was $-27.6 \pm 3.6 \text{ mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$, when cultured with no sediment, 0 psu and 150 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ conditions. After the long period of cultivation, the highest was $60.3 \pm 6.7 \text{ mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ when cultured with no sediment, 25 psu and 700 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ condition and the lowest was $-21.1 \pm 4.6 \text{ mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$, when cultured with a high sediment, 31 psu and 700 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$

condition (Fig. 4). Stepwise multiple regression analysis showed that the net photosynthesis rates indicates a positive correlation with the salinity but a negative correlation with the sediment after the short period of cultivation; and no correlations after the long period of cultivation (Table 1). There was no correlation between the respiration rates and other factors in the short period of cultivation, but a positive correlation with light intensity and sediment (Table 1).

Table 1. Partial correlation coefficients of the effects of environmental parameters on biomass, pigment content, net photosynthesis, respiration and agar content of *Gracilaria tenuistipitata*

Variables	Partial correlations coefficients			F	P-value	R ²	
	Salinity	Light Intensity	Sediment				
Biomass (%WG)	0.776	0.472	–	289.100	0.000	0.845	
Agar (%DW)	–0.273	0.210	–0.288	7.533	0.000	0.179	
Pigment contents (mg g ⁻¹ FW)	Chl <i>a</i>	–0.312	–0.315	–	11.449	0.000	0.179
	PE	–	–	–	–	–	–
	PC	–	–0.276	0.195	6.397	0.002	0.109
Net Photosynthesis rate (mg O ₂ g ⁻¹ DW h ⁻¹)	Short	0.263	–	–0.268	5.665	0.005	0.133
	Long	–	–	–	–	–	–
Respiration rate (mg O ₂ g ⁻¹ DW h ⁻¹)	Short	–	–	–	–	–	–
	Long	–	0.262	0.280	8.327	0.000	0.137

Chl *a*, chlorophyll *a*; Long, 20 days cultivation; DW, dry weight; FW, fresh weight; PC, phycocyanin; PE, phycoerythrin; Short, 3 days cultivation; WG, weight gain.

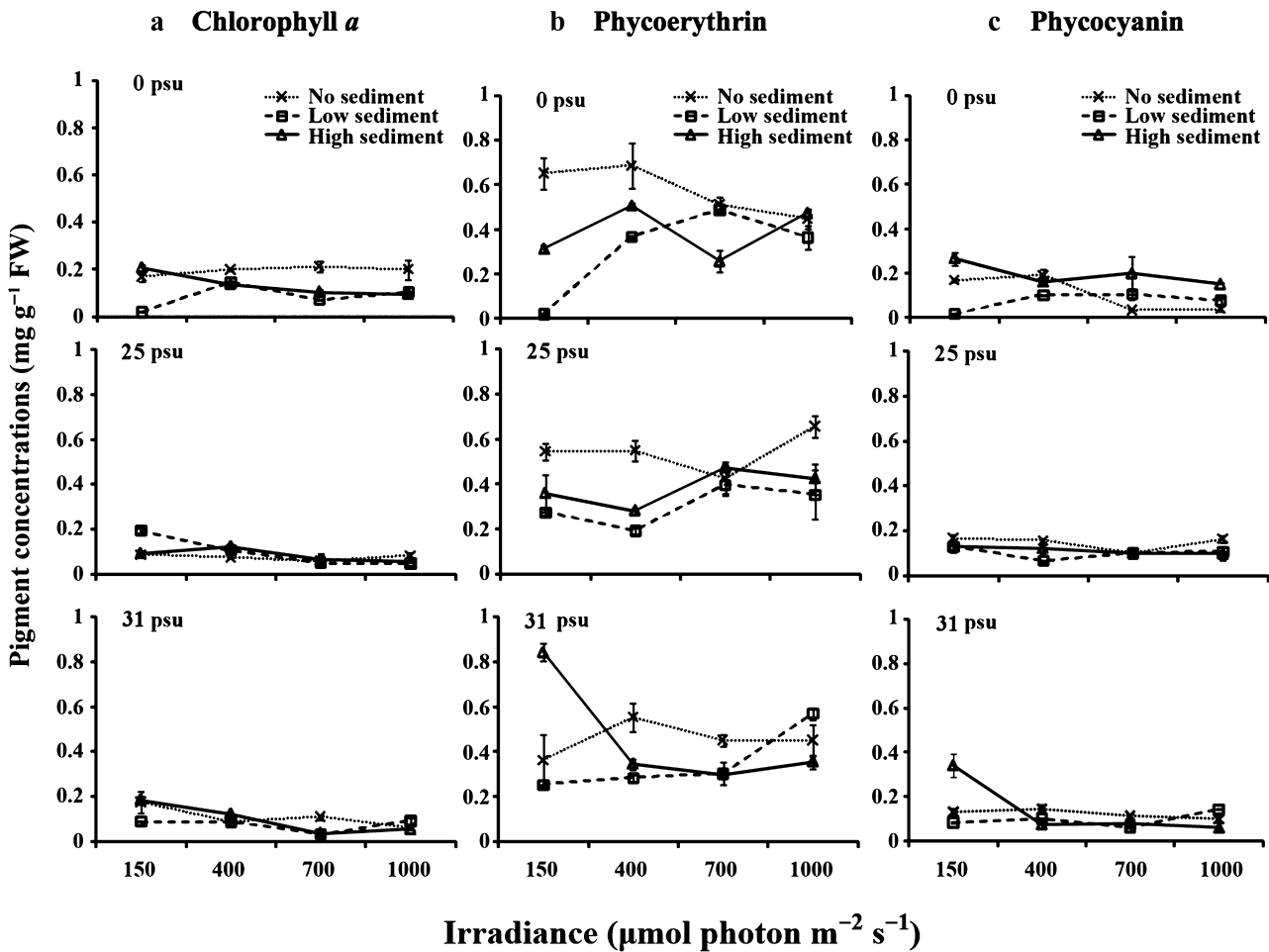


Fig. 3. Effects of salinity, light intensity and sediment on pigment concentrations (mg g⁻¹ FW) of *Gracilaria tenuistipitata* after 20 days. (a) Chlorophyll *a*; (b) phycoerythrin; (c) phycocyanin (*n* = 5, mean ± SE).

DISCUSSION

There have been numerous studies on physiological responses to combinations of abiotic factors such as

salinity and light (Israel *et al.* 1999; Li-hong *et al.* 2002; Chirapart & Lewmanomont 2004; Chirapart *et al.* 2006; Skriptsova & Nabivailo 2009). This is the first study that has included sediments in the treatment.

Net Photosynthesis

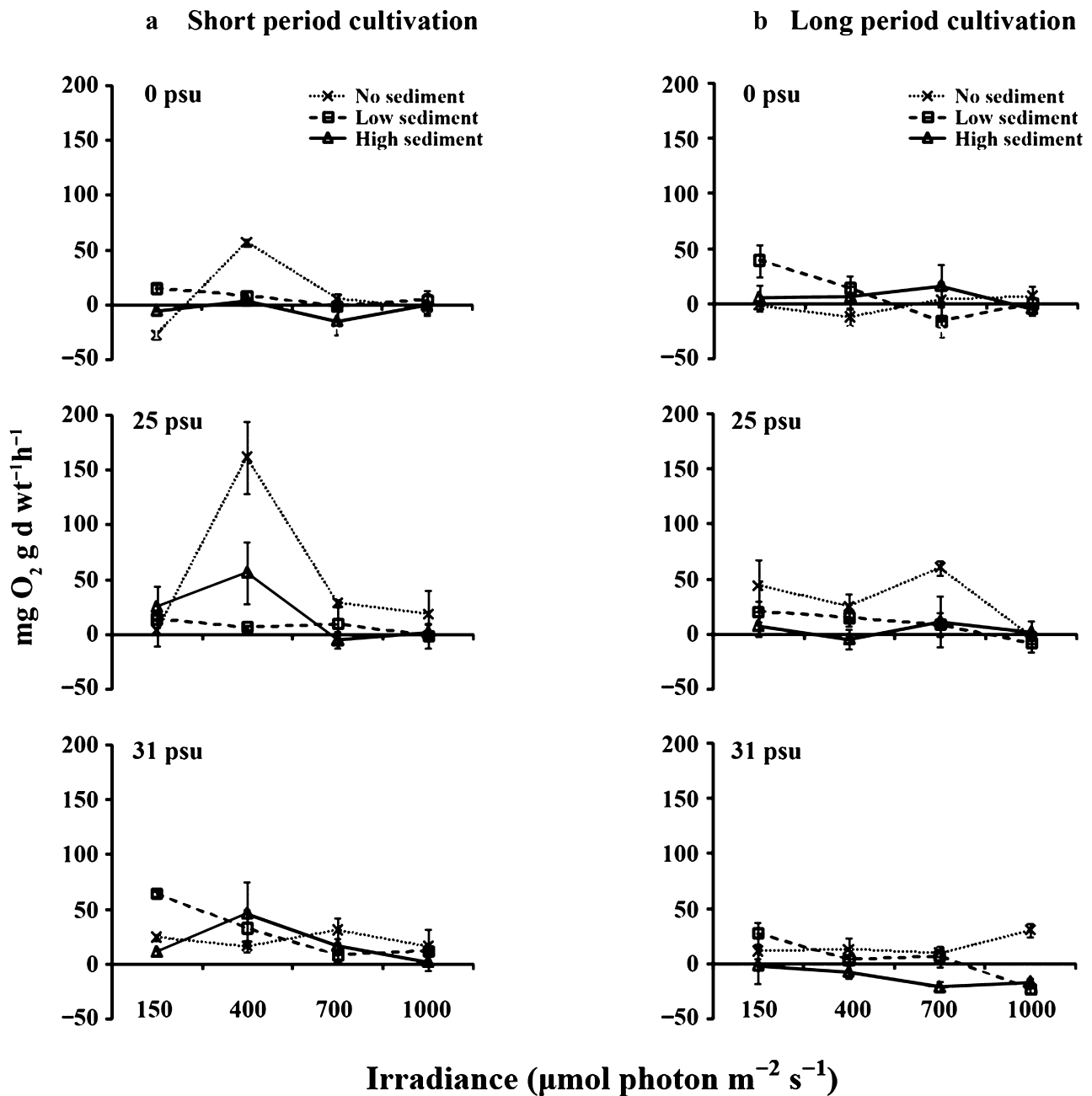


Fig. 4. Effects of salinity, light intensity and sediment on net photosynthesis of *Gracilaria tenuistipitata* after 3 and 20 days. (a) Short cultivation, 3 days; (b) long cultivation, 20 days ($n = 5$, mean \pm SE).

Variations in these three factors are common for this seaweed that lives in estuarine environments in many places such as in China and the Philippines. Sedimentation has become much more important with the increases in development and deforestation over recent decades. This study revealed that *G. tenuistipitata* showed resilience to stress under different salinities, light intensities and sediments. Salinity was, however, the most important factor affecting productivity.

In this study, the best condition for the highest daily specific growth rate (6.9% per day) was at 25 psu. This salinity was lower than the study by Yongjian *et al.* (2009), which reported a 10.25% increase per day at 24 psu. *Gracilaria tenuistipitata* grew over a wide range of salinities from as high as 39 psu (Israel *et al.* 1999), and this indicated that it was a highly tolerant species. At 0 psu, the biomass and photosynthesis rate was lowest. Other work has shown that osmotic pressure, hypo- and

hyper-salinities have caused significant reductions in the growth of many red algae as well as *Gracilaria* (Kirst 1989; Dawes *et al.* 1999; Wong & Chang 2000; Phooprong *et al.* 2007). This also led to a change in chemical structure such as pigments of the photosystem, rates of protein synthesis and storage products (Kirst 1989). The decrease of agar content was caused by inhibition of photosynthesis, carbon fixation and for the use of energy to balance the ion-exchange at lower salinities (Macler 1988; Kirst 1989; Li-hong *et al.* 2002).

Gracilaria tenuistipitata tolerated a wide range of light intensities from 150–700 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$. Increased light intensity promoted an increase in biomass but also increased respiration and decreased agar production. Generally, the optimal light for this species is 20–300 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Yongjian *et al.* 2009) but in this study, the maximum biomass was found at a higher light intensity of 700 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ and photoinhibition was observed at 1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. This is similar to other species, *G. cornea* responded optimally to irradiances between 100–800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for growth and photoinhibition occurred above 1000 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (Dawes *et al.* 1999). The pigment content (chlorophyll *a* and phycocyanin) decreased with increasing light intensity. In this study, the highest phycoerythrin content was 0.8 mg g^{-1} FW at 150 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$, higher than in the study by Beer & Levy (1983) that reported a high of 0.65 mg g^{-1} FW at 140 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$. The changes in pigmentation were due to photoacclimation. There was an increase either in the number of photosynthetic units or in the size of each photosynthetic unit when cultured under low light intensity and this was associated with an increased dark colour. The increase of metabolic processes devoted to their resources including the synthesis of photosynthetic units (Beer & Levy 1983; Lobban *et al.* 1985; Falkowski & Raven 1997), is well known in red algae (Beer & Levy 1983; Carnicas *et al.* 1999; Godinez-Ortega *et al.* 2008). Light could also influence the life history and life phase of this species (Barufi *et al.* 2010), but reproduction was not fully investigated in this study.

Sedimentation reduces underwater irradiance and can cover and bury the seaweed (Chapman & Fletcher 2002; Airoldi 2003). The photosynthesis and agar production decreased with increased amounts of sediment, whereas phycocyanin and respiration increased. This indicated that pigment production can adapt to variations in light intensity (Izagirre *et al.* 2009), as perhaps indicated by the dark red thalli. In this study, the highest phycocyanin was 0.3 mg g^{-1} FW at 150 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$. Sediment is known to inhibit photosynthetic carbon fixation (Post & Arieli 1997; Chapman & Fletcher 2002). Various changes, such as the establishment of an artificial reef and a waterfront pavilion around the lagoon

during this study, have increased sedimentation from the soft bottom clays. Sediment clearly influences the rates of photosynthesis and respiration of *G. tenuistipitata*. There was a slight increase of pigments since the sediments reduced the light. In conditions of high sediment, the sediment covers the algal surface causing anoxia, but in the lab experiments the sediment was not so crucial since aeration was provided throughout the experiment, causing it to be suspended most of the time. In contrast, in the field, the sediment accumulation was high and the bottom was covered with a sediment deeper than 40 cm. Anoxic conditions clearly occurred as indicated by the strong smell of hydrogen sulfide (H_2S). These conditions were not suitable for growth or for recruitment of the new juveniles produced months earlier. Recruitment is therefore limited on the soft-bottom but is often observed on the fishnets or any other hard substrate within the lagoon.

From this study, two crops of *G. tenuistipitata* could be produced annually by cultivation (3–4 months each per crop) in the Songkhla lagoon. The first crop, from mid-January to the end of April (dry season), with a salinity of 20 to 30 psu, and a light intensity of 700 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ and the second crop from mid-July to the end of September (first rainy season), with a salinity 17 to 30 psu, a light intensity 400–700 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ such conditions that will provide high growth and production of agar. Furthermore, baseline information from this study could be introduced to facilitate inland pond cultivation or in abandoned shrimp farm ponds.

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