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Sargassum henslowianum as a potential biofilter in mariculture farms of a subtropical eutrophic bay

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ABSTRACT

Mariculture has caused negative environmental impacts on the marine ecosystem in Dapeng Cove, Daya Bay, South China Sea. In this study, the brown macroalgae *Sargassum henslowianum* was introduced into fish and oyster farms as a biofilter to evaluate its bioremediation potential. We examined the temporal dynamics of seaweed growth, tissue nutrients (N and P), and heavy-metal contents (Cu and Zn) over a 100-day culture period (December 2012 to March 2013). The thalli of seaweed grown in the fish farm suffered greater from fouling and predation than those grown in the oyster farm. The specific growth rates by wet weight of *S. henslowianum* for the first three culture periods were all significantly higher in the oyster farm (at 6.30, 6.90, and 2.18% d^{-1}) than the rates in the fish farm (at 2.77, 4.19, and −1.16% d^{-1}). The tissue nutrients and heavy-metal contents of the seaweed at both sites all varied significantly with time. The estimated N bioaccumulation efficiency of seaweed at the oyster farm increased with time (from −34.44 to 62.91 mg $thallus^{-1}$ d^{-1}) until late February, but values for the seaweed at the fish farm (−4.70 to 7.19 mg $thallus^{-1}$ d^{-1}) increased only until late January. N bioaccumulation efficiency was significantly higher for seaweed in the oyster farm than for seaweed in the fish farm up to February 23; similar trends were found for the P, Cu, and Zn bioaccumulation efficiencies. We conclude that large-scale cultivation of *S. henslowianum* in the oyster farms of Dapeng Cove should be the first choice to alleviate eutrophication and improve water quality, and the seaweed biomass should be harvested by late February, allowing it to accumulate the optimum amount of nutrients and heavy metals from the water column.

1. Introduction

Aquaculture is increasingly significant for meeting the human need for animal protein; the global production of aquatic animals from aquaculture in 2014 amounted to 73.8 million tonnes (mt), of which more than 60% was from China (45.5 mt) (FAO, 2016). Fish and mollusk mariculture in China has accelerated in recent years, with total their production amounting to 1.2 mt and 14.6 mt, respectively, in 2014 (China Fisheries Yearbook, 2016). Fish and mollusk mariculture can also reduce the pressure on fishery resources; however, aquaculture activities can cause negative environmental impacts on marine ecosystems. For example, fish mariculture adds a continuous or pulsed release of nutrients, which can inevitably promote eutrophication in coastal waters (Troell et al., 1997; Chopin et al., 2001; Islam, 2005; Zhou et al., 2006; He et al., 2008; Yu et al., 2014a,b, 2016). Heavy metals, mainly zinc (Zn) and copper (Cu), from fish feeds and the antifouling agents used with net-pen systems can accumulate within the vicinity of fish farms and affect the water column and biota (Mendiguchía et al., 2006; Sutherland et al., 2007; Kalantzi et al., 2013; Yu et al., 2014a). Mollusk mariculture can form nutrient hot spots characterized by high carbon and nutrient fluxes, which can have a negative effect on the coastal environment and the health of the cultivated organisms themselves (Newell, 2004; Mao et al., 2006; Nizzoli et al., 2011; Yu et al., 2016); heavy-metal contamination has also been found in mollusk farms (Han et al., 1996; Kang et al., 2012; Chen et al., 2014).

Dapeng Cove is a typical mariculture area of Daya Bay, South China Sea; fish cage farming and oyster longline culture are two typical types

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of mariculture employed in this bay, and their history exceeds 20 years (Yu et al., 2016). Although the mariculture activities of Dapeng Cove have contributed greatly to local economic development, the environmental quality of the bay has deteriorated as a result of nutrient and heavy metal contamination during recent years, such as through the high nutrient load now found in sediments at the fish and oyster farms (Huang et al., 2010; Jiang et al., 2017), and eutrophication around mariculture farms during summer (Peng et al., 2012). Moreover, high levels of heavy metals (especially Cu and Zn) in the sediments have also been recorded for Dapeng Cove (Li, 2003; Ke et al., 2007; Yu et al., 2010; Liu et al., 2018).

The pollution of Dapeng Cove poses a threat to local public health, and efficient and cost-effective treatment means for the pollution emanating from aquaculture are required for the sustainable use of this bay. In recent years, the use of seaweeds as a biofilter has been identified as a sustainable approach to reducing pollution caused by mariculture. With this technology, seaweeds act as biofilters that efficiently assimilate large amount of nutrients produced by mariculture, while heavy metals from the surrounding environment can also be absorbed by the seaweed biomass (Chopin et al., 2001; Mao et al., 2006; Zhou et al., 2006; He et al., 2008; Tsagkamilis et al., 2010; Yu et al., 2014a, 2016; Ratcliff et al., 2016).

In a previous study, we identified native *Sargassum* species as ideal biofilters for co-culturing in the fish farms of Dapeng Cove because of both their bioremediation potential and commercial value (Yu et al., 2014a, 2016). Among various species, the brown macroalgae *S. henslowianum* proved to have a high bioaccumulation capacity for adsorbing inorganic nutrients and heavy metals (Yu et al., 2014a). In the present study, *S. henslowianum* was introduced into fish and oyster farms in Dapeng Cove, and the temporal dynamics of its growth, tissue nutrients, and heavy metal contents in the thalli were examined across a 100-day culture period, allowing us to evaluate the bioremediation potential of this seaweed.

2. Materials and methods

2.1. Study sites

The study was carried out at a fish farm (22°33′59″N, 114°31′18″E) and oyster farm (22°33′30″ N, 114°30′58″E) in Dapeng Cove, Guangdong, southern China, between 5 December 2012 and 29 March 2013. The fish farm occupied a total area of 30 ha; the fishes cultured by this farm (mainly *Lutjanus erythopterus*, *Epinephelus fario*, and *Rachycentron canadum*) were mainly fed with trash fish, and yielded an annual production of ~450 t (Yu et al., 2014b, 2017). The oyster farm occupied a total area of 200 ha; the oysters (*Crassostrea angulata*) were cultured on long-lines (length 200–300 m, set at 2.4-m intervals and a depth of 1 m), with total annual production reaching 1.8×10^4 t (Yu et al., 2016, 2017). Current velocities at both sites were < 10 cm s^{-1}; the water depths were 4 m and 5 m at the oyster farm and fish farm, respectively.

2.2. Seaweed collection and cultivation

Sargassum henslowianum thalli were collected during low tide from seaweed beds in Daya Bay, along the coast at Yangmeikeng (22°32′34″ N, 114°35′28″ E), about 8 km east of Dapeng Cove. The thalli collected were transferred to the laboratory at the Marine Biology Research Station of Daya Bay (114°31′12″ N, 22°33′10″ E), where they were washed thoroughly with filtered seawater to remove detritus and epiphytes. Seaweeds of similar size with intact leafy fronds and creeping stolons were selected for the experiment. Six thalli were nipped on the creeping stolons at 3–5 cm intervals, and added to 1.2-m-long, three-strand culture ropes, with a 0.5-kg weight fixed to one end of each rope. Together, the ropes and gas vesicles of the seaweed allowed all the cultured thalli to maintain a vertical position in the water column.

The *S. henslowianum* were then cultivated in empty fish cages (6-mm mesh) and along blank oyster longlines, at 1-m intervals and a depth of ~1 m. We employed 50 ropes at each study site; of these, 10 marked ropes were used for the growth measurements, and the remainder were used for the tissue measurements. The initial average length and wet weight of the marked thalli grown in the fish farm were 47.06 ± 8.46 cm $thallus^{-1}$ and 24.84 ± 8.46 g $thallus^{-1}$, respectively; the averages of those grown in the oyster farm were 46.30 ± 6.60 cm $thallus^{-1}$ and 24.78 ± 7.57 g $thallus^{-1}$, respectively. The seaweeds were all left undisturbed until sampling. After sampling, some thalli were brushed and washed with distilled water, dried at 60 °C for 2 days, and then ground to powder and stored in plastic ziplocked bags at 4 °C, for the determination of tissue composition.

2.3. Seaweed sampling and growth measurements

After deployment, the *S. henslowianum* were sampled every 2–4 weeks. All the marked seaweeds at both sites were retrieved for measurement of their growth. Specific growth rate (SGR, % d^{-1}) of the seaweed was calculated as:

$$SGR = 100 (\ln N_t - \ln N_0) / \Delta t$$

where N_t is the mean length (cm) or wet weight (g) at time t; N_0 is the initial mean length (cm) or wet weight (g); and Δt is the length of the culture period (days, d).

After taking the growth measurements, the marked *S. henslowianum* continued to be cultured at their original culture sites. Meanwhile, all the unmarked seaweeds were shaken to remove silt on their surfaces, and then seaweeds from five ropes at each site were collected and prepared for determinations of tissue composition, as described above.

2.4. Tissue composition and bioremediation efficiency

The tissue nitrogen (N) contents of the seaweed samples were measured using a Flash EA 2000 Elemental Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Tissue phosphorus (P) contents were measured following the method of Zhou et al. (2003, 2006). Heavy-metal (Cu and Zn) contents were measured after acid digestion (HNO_3 plus $HClO_4$), using a Hitachi Z-2000 atomic absorption spectrometer (Hitachi, Tokyo, Japan).

Bioremediation efficiency (BE, mg $thallus^{-1}$ d^{-1} for nutrients or μg $thallus^{-1}$ d^{-1} for heavy metals) was calculated based on the nutrient or heavy-metal accumulation capacities per thallus per day, for each culture period, as follows:

$$BE = (W_t C_t - W_0 C_0) / \Delta t$$

where W_t is the mean dry weight (g) at time t; W_0 is the initial mean dry weight (g); C_t is the mean nutrient content (mg g^{-1}) or heavy-metal content (μg g^{-1}) at time t; C_0 is the initial mean nutrient content (mg g^{-1}) or heavy-metal content (μg g^{-1}); and Δt is the length of the seaweed culture period (d).

2.5. Environmental parameters

Environmental parameters at the fish and oyster farms were monitored at slack tide during each sampling period. Water temperature, dissolved oxygen (DO), salinity, and pH at both sites were monitored directly at a depth of 0.5 m, using a YSI 6920 probe (YSI, Yellow Springs, OH, USA). Triplicate water samples were taken at the same depth for determinations of the nutrients and heavy metals in the water at each site. Nutrient concentrations (NH_4-N, NO_2-N, NO_3-N, and PO_4-P) were determined according to Grasshoff et al. (1983). Heavy-metal (Cu and Zn) contents were determined with a Hitachi Z-2000 atomic absorption spectrometer (Hitachi, Tokyo, Japan).

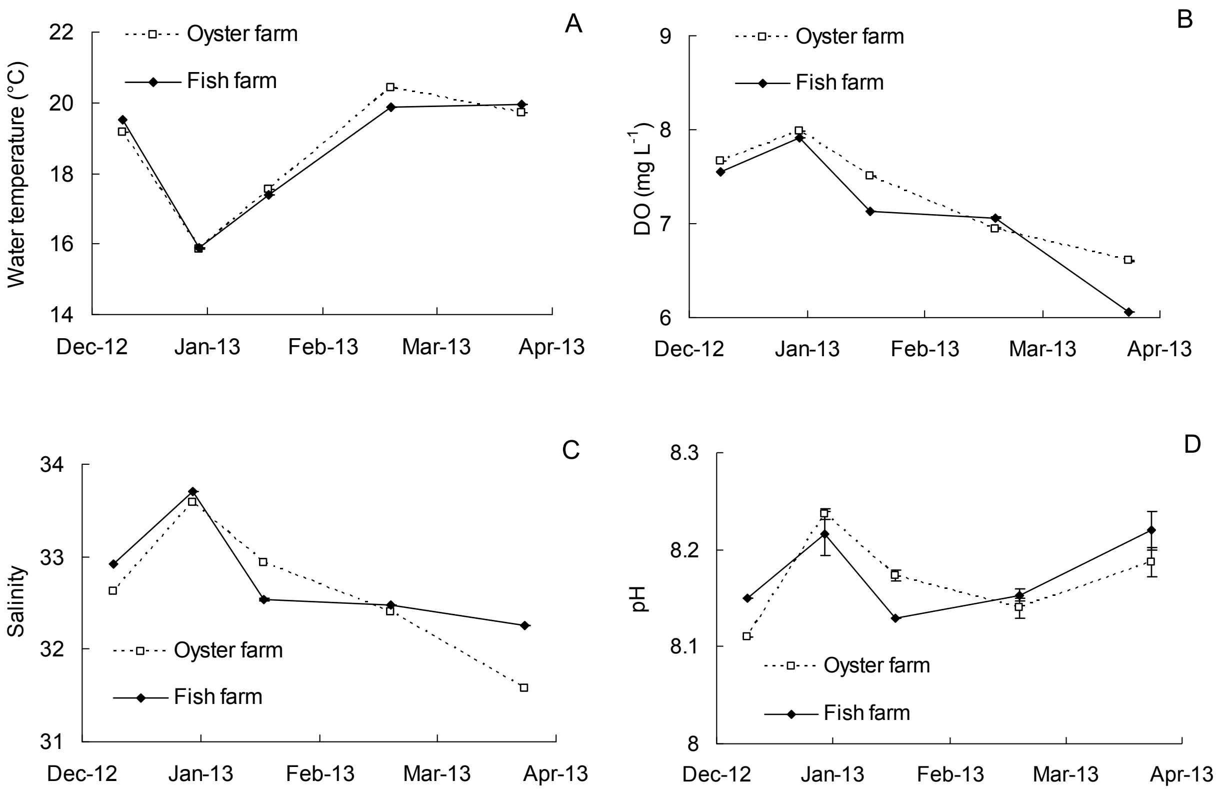

Fig. 1. Variations in water parameters at the fish and oyster farms where *Sargassum henslowianum* was cultivated in Dapeng Cove, Daya Bay, South China Sea, during the study period: (A) water temperature, (B) DO, (C) salinity, and (D) pH. Data are presented as mean ± SD ($n = 3$). In most cases, the error bars are smaller than the symbols.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 19.0 for Windows (IBM Corp., Armonk, NY, USA). Data as percentages were arcsine transformed to obtain normality prior to analysis but presented as non-transformed data. Data from the same experimental site at different sampling times were analyzed with one-way ANOVA, followed by comparisons of means by the Student–Newman–Keuls (SNK) test. Data for the two study sites at the same sampling time were compared with a Student's *t*-test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Environmental parameters

Water temperature at the two sites ranged from 15.84 to 20.44 °C during the study period (Fig. 1A). The water temperature decreased continually with time from the beginning of the experiment, and reached the lowest value as of the January 4 sampling, after which it increased and leveled off at ~20 °C as of the February samplings. Water temperature was similar at both sites for a given sampling time. DO concentrations were nearly saturated or oversaturated during the whole experimental period (Fig. 1B), and the DO level at the oyster farm was always higher than that at the fish farm for each sampling time, with the exception of February 23. Salinity levels at both sites were relatively constant across the study period (range 31.58–33.71; Fig. 1C), but with comparatively higher values during the December and January samplings, and lower values during the February and March samplings. Salinity at the oyster farm was always lower than at the fish farm, with the exception of the January 22 sampling, when salinity was slightly higher in the oyster farm (32.94) than in the fish farm (32.54). pH at the two sites ranged from 8.11 to 8.24 across the study period (Fig. 1D), with comparatively high values measured during the January 4 and March 29 samplings, although the values were similar for both sites at each sampling time.

The changes of dissolved inorganic nitrogen (DIN) (NH_4-N, NO_2-N and NO_3-N) and dissolved inorganic phosphate (DIP) (PO_4-P) concentrations at the fish and oyster farms are shown in Fig. 2. The NH_4-N levels at both study sites all showed increasing trends with time (Fig. 2A); in most cases, the levels at the fish farm (range 2.45–7.37 μmol L^{-1}) were higher than those at the oyster farm (range 1.83–9.69 μmol L^{-1}) at each sampling time, with the exception of the March samplings, when the NH_4-N levels were 0.3-times higher at the oyster farm than at the fish farm. The NO_2-N and NO_3-N levels at the fish farm were very similar for both sites at the December and January samplings, while the levels at the oyster farm were higher than at the fish farm during the February and March samplings (Fig. 2B and C). The NO_3-N levels at both sites were comparatively low at the December 15, January 4, and February 23 samplings. The PO_4-P levels at both sites showed a general increasing trend with time, and the values for the fish farm (range 0.39–0.64 μmol L^{-1}) were very similar to those for the oyster farm (range 0.38–0.66 μmol L^{-1}) at each sampling time (Fig. 2D); however, the values for the March samplings were ~0.5-times higher than those for the December and January samplings at both sites.

The DIN/DIP molar ratios of the fish farm ranged from 22.13 to 48.90 over the study period, with the maximum value observed for January 22; those values for the oyster farm ranged from 22.28 to 50.29, and the maximum value was likewise observed at the end of January (Fig. 2E). In most cases, the DIN/DIP ratios of both sites were > 24. The DIN/DIP ratios for the two study sites were similar for each sampling period, with the exception of the March 29 sampling, when the value for the oyster farm was 0.6-times higher than that for the fish farm.

The Cu concentrations varied greatly with time at both sites (Fig. 3A). The minimum values in the fish farm (1.60 ± 0.14 μg L^{-1})

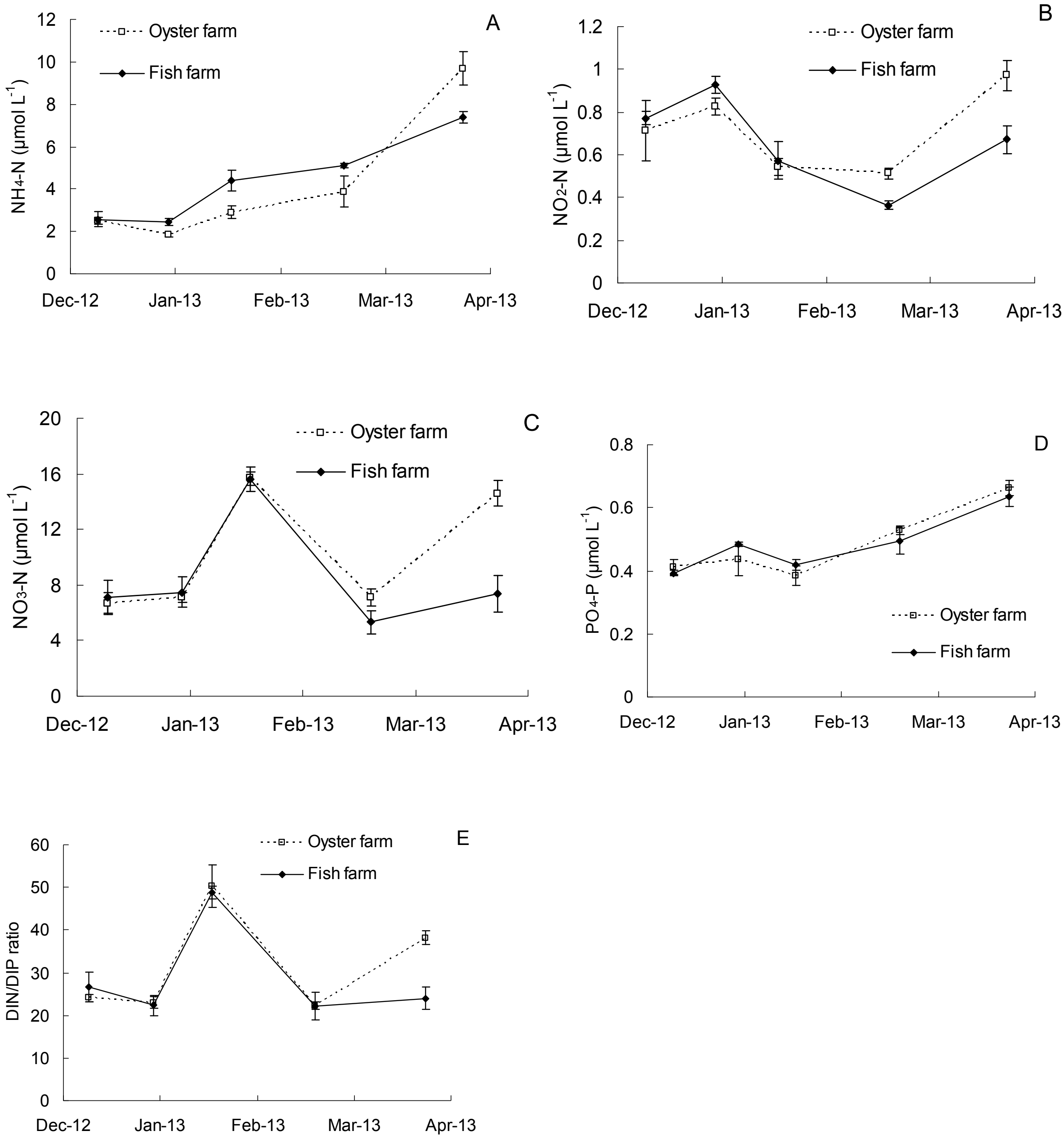

Fig. 2. Variations in nutrient concentrations at the fish and oyster farms of Dapeng Cove, China, during the study period: (A) NH_4-N, (B) NO_2-N, (C) NO_3-N, (D) PO_4-P, and (E) DIN/DIP. Data are given as mean ± SD ($n = 3$).

and oyster farm (1.70 ± 0.14 μg L^{-1}) were all observed during the January 4 sampling, while the maximum values were all observed during the January 22 sampling (3.90 ± 0.00 and 6.15 ± 0.07 μg L^{-1} for the fish farm and oyster farm, respectively). The Cu level was lower in the fish farm than in the oyster farm at each sampling time. The Zn concentrations ranged from 7.00 to 13.50 μg L^{-1} at the fish farm over the study period, while the values ranged from 5.00 to 11.00 μg L^{-1} at the oyster farm. The Zn concentrations of the oyster farm observed at the December 15 and February 23 samplings were higher than those at the fish farm; in contrast, the Zn concentrations were all lower at the oyster farm than at the fish farm during the other sampling times.

3.2. Seaweed growth

The *S. henslowianum* at both sites all grew well during the first two culture periods, and all thalli were observed as intact until the January 22 sampling (Fig. 4B and E). Following that, fouling organisms (tubeworm *Spirorbis* sp.) and herbivorous predators (isopod *Sphaeroma* sp.) became plentiful on the surfaces of the seaweeds, especially those in the fish farm (Fig. 4G); the thalli became brittle and were easily broken off as of the February 23 sampling; and the lengths of the seaweeds shorted during the last culture period (Fig. 4D). In addition, the thalli of seaweed at the fish farm were continuously covered with a layer of silt (Fig. 4E).

The specific growth rates by length (SGR_L) of the *S. henslowianum* cultured in the fish and oyster farms all varied significantly with time (one-way ANOVA, $p < 0.001$) (Fig. 5A), and the maximum values for both sites were observed during the second culture period (3.04 ± 1.19% d^{-1} for seaweed at the oyster farm, and 2.82 ± 0.77% d^{-1} at the fish farm). Negative growth rates in length were observed

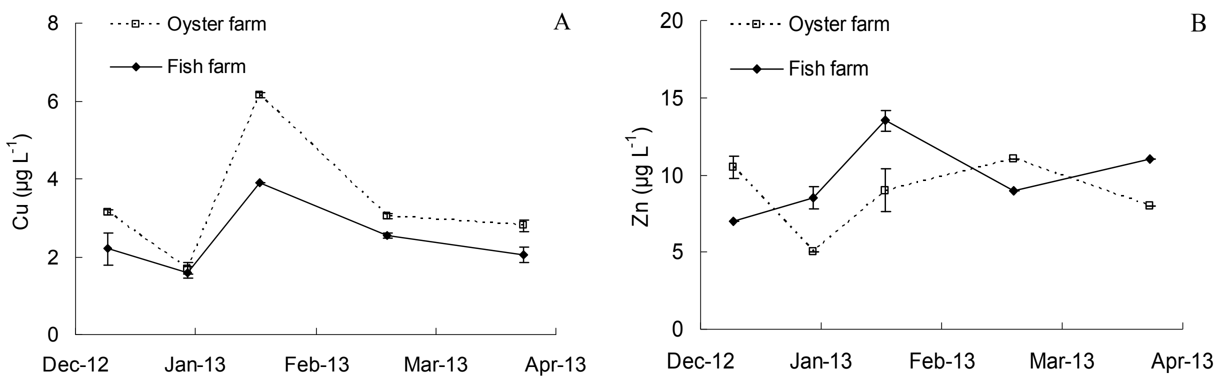

Fig. 3. Variations in heavy-metal concentrations at the fish and oyster farms of Dapeng Cove, China, during the study period: (A) Cu, and (B) Zn. Data are given as mean ± SD (n = 3).

Fig. 4. Growth measures and examples of fouling and predation of *Sargassum henslowianum* cultivated in the fish and oyster farms at Dapeng Cove, South China Sea: (A) thalli at deployment, (B–D) thalli cultured in the oyster farm, and (E–G) thalli cultured in the fish farm. Photographs were taken on (A) 15 December 2012, (B and E) 22 January 2013, (C and F) 23 February 2013, and (D and G) 29 March 2013. Arrows point to fouling organisms (tubeworm *Spirorbis* sp.) or herbivorous predators (isopod *Sphaeroma* sp.) on *S. henslowianum* cultured in the fish farm.

during the last period of this study. No significant differences were observed among the values of SGR_L for each sampling period (*t*-test, $p > 0.05$). The specific growth rates by wet weight (SGR_W) of *S. henslowianum* at both sites also varied significantly with time (one-way ANOVA, $p < 0.001$) (Fig. 5B), with comparatively high values observed during the first two culture periods, and negative growth rates found for seaweeds at both sites during the last culture period. The SGR_W of *S. henslowianum* in the oyster farm during the first three culture periods (at 6.30, 6.90, and 2.18% d^{-1}) were all significantly higher than the rates in the fish farm (at 2.77, 4.19, and −1.16% d^{-1}) (*t*-test, $p < 0.001$, $p < 0.001$, and $p = 0.008$, respectively), for the same periods; no significant difference was observed among the values for the last culture period (*t*-test, $p = 0.418$). In this study, the maximum mean fresh-weight biomass increments for the seaweeds cultured in the oyster farm and fish farm were about 23- and 3-times, respectively, over the whole study period.

3.3. Tissue composition

The tissue N and P contents and the tissue N/P ratios of the *S. henslowianum* at the study sites are shown in Table 1. The N contents of *S. henslowianum* increased at both sites with time after deployment, and the maximum values were all observed during the February 23 sampling. The N contents of the *S. henslowianum* was significantly higher at

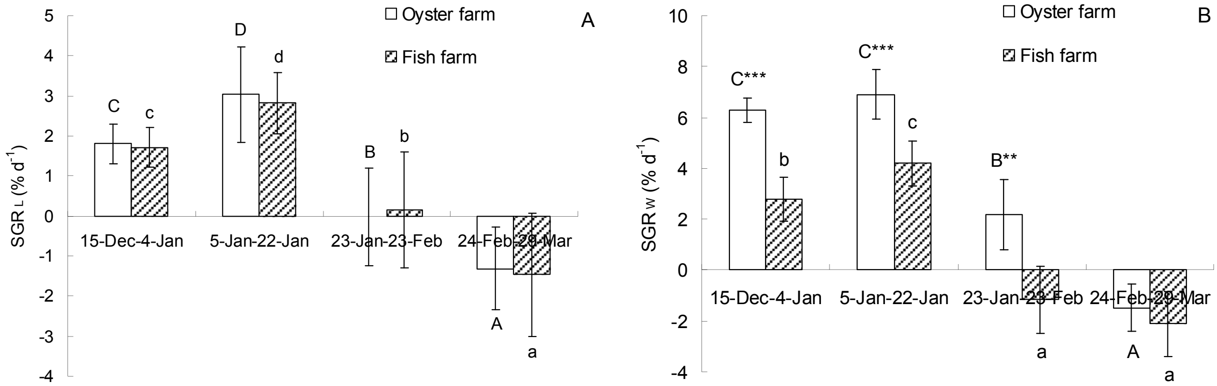

Fig. 5. Variations in growth parameters of the *Sargassum henslowianum* cultured in the fish and oyster farms of Dapeng Cove, China, over the study period: (A) specific growth rate by length (SGR_L), and (B) specific growth rate by wet weight (SGR_W). Data are given as mean ± SD ($n = 10$). Different superscript letters indicate significant differences among sampling periods at the same site; asterisks (*) indicate significant differences between sites at the same sampling period ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

the oyster farm than at the fish farm during the February and March samplings (*t*-test, $p = 0.014$ and $p = 0.024$, respectively). The P contents of the seaweeds cultured at both study sites varied significantly with time (one-way ANOVA, $p < 0.001$). The P contents of the seaweeds cultured in the oyster farm were significantly lower than that of seaweeds in the fish farm at the January 4 and January 22 samplings (*t*-test, $p = 0.045$ and $p = 0.035$, respectively), whereas the P contents of seaweeds in the oyster farm were significantly higher than that of seaweeds in the fish farm during the February and March samplings (*t*-test, both $p < 0.001$).

The tissue N/P ratios of the *S. henslowianum* cultured in both sites also varied significantly with time (one-way ANOVA, $p < 0.001$) (Table 1). The N/P ratios of seaweeds in the oyster farm (range 23.50–44.38) increased continuously with time and reached a maximum (44.38 ± 3.52) as of the February 23 sampling, and a similar trend was found in the fish farm. The tissue N/P ratios of the seaweeds cultured in the oyster farm were lower than those of the fish farm at the January 4 and January 22 samplings (*t*-test, $p = 0.031$ and $p = 0.016$, respectively), whereas the tissue N/P ratios were lower at the oyster farm than at the fish farm during the February sampling (*t*-test, $p = 0.028$).

The tissue Cu and Zn contents of the *S. henslowianum* cultured at the two sites varied significantly with time (one-way ANOVA, $p < 0.001$) (Table 2). Both the Cu and Zn contents of seaweed in the oyster farm increased with time after deployment, and the maximum values (9.17 ± 0.21 μg g^{-1} for Cu, and 31.00 ± 1.00 μg g^{-1} for Zn) were observed near the end of the study; similar trends were found for the seaweed in the fish farm. The Cu contents was significantly lower for seaweeds in the oyster farm as compared with in the fish farm for the January 4, January 22, and March 29 samples (*t*-test, $p = 0.003$, $p < 0.001$, and $p < 0.001$, respectively), but significantly higher for the February 23 samples (*t*-test, $p < 0.001$). The Zn contents was significantly lower for seaweeds in the oyster farm as compared with in the fish farm for the January 4, January 22, and March 29 samples (*t*-test, $p < 0.001$), but did not differ significantly between the two sites for the February 23 samples (*t*-test, $p > 0.05$).

3.4. Bioaccumulation efficiency

The bioaccumulation efficiencies in terms of the nutrients N and P are shown in Fig. 6. The estimated N bioaccumulation efficiency of *S. henslowianum* at both sites varied significantly with time (one-way ANOVA, $p < 0.001$). The N bioaccumulation efficiency of seaweed in the oyster farm (range −34.44 to 62.91 mg $thallus^{-1}$ d^{-1}) increased with time up to the February 23 sampling; negative values were observed thereafter (Fig. 6A). The estimated N bioaccumulation efficiency of seaweed in the fish farm (range −4.70 to 7.19 mg $thallus^{-1}$ d^{-1}) increased during the first two culture periods, but then decreased sharply after the January 22 sampling. The N bioaccumulation efficiencies of the *S. henslowianum* cultured in the oyster farm were significantly higher than those estimated for seaweed in the fish farm at each sampling period until February 23 (*t*-test, $p < 0.001$); no significant difference was found between the two sites for the last sampling period (*t*-test, $p > 0.05$). Similar trends were observed for the P bioaccumulation efficiencies (Fig. 6B).

The maximum N bioaccumulation capacities of *S. henslowianum* per culture rope (six thalli) in the oyster farm and fish farm were 16,530.20 mg and 1,166.05 mg, respectively, for the whole study period, while the maximum P bioaccumulation capacities for seaweed at the two sites were 696.25 mg and 55.09 mg per culture rope, respectively.

The Cu and Zn bioaccumulation efficiencies of the seaweeds at both sites also varied significantly with time (one-way ANOVA, $p < 0.001$) (Fig. 7). The Cu bioaccumulation efficiencies of *S. henslowianum* in the oyster farm (range −1.22 to 11.33 μg $thallus^{-1}$ d^{-1}) increased with time until the February 23 sampling, and then decreased sharply during

Table 1
Tissue N and P contents and the tissue N/P ratios of *Sargassum henslowianum* cultivated at a fish farm and oyster farm in Dapeng Cove, Daya Bay, South China Sea, over the 100-day study period. Different superscript letters indicate significant differences among sampling periods at the same site ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

Sampling date	N (%)		P (μg g^{-1})		N/P ratio	
	Oyster farm	Fish farm	Oyster farm	Fish farm	Oyster farm	Fish farm
15 December 2012	2.03 ± 0.09^{A}	2.03 ± 0.09^{a}	1916.89 ± 7.03^{C}	1916.89 ± 7.03^{d}	23.50 ± 1.11^{A}	23.50 ± 1.11^{a}
4 January 2013	2.38 ± 0.05^{A}	2.28 ± 0.10^{ab}	1667.75 ± 28.64^{B*}	1718.04 ± 9.66^{c}	31.67 ± 0.56^{C*}	29.38 ± 1.08^{b}
22 January 2013	2.74 ± 0.20^{A}	2.53 ± 0.11^{b}	1418.62 ± 51.78^{A*}	1519.18 ± 20.44^{b}	42.70 ± 2.16^{D*}	36.81 ± 1.36^{c}
23 February 2013	3.23 ± 0.22^{B*}	2.65 ± 0.11^{b}	1614.55 ± 27.80^{B***}	1123.65 ± 5.32^{a}	44.38 ± 3.52^{D*}	52.22 ± 1.99^{d}
29 March 2013	3.04 ± 0.19^{B*}	2.19 ± 0.37^{ab}	2469.43 ± 4.92^{D***}	2056.14 ± 32.61^{e}	27.32 ± 1.77^{B}	23.60 ± 3.63^{a}

Table 2
Tissue heavy-metal (Cu and Zn) contents of thalli of *Sargassum henslowianum* cultivated at a fish farm and oyster farm in Dapeng Cove, Daya Bay, South China Sea, over the 100-day study period. Different superscript letters indicate significant differences among sampling periods at the same site (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Sampling date	Cu ($\mu g\ g^{-1}$)		Zn ($\mu g\ g^{-1}$)	
	Oyster farm	Fish farm	Oyster farm	Fish farm
15 December 2012	3.90 ± 0.26^{A}	3.90 ± 0.26^{a}	8.93 ± 0.15^{A}	8.93 ± 0.15^{a}
4 January 2013	$5.47 \pm 0.17^{B**}$	6.27 ± 0.13^{c}	$12.34 \pm 0.31^{B***}$	18.26 ± 0.34^{c}
22 January 2013	$6.05 \pm 0.05^{C***}$	6.55 ± 0.05^{c}	$13.50 \pm 0.50^{C***}$	21.50 ± 0.50^{d}
23 February 2013	$6.33 \pm 0.23^{C**}$	4.87 ± 0.12^{b}	16.33 ± 0.58^{D}	16.33 ± 1.15^{b}
29 March 2013	$9.17 \pm 0.21^{D**}$	11.47 ± 0.40^{d}	$31.00 \pm 1.00^{E***}$	48.67 ± 1.15^{e}

the last period. The Cu bioaccumulation efficiencies of the seaweeds in the fish farm (range −0.82 to 1.78 μg thallus^{-1} d^{-1}) increased with time over the first two culture periods, but decreased between January 23 and February 23, and increased again during the last culture period (Fig. 7A). The Cu bioaccumulation efficiencies of the *S. henslowianum* grown in the oyster farm were all significantly higher than those observed for the fish farm at each sampling period until February 23 (*t*-test, all $p < 0.001$), whereas no significant difference was found between the two sites for the last sampling period (*t*-test, $p > 0.05$). Similar trends were found for the Zn bioaccumulation efficiencies (Fig. 7B). Overall, the temporal variation in Cu and Zn bioaccumulation efficiencies of the *S. henslowianum* were generally similar to those of the nutrients N and P.

4. Discussion

The present study showed that the growth and tissue composition of brown macroalgae *Sargassum henslowianum* cultured in a marine fish farm and an oyster farm all varied greatly with time. In most cases, the seaweeds at the oyster farm obtained a higher growth rate and had greater nutrients and heavy-metals bioaccumulation efficiencies than the seaweeds at the fish farm.

4.1. Growth

In this study, the growth rates by wet weight (SGR_W) of the *S. henslowianum* in the first two culture periods were comparable to or greater than the levels of biofiltration achieved in treating aquaculture effluents by *Gracilaria lemaneiformis* (6.73–11.03% d^{-1}), *G. chilensis* (7% d^{-1}), carrageenophytes (2.75–4.41% d^{-1}), and *S. hemiphyllum* (0.90–7.16% d^{-1}) (Troell et al., 1997; Zhou et al., 2006; Rodrigueza and Montaño, 2007; Yu et al., 2016). However, the growth rate of the seaweed at both mariculture sites decreased as of the January 22 sampling. Overall, the *S. henslowianum* grew better at the oyster farm than at the fish farm.

Growth of seaweeds is influenced by various factors, particularly light, water temperature, salinity and nutrient availability (Cronin and Hay, 1996; Yokoya et al., 1999; Zhou et al., 2006; Reid et al., 2013; Chen and Zou, 2014; Yu et al., 2016). In this study, salinity at both sites was within the optimal range for most benthic species of *Sargassum* (24–42) (Hanisak and Samuel, 1987); furthermore, the pH and DO at both sites were within the normal range of natural seawater. Therefore, these factors probably did not have a significant effect on differences in the growth of the *S. henslowianum*.

The growth rate of *Sargassum* species is reported as usually increasing with nutrient availability and water temperatures (Hanisak and Samuel, 1987; Troell et al., 1997; Yu et al., 2014a, 2016). For *S. henslowianum*, the growth rate increased as the water temperature rose from 15 to 25 °C (Chen and Zou, 2014). In this study, the water temperatures were lowest before January 4, and the measured nutrients (NH_4-N, NO_3-N and PO_4-P) were likewise at low levels during this period; between December 15 and January 4, however, the growth rates of the seaweeds were significantly higher than during the last two culture periods (see Fig. 5). This finding suggests that other factors, rather than water temperature and nutrient availability, had a stronger negative effect on the growth of *S. henslowianum*.

It has been found that self-shading has an adverse effect on the growth of seaweeds, and that macroalgal communities may switch from active production to rapid decomposition when the biomass density exceeds a critical threshold (Viaroli et al., 1996; Carton-Kawagoshi et al., 2014; Yu et al., 2016). In this study, the biomass densities of *S. henslowianum* at both sites decreased during the last culture period; this could be mainly attributed to light limitation from self-shading, or perhaps the thalli had reached a declining growth phase after February 23.

Fouling and herbivorous predators pose a significant hazard to the

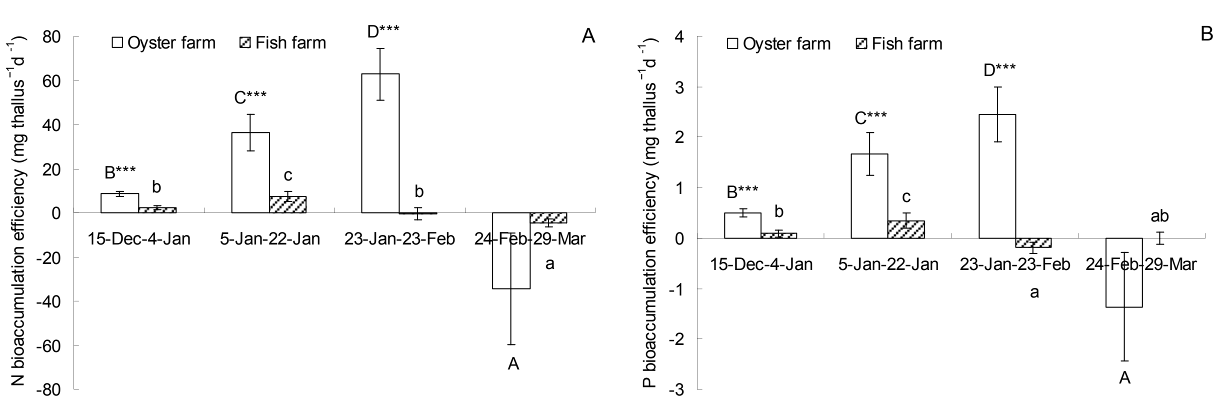

Fig. 6. Estimated nutrient bioaccumulation efficiencies of the *Sargassum henslowianum* cultured in the fish and oyster farms of Dapeng Cove, China, over the study period: (A) N bioaccumulation efficiency, and (B) P bioaccumulation efficiency. Data are given as mean ± SD ($n = 10$). Different superscript letters indicate significant differences among sampling periods at the same site; asterisks (*) indicate significant differences between sites at the same sampling period (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

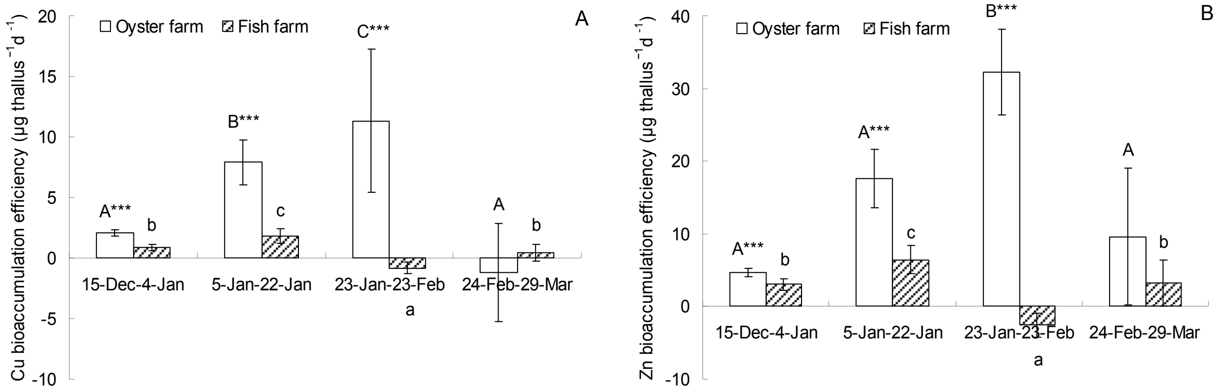

Fig. 7. Estimated heavy-metal bioaccumulation efficiencies of the *Sargassum henslowianum* cultured in the fish and oyster farms of Dapeng Cove, China, over the study period: (A) Cu bioaccumulation efficiency, and (B) Zn bioaccumulation efficiency. Data are given as mean ± SD ($n = 10$). Different superscript letters indicate significant differences among sampling periods at the same site; asterisks (*) indicate significant differences between sites at the same sampling period (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

growth of seaweeds (Fletcher, 1995; Hansen et al., 2006; Yu et al., 2016). The silt flux at the fish farm in Dapeng Cove has been found to be much higher than at other sites, while the silt concentration at the oyster farm was relatively low due to the filter-feeding activity of the oyster population (Yu et al., 2016). In this study, the *S. henslowianum* maintained in the fish farm suffered greater fouling (by siltation and tubeworms; see Fig. 4E and G) and herbivory (by isopods; see Fig. 4G) than the seaweed grown in the oyster farm; consequently, in most cases, the thalli cultured at the fish farm exhibited lower growth rates than those grown at the oyster farm.

4.2. Tissue composition

The tissue nutrient composition (N and P) of seaweeds is influenced by various environmental factors and also reflects the ability of seaweeds to sequester nutrients as a consequence of nutrient input, biological demand of the seaweed, and biomass dilution due to growth (Hanisak, 1979; Fong et al., 1998; Campbell et al., 1999; Zhou et al., 2006; Yu et al., 2014a, 2016). Seaweeds grown in high-nutrient waters display an obvious increase in their tissue nutrient contents (Rönnberg et al., 1992; Zhou et al., 2006; Yu et al., 2016). In this study, the N levels in seawater from the oyster and fish farms were comparatively high, and therefore it was unsurprising to find that the N tissue contents of *S. henslowianum* at both sites increased with time after deployment. The P level in seawater at both sites increased with time; however, the P tissue contents of *S. henslowianum* all decreased before the January 22 sampling, and this appeared to be caused by dilution during a period of high growth.

The tissue atomic ratio of N/P provides a useful index for evaluation of the nutrient status of macroalgae, and a tissue N/P ratio of 30 was found to be optimum for most marine benthic algae (Atkinson and Smith, 1983; Harrison and Hurd, 2001). A tissue N/P ratio greater than 11–24 is indicative of P limitation for macroalgae, whereas a ratio lower than 8–16 indicates N limitation (Wheeler and Björnsäter, 1992). In this study, the N contents and N/P ratios in tissues of seaweeds cultured at both sites increased with time after deployment, and the tissue N/P ratios were > 24 in most cases, which together with the high DIN/DIP ratios of seawater indicated P rather than N as the limiting factor for the *S. henslowianum* grown in the study area.

Marine macroalgae can accumulate metals from the dissolved ionic phase in seawater, thus the metal contents of seaweed tissues most likely reflects the dissolved metal concentrations in the surrounding seawater (Páez-Osuna et al., 2000; Stengel et al., 2005). It has been reported that all environmental factors regulating seaweed growth could also influence the accumulation of metals in their tissue, and the accumulation can even occur during slow growth. Alternately, a dilution of the metals accumulated may occur as a result of a higher growth rate and fast tissue expansion, thus lowering the tissue metal concentration (Stengel et al., 2005). In this study, the Cu and Zn contents in the tissues of thalli increased with time before the January 22 sampling, a period of high growth rate for the *S. henslowianum*. The Cu concentrations were invariably lower in the seaweed grown at the fish farm as compared with at the oyster farm for a given sampling time, across the entire study period, while in most cases the tissue Cu contents were higher in *S. henslowianum* cultured in the fish farm than it was in seaweeds at the oyster farm. Therefore, the temporal variation in tissue heavy metals in the seaweed at both sites cannot be attributed only to the bioavailable concentrations in the seawater and likely dilution causd by growth, but is probably influenced by other underlying factors, such as the seaweed's metal-binding ability and metabolic activity; this is a topic for further study.

4.3. Bioaccumulation efficiency

Among different measures to control environmental pollution, seaweed cultivation is receiving more and more attention because of the low cost of cultivation and its high pollutant removal efficiency (Chopin et al., 2001; Mao et al., 2006; Zhou et al., 2006; He et al., 2008; Tsagkamilis et al., 2010; Ratcliff et al., 2016; Yu et al., 2016). In this study, a more rapid growth rate combined with higher tissue nutrient contents allowed the *S. henslowianum* grown in the oyster farm to achieve better nutrient bioaccumulation efficiencies than the seaweed in the fish farm, during the first three culture periods. The sharp decrease in the nutrient bioaccumulation efficiencies of the seaweeds in the oyster farm after February was mainly a consequence of negative growth during the last culture period. Nutrient loading from cage aquaculture has been reported as 132.5 mg of N and 25.0 mg of P per 1 g of fish produced (Islam, 2005). In this study, based on the maximum N and P bioaccumulation capacities of the *S. henslowianum* across the whole culture period, we calculated that the seaweeds of each rope in the oyster farm could uptake the N or P loading from 124.8 g or 27.9 g fish mass produced, and these values were much higher than those estimated for the fish farm (8.8g or 2.2 g, respectively).

Seaweeds (either live or dead biomass) have been shown to be efficient biosorbents with an ability to bind a variety of metals (Yu et al., 1999; Anastopoulos and Kyzas, 2015; Murphy et al., 2007). Brown macroalgae have a high binding capacity of their polysaccharides for divalent metal species (Giusti, 2001; Stengel et al., 2005). In this study, although the tissue Cu and Zn contents of *S. henslowianum* grown in the oyster farm were mostly comparatively low, the metal bioaccumulation efficiencies were higher for the seaweeds in the oyster farm as compared with those in the fish farm before February 23; this was mainly attributable to the comparatively higher biomass of the seaweeds in the oyster farm during this period.

We conclude that large-scale cultivation of *S. henslowianum* in the oyster farms of Dapeng Cove should be the first choice to alleviate eutrophication and improve water quality, and the seaweed biomass should be harvested before the end of February, allowing them to accumulate an optimum amout of nutrients and heavy metals from the water column. Furthermore, as non-living seaweed biomass is more practical and favorable for the biosorption of heavy metals (Yu et al., 1999; Anastopoulos and Kyzas, 2015), we speculate that the harvested *S. henslowianum* biomass can be treated and further used as an ideal biosorbent for heavy-metal removal in wastewater treatment.

CRediT authorship contribution statement

Zonghe Yu: Writing - original draft, Conceptualization, Methodology, Investigation, Funding acquisition. **Hongyan Sun:** Data curation, Investigation, Writing - review & editing. **Wen Huang:** Data curation, Investigation. **Chaoqun Hu:** Investigation, Data curation. **Yi Zhou:** Funding acquisition, Supervision, Methodology, Investigation, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.110615.

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