Journal of Applied Phycology (2019) 31:1425–1432
https://doi.org/10.1007/s10811-018-1601-4

Integration of white shrimp (*Litopenaeus vannamei*) and green seaweed (*Ulva prolifera*) in minimum-water exchange aquaculture system

Hong-xing Ge[1,2,3,4] · Qian Ni[3] · Jian Li[2] · Ji-tao Li[2] · Zhao Chen[2] · Fa-zhen Zhao[2]

Received: 2 June 2018 / Revised and accepted: 8 August 2018 / Published online: 25 August 2018
© Springer Nature B.V. 2018

Abstract
Ulva prolifera is one of the most common macroalgae and is distributed widely off the coast of China. It is well known for its rapid growth rate and good reproduction and it has fast nitrogen removal efficiency. Integration of seaweed cultivation in shrimp farming water is considered a potential aquaculture practice since seaweeds can convert dissolved inorganic nitrogen into biomass and the biomass can be harvested easily. This study investigated the effect of integrating green seaweed (*Ulva prolifera*) with *Litopenaeus vannamei* (500 shrimp m^{-3}) at varying levels of water exchange daily on water quality and shrimp growth performance. The four daily water exchange quantities were 5% (T1), 10% (T2), 15% (T3), and 20% (T4). The appropriate range of the stocking density of *U. prolifera* was 800 mg L^{-1}. No significant differences were observed in total ammonia nitrogen (TAN) concentration between T2 and T3 ($P > 0.05$) from beginning to end. The concentrations of nitrite and nitrate in all treatment remained constant at low levels from beginning to the end. On day 35, there were no significant differences in survival rate of shrimp among T2, T3, and T4. No significant differences in FCR were observed in group T2, as compared to T1, T3, or T4. No significant differences in cumulative weight of *U. prolifera* were observed among T1, T2, and T3; however, they were significantly larger than that of T4. The study demonstrates that integrating *U. prolifera* (800 mg L^{-1}) with *L. vannamei* (500 shrimp m^{-3}) with 10% water exchange can control the water quality and enhance shrimp growth.

Keywords Seaweed · White shrimp · Water quality control · Growth performance

Introduction

Super-intensive shrimp farming has been a great success (Holl et al. 2011; Ge et al. 2016a; Pungrasmi et al. 2016). However, the environmental impacts of aquaculture activities in recent years have drawn great attention (Xu and Pan 2012; van Rijn 2013; Zhu et al. 2015). Ammonia and nitrite in aquaculture wastewater cannot only pollute the environment, but also can cause serious damage to shrimp. Reducing the negative influences of aquaculture on environment is key to ensuring the long-term sustainability and the further development of the industry (Vinatea et al. 2010; Furtado et al. 2015). Therefore, nitrosobacteria and nitrifying bacteria filtration in recirculating aquaculture system (RAS) have been employed to control ammonia (Guerdat et al. 2010; Kuhn et al. 2010a). However, nitrate nitrogen accumulation can also reduce survival and growth of shrimp (Kuhn et al. 2010b; Furtado et al. 2015). Moreover, nitrate in the wastewater without treatment may lead to environmental pollution. However, the removal of nitrate in wastewater is complicated and costly (Cahill et al. 2010; Furtado et al. 2015).

Seaweeds are another promising method to control inorganic nitrogen (Baloo et al. 2014; Ge et al. 2016a). *Ulva prolifera* is a cosmopolitan green seaweed (Sun et al. 2015)

✉ Jian Li
lijian@ysfri.ac.cn

[1] Jiangsu Key Laboratory of Marine Bioresources and Environment, Huaihai Institute of Technology, Lianyungang 222005, Jiangsu Province, China

[2] Key Laboratory for Sustainable Development of Marine Fisheries, Ministry of Agriculture; Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, Shandong Province, China

[3] Jiangsu Key Laboratory of Marine Biotechnolog, Huaihai Institute of Technology, Lianyungang 222005, Jiangsu Province, China

[4] Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Huaihai Institute of Technology, Lianyungang 222005, Jiangsu Province, China

which can use multiple nitrogen sources (Sun et al. 2015; Li et al. 2018a) and acclimatize to the changes of temperature by morphology-driven physiological and biochemical variation (Gao et al. 2016). These characteristics allow *U. prolifera* to adapt to the environment of shrimp farming (such as high water temperature, nutrient enrichment, and so on) and act as a biofilter to control ammonia concentration (Baloo et al. 2014). Therefore, green seaweeds such as *U. prolifera* have drawn great attention (Liu et al. 2009; Yabe et al. 2009). The dry matter of *U. prolifera* has high carbohydrate (62%) and protein (27%) and low lipid (0.3%) contents (Li et al. 2015; Shao et al. 2017). The polysaccharide from *Ulva* spp. has biological activities such as immunomodulatory (Kim et al. 2011; Akbary and Aminikhoei 2018) antioxidant (Shi et al. 2017; Li et al. 2018b), antimicrobial (Berri et al. 2016; Shao et al. 2017), and anticancer (Jiao et al. 2009; Murphy et al. 2014) activities. In addition, *Ulva* co-cultured with shrimp can convert ammonia, nitrite, and nitrate into biomass, and the biomass can be harvested and processed into animal feed or for human consumption (Wang et al. 2007a, b; Zhang et al. 2011; Bikker et al. 2016; Qiu et al. 2018). However, inorganic nitrogen concentration in wastewater is high and seaweed can only partially remove the inorganic nitrogen. In the present study, *U. prolifera* was selected as a biofilter. The appropriate quantity of integrating *U. prolifera* with white shrimp was determined first, and then, the effects of integrating *U. prolifera* with white shrimp (500 shrimp m^{-3}) at varying water exchange levels on water quality and shrimp growth performance were explored. The growth performance of *U. prolifera* was also monitored.

Materials and methods

Source of seaweed and shrimp

The green seaweed *U. prolifera* was provided by Xiangshan Xuwen Seaweed Development Co., Ltd. in Zhejiang province. The seaweed was cultured in sterile culture and the branch length was 2.51–3.03 cm. Specific pathogen-free *L. vannamei* (PLs 5) was obtained from a commercial breeding station (Chia-Tai, Hainan) and the PLs were cultured at a stocking density of 1000 shrimp m^{-2} in a nursery tank until the shrimp reached a mean weight of 3.45 g.

Experimental set up

Preliminary experiment: Effect of *U. prolifera* on water quality of white shrimp reared in the zero water exchange system zero exchange water

To determine appropriate range of *U. prolifera* in the shrimp culture system, a preliminary trial of controlling water quality by *U. prolifera* was conducted. Seventy-five shrimp were stocked at a density of 500 shrimp m^{-3} in PVC tanks ($r = 0.245$ m, h = 1 m, water depth = 0.8 m). The initial weight of shrimp was 3.01 ± 0.11 g. The preliminary trial consisted of six treatments in triplicate and the densities of *U. prolifera* were 0, 200, 400, 600, 800, and 1000 mg L^{-1}. According to Khoi and Fotedar (2011) and Wang et al. (2010), to make full use of light and avoid eating by shrimp, *U. prolifera* were cultured in floating cages which were a strung confinement of PVC rods ($30.0 \times 25.0 \times 20.0$ cm) (Fig. 1). The outer walls of the cages were wrapped with multifilament nets with mesh number of 20. Shrimp cultured without *U. prolifera* were used as the control. During the trial, no water was exchanged. The seawater (salinity 32.5) was disinfected with chlorine bleach, and then, the waste chlorides were removed by deep aeration. Water temperature and pH were 28.5 °C and 8.7, respectively. Dissolved oxygen (DO) remained above 5.0 mg L^{-1} during the experiment. Light was provided by LED and natural light, and light intensity of water surface remained above 30 μmol photons m^{-2} s^{-1} with a 16 h light/8 h dark cycle (Khoi and Fotedar 2011). Shrimp were fed four times a day at a rate of 5% body weight with a commercial feed containing 42% crude protein and 6.5% crude fat (Fuxing, Xiamen) and cultured for 96 h. The daily feed amount was adjusted to the biomass in the tanks and the feeding level was determined at 5% on wet body weight weekly. At 1 h after feeding, uneaten food and feces were siphoned out and filtered through a filter with a mesh number of 200 and then the filtrate water was returned to the same PVC tank. To replace evaporative losses, water was added as needed. Water samples were collected at 24, 48, 72, and 96 h to determine total ammonia nitrogen (TAN), nitrite nitrogen (NO_2–N), and nitrate nitrogen (NO_3–N) following Ge et al. (2016a).

Main experiment set up

The appropriate range of the stocking density of *U. prolifera* was maintained considering the water quality (especially ammonia removal efficiency) and the utilization efficiency of light. Considering this, at the main experiment, the stocking density of *U. prolifera* was 800 g fresh weight m^{-3} and the density of shrimp was 500 shrimp m^{-3}. The initial weight of shrimp was 3.45 ± 0.13 g. The initial ratio of seaweed biomass to shrimp biomass was 800 g m^{-3} of fresh seaweed for 1.725 kg shrimp m^{-3}. To test the effects of *U. prolifera* and the different quantity of water exchange on water quality control and shrimp growth, four treatments were set up in triplicate: 5% (T1), 10% (T2), 15% (T3), and 20% (T4) of exchanged water daily as well as partial water which was exchanged at 1 h after each feeding. The materials and

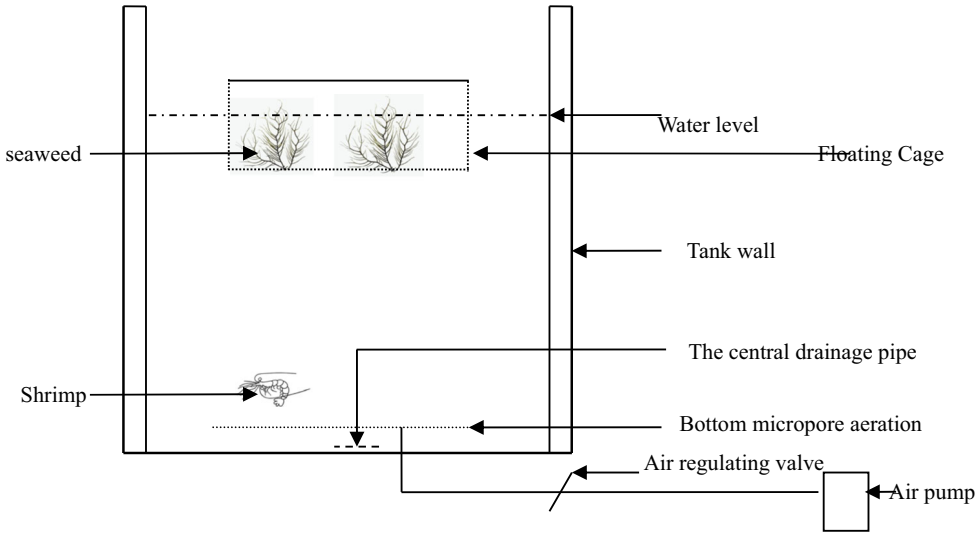

Fig. 1 Plain view of the aquaculture system

aquaculture facilities were the same as the preliminary experiment. To remove uneaten food and feces from the floating cages and *U. prolifera*, the floating cages and *U. prolifera* were removed from the tanks every 3 days, flushed with water, and then weighted to maintain the fresh weight *U. prolifera* at 800 mg L^{-1}. Then, the floating cages and *U. prolifera* were replaced in the same tank. The feeding management of shrimp was the same as the preliminary experiment.

Data collection

During the experiment, water temperature, dissolved oxygen (DO), salinity, and pH value were determined daily with an YSI Model Handheld Instrument (YSI Incorporated, USA) at 18:00. Total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), and nitrate nitrogen (NO_3-N) were determined weekly following Ge et al. (2016b). After the rearing test, all shrimp in each tank were counted and weighed and *U. prolifera* in tank was weighed. The metrics were calculated according to the equations: growth rate (g $week^{-1}$) = weight gain (g)/culture weeks; cumulative weight (g) = final weight (g)–initial weight (g); survival (%) = [(number of initial shrimp–number of dead shrimp)/number of initial shrimp] × 100; FCR = total dry feed intake (g)/weight gain (g), and specific growth rate (SGR, % day^{-1}) = 100 × ln [final weight/initial weight]/culture time (day).

Statistical analysis

All data are expressed as mean ± standard deviation (SPSS 17.0). A one-way analysis of variance (ANOVA) and Tukey's tests were applied to determine significant difference among treatments. If significant differences were found, Duncan's multiple comparison was applied at a 5% significance level.

Results

Effect of *U. prolifera* on water quality of white shrimp reared in the zero water exchange system

A large number of shrimp in the control group and some *U. prolifera* in the group of 1000 mg L^{-1} were found dead in 96 h. During the experiment, the concentration of nitrite nitrogen and nitrate nitrogen was too low to determine, while TAN concentration in all treatments increased over time (Fig. 2). TAN in the control rose sharply from start to finish. At the same time, TAN concentration decreased with the increase of *U. prolifera* quantity and TAN concentration in the 400-, 600-, and 800-mL L^{-1} groups was significantly lower than those of the control group all the time ($P < 0.05$). The concentrations of TAN in the 1000-mg L^{-1} group from 24 to 72 h were significantly lower than those of the control group ($P < 0.05$). However, TAN in the 1000-mg L^{-1} group rose sharply from 72 to 96 h. TAN in the 800 mg-L^{-1} group that was lower than in the other groups from beginning to end, and at 48 and 72 h, TAN concentrations were significantly lower than those of the 200-, 400-, and 600-mg L^{-1} groups ($P < 0.05$).

Effect of *U. prolifera* and quantity of water exchange on water quality

Water quality during the experiment is shown in Table 1. Salinity, water temperature, pH, and dissolved oxygen (DO) were relatively stable. The concentration of nitrite and nitrate in all treatment remained at very low level from beginning to the end.

TAN in all treatments showed an increasing trend over time (Fig. 3). At the same time point, TAN concentration showed a decreasing trend as the water exchange quantity increased. On

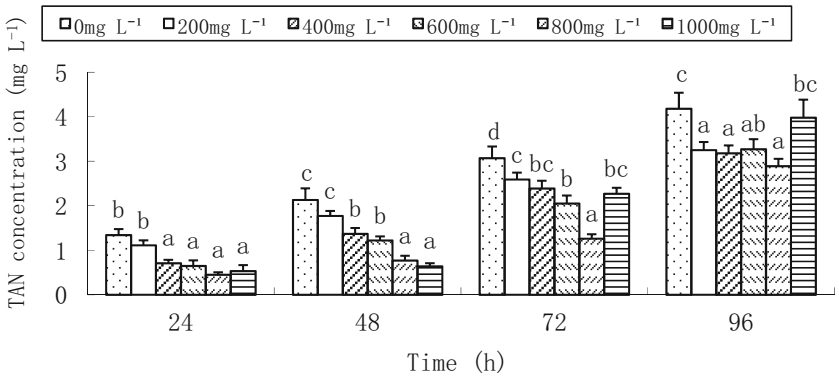

Fig. 2 Changes of TAN concentration in different density of *U. prolifera* (results presented are mean value and standard deviation, $n = 3$. Different letters indicate statistical difference at $P < 0.05$.

days 14 and 21, there were no significant differences in TAN concentration among the treatments ($P > 0.05$). On day 28, TAN in T4 was not significantly different from that in T3; however, it was significantly lower than in T1 and T2 ($P < 0.05$). On day 35, the TAN concentrations were 2.75 ± 0.21, 2.47 ± 0.16, 2.22 ± 0.21, and 2.06 ± 0.18 mg L^{-1}, respectively, and the TAN concentrations in T3 and T4 were significantly lower than T1 ($P < 0.05$); however, no significant differences in TAN concentration were observed in T3 and T4 compared to group T2 ($P > 0.05$). From beginning to end, no significant differences were observed in TAN concentration between T2 and T3 ($P > 0.05$).

Effect of *U. prolifera* and quantity of water exchange on shrimp growth performance

Shrimp growth performance at the end of the experiment is shown in Table 2. Maximum final weight of shrimp was recorded in group T4, followed by T3, T2, and T1. No significant differences were observed among groups T1, T2, and T3 ($P > 0.05$); however, they were significantly lower than that of shrimp in T4 ($P < 0.05$). As is the case with shrimp final weight, maximum growth rate was observed in T4 (1.35 ± 0.07 g) which was significantly faster than those of other groups. There were no significant differences ($P > 0.05$) in survival between groups T1 and T2; however, survival of T1 was significantly lower than in groups T3 and T4 ($P < 0.05$). No significant differences were observed among T2, T3, and T4 ($P > 0.05$). Maximum FCR was recorded in group T1, followed by T2, T3, and T4. No significant differences in FCR were observed in group T2, as compared to T1, T3, or T4 ($P > 0.05$). Significant differences in water consumption were observed among the treatments ($P < 0.05$).

U. prolifera growth performance

On day 35, the cumulative weight of *U. prolifera* showed a decreasing trend as the water exchange quantity increased (Table 3). No significant differences in cumulative weight of *U. prolifera* were observed among T1, T2, and T3 ($P > 0.05$); however, they were significantly larger than T4 ($P < 0.05$). There were no significant differences in SGR and growth rate between T3 and T4 ($P > 0.05$); however, SGR and growth rate of T4 were significantly slower than those of T1 and T2 ($P < 0.05$).

Discussion

Ammonia, nitrite, and nitrate are the main forms of dissolved inorganic nitrogen, and ammonia and nitrite are very poisonous to shrimp (Khoi and Fotedar 2011; Ge et al. 2016a). In the course of production of farming shrimp, ammonia mainly originates from excreta and uneaten feed and ammonia is oxidized to nitrite and nitrate by nitrosobacteria and nitrifying bacteria (Guerdat et al. 2010). According to Kuhn et al.

Table 1 Water quality in the tanks of four treatments in the polyculture of *L. vannamei* and *U. prolifera* during the 35-day experiment

	T1	T2	T3	T4
Salty (‰)	31.28 ± 0.25	31.19 ± 0.19	31.15 ± 0.13	31.12 ± 0.10
Temperature (°C)	28.75 ± 0.31	28.67 ± 0.33	28.52 ± 0.42	28.35 ± 0.54
pH value	8.76 ± 0.16	8.72 ± 0.21	8.70 ± 0.25	8.67 ± 0.28
DO (mg L^{-1})	5.53 ± 0.24	5.53 ± 0.25	5.49 ± 0.24	5.47 ± 0.23
NO_2-N (mg L^{-1})	0.20 ± 0.17	0.22 ± 0.18	0.18 ± 0.17	0.17 ± 0.15
NO_3-N (mg L^{-1})	0.13 ± 0.11	0.12 ± 0.11	0.09 ± 0.07	0.08 ± 0.07

Notes: Values (mean ± SD of three replicates) in the same row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant differences between treatments

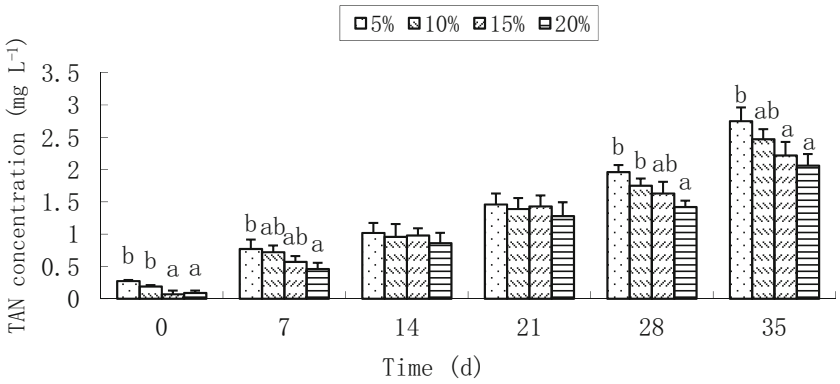

Fig. 3 TAN in the tanks of four treatments in the polyculture of *L. vannamei* and *U. prolifera* during the 35-day experiment (results presented are mean and standard deviation $n = 3$). Different letters indicate statistical differences $p < 0.05$.

(2010a), there are significant correlation between nitrate and nitrifying bacteria. In the present study, nitrite and nitrate were not detected. This may be a result of the seawater being disinfected with chlorine bleach killing all bacteria (Pintar et al. 2004). It can also indicate that the ammonia nitrogen removal may result from *U. prolifera* taking up ammonia nitrogen as shown by Baloo et al. (2014). The TAN concentration decreased as the *U. prolifera* quantity increased, indicating that increasing the quantity of *U. prolifera* can increase ammonia removal rate. However, TAN in the 1000-mg L^{-1} group rose sharply from 72 to 96 h. This may be due to some *U. prolifera* dying because of less or no light, dissolved oxygen and some other conditions. The dead macroalgae cannot remove ammonium and could actually release ammonia. TAN in the 800-mg L^{-1} group was lower than those of the other group from beginning to end. This indicated that, when the quantity of *U. prolifera* is 800 mg L^{-1}, the alga has the maximum efficiency of nitrogen removal. However, TAN concentration in all treatments showed an increasing trend over time indicating that *Ulva* cannot remove nitrogen completely. Therefore, to maintain water quality, some water should be exchanged (Brito et al. 2014).

Temperature, salinity, pH, and dissolved oxygen were within the optimum range for shrimp (Wik et al. 2009; Esparza-Leal et al. 2016) and also within the normal ranges of *U. prolifera* (Neori et al. 2004; Cruz-Suárez et al. 2010). The concentration of nitrite and nitrate in all treatment remained at very low level throughout. Ammonia is one of the most important factors that impacts the growth and development of shrimp (Vinatea et al. 2010; Ge et al. 2016a). Therefore, ammonia nitrogen removal is a key problem of controlling water quality in shrimp culture (Holl et al. 2011). To remove ammonia nitrogen from shrimp aquaculture ponds, three main practical approaches are emerging: water exchange (Cuzon et al. 2004), bacterial nitrification into nitrate (Kuhn et al. 2010a; Furtado et al. 2015), and seaweed assimilation into biomass (Cahill et al. 2010; Ge et al. 2016b). In this present study, at the same time point, TAN concentration decreased as the water exchange increased. This indicates that water exchange can control water quality. However, water exchange not only increases water consumption (Azim and Little 2008), but also raises the risk of pathogen transmission (Han et al. 2015). Using the seaweed technology for wastewater purification cannot only control water quality (Cahill et al. 2010), but also have economical and social benefits (Cruz-Suárez et al. 2010). Ge et al. (2016a) reported that microalgae co-cultured with shrimp with little water exchange can control ammonia under 0.5 mg L^{-1}. Wang et al. (2007a, b) had similar results showing that *Ulva* can remove 68% ammonia nitrogen of sea cucumber culture water. In the present study, on day 35, the TAN concentrations were 2.75 ± 0.21, 2.47 ± 0.16, 2.22 ± 0.21, and 2.06 ± 0.18 mg L^{-1} showing that applying some water exchange in combination with *U. prolifera* purification can control ammonia nitrogen within safe concentrations for

Table 2 Shrimp growth performance of four treatments for 35 days

	T_1	T_2	T_3	T_4
Initial weight (g)	3.45 ± 0.13	3.45 ± 0.13	3.45 ± 0.13	3.45 ± 0.13
Final weight (g)	9.61 ± 0.20^{a}	9.73 ± 0.21^{a}	9.82 ± 0.28^{a}	10.21 ± 0.36^{b}
Survival rate (%)	85.78 ± 4.68^{a}	87.56 ± 2.04^{ab}	93.33 ± 1.33^{b}	93.78 ± 2.04^{b}
Growth rate (g week $^{-1}$)	1.23 ± 0.04^{a}	1.26 ± 0.04^{a}	1.27 ± 0.06^{a}	1.35 ± 0.07^{b}
FCR	2.05 ± 0.25^{b}	1.84 ± 0.10^{ab}	1.61 ± 0.05^{a}	1.48 ± 0.06^{a}
Water consumption (L kg^{-1})	663.42 ± 35.99^{a}	1032.64 ± 92^{b}	1344.50 ± 19.21^{c}	1645.92 ± 35.51^{d}

Notes: Values (mean ± SD of three replicates) in the same row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant differences between treatments

Table 3 *U. prolifera* growth performance of four treatments for 35 days

	T1	T2	T3	T3
Initial weight (g)	120	120	120	120
Cumulative weight (g)	449.20 ± 17.17^{b}	454.93 ± 26.00^{b}	426.03 ± 24.45^{ab}	380.27 ± 12.55^{a}
SGR (%)	3.77 ± 0.11^{b}	3.80 ± 0.16^{b}	3.62 ± 0.16^{ab}	3.29 ± 0.09^{a}
Growth rate (g week $^{-1}$)	89.84 ± 3.43^{b}	90.99 ± 5.20^{b}	85.21 ± 4.89^{ab}	76.05 ± 2.51^{a}

Notes: Values (mean ± SD of three replicates) in the same row with different letters are significantly different ($P < 0.05$). Absence of letters indicates no significant differences between treatments

shrimp. From beginning to end, no significant differences were observed in TAN concentration between T2 and T3. This indicated that 10% water exchange and 800 mg L^{-1} *U. prolifera* can control ammonia nitrogen within safe concentration for white shrimp (500 shrimp m^{-3}).

At the end of the feeding experiment, the shrimp survival rates were between 85.78 and 93.78% indicating that *U. prolifera* did not harm shrimp. This is similar to the results of Castro et al. (2004) and Cruz-Suárez et al. (2010). Furthermore, Khoi and Fotedar (2011) found that *U. lactuca* was co-cultured with king prawn that can promote the growth of king prawn. Maximum growth rate of shrimp was recorded in group T4, followed by T3, T2, and T1. This is mainly because better water quality contributes to the growth of shrimp (Copertino et al. 2009) and is consistent with the present result that *U. prolifera* present in the tank can remove ammonia nitrogen.

In the present study, minimum FCR was 1.48 ± 0.06. The decreased FCR may result from that shrimp cultured with *U. prolifera* may improve the utilization of artificial feed (Copertino et al. 2009), it may also be because that the seaweed acted as a nutritional supplement for shrimp (Cruz-Suárez et al. 2010). In the present study, shrimp survival rate, final weight, and growth rate showed an increasing trend as the exchange water quantity increased indicating improved water quality (Cuzon et al. 2004; Ge et al. 2016a). In addition, water exchange can promote the sloughing of the cuticle leading to growth (Bray et al. 2006; Kuhn et al. 2010b) thus enhancing feed utilization (Samocha et al. 2015). In the present study, maximum shrimp final weight, growth rate, and survival rate were found in T4; however, they were not significantly greater than those in T3. In addition, water consumption of T4 was 22.42% higher than that of T3. Increasing water exchange not only consumes large amount of water, but also causes pollution of the environment. There were no significant differences in shrimp survival rate, final weight, growth rate, and FCR between T2 and T3 indicating that 10% water exchange and 800 mg L^{-1} *U. prolifera* can improve the survival rate and growth of shrimp.

Ulva prolifera possesses high nutrient value (Zhao et al. 2011; Teng et al. 2013). Moreover, the macroalgae can be used as an animal feed (Cruz-Suárez et al. 2010) or feed additive (Castro et al. 2004). In the present study, the cumulative weight of *U. prolifera* decreased as the water exchange quantity increased, probably due to decreased nutrients in the water at higher exchange rates. In the present study, the water temperature was as high as 28.75 °C; however, the seaweed grew fast. This indicated that *U. prolifera* could adapt to high water temperature and this is consistent with Mantri et al. (2011) who showed that higher temperature enhances *U. prolifera* growth. Wang et al. (2007a, b) reported that the growth rate of *U. pertusa* was 3.3%. However, in the present study, the growth rates of *U. prolifera* were 3.77, 3.80, 3.62, and 3.29%, respectively. This is probably because that the water temperature in the present study was higher than that in Wang et al. (2007a, b) and Gao et al. (2016). No significant differences in growth rate and cumulative weight of *U. prolifera* were observed among T1, T2, and T3.

Conclusion

This study investigated the effect of integrating *U. prolifera* with *L. vannamei* at varying rates of daily water exchange on water quality and shrimp growth. It was found that 10% water exchange daily and 800 mg L^{-1} *U. prolifera* can control ammonia nitrogen within safe concentration for white shrimp (500 shrimp m^{-3}) and can also improve the survival and growth of shrimp.

Acknowledgements The authors are grateful to all the laboratory members for experimental material preparation and technical assistance.

Funding This study was supported by the earmarked fund for Modern Agro-industry Technology Research System (No.CARS-48), the Program of Shandong Leading Talent (No.LNJY2015002), Su bei science and technology special program (No.SZ-LYG2017029), the Huaihai Institute of Technology start-up funds (No. KQ17022), Open-end Funds of Jiangsu Key Laboratory of Marine Biotechnology (No. HS2017002) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References

Akbary P, Aminikhoei Z (2018) Effect of water-soluble polysaccharide extract from the green alga *Ulva rigida* on growth performance, antioxidant enzyme activity, and immune stimulation of grey mullet *Mugil cephalus*. J Appl Phycol 30:1345–1353

Azim ME, Little DC (2008) The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283:29–35

Baloo L, Azman S, Said MIM, Ahmad F, Mohamad M (2014) Biofiltration potential of macroalgae for ammonium removal in outdoor tank shrimp wastewater recirculation system. Biomass Bioenergy 66:103–109

Berri M, Slugocki C, Olivier M, Helloin E, Jacques I, Salmon H, Demais H, Le Goff M, Collen PN (2016) Marine-sulfated polysaccharides extract of *Ulva armoricana* green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. J Appl Phycol 28:2999–3008

Bikker P, van Krimpen MM, van Wikselaar P, Houweling-Tan B, Scaccia N, van Hal JW, Huijgen WJJ, Cone JW, López-Contreras AM (2016) Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. J Appl Phycol 28:3511–3525

Bray WA, Williams RR, Lightner DV, Lawrence AL (2006) Growth, survival and histological responses of the marine shrimp, *Litopenaeus vannamei*, to three dosage levels of oxytetracycline. Aquaculture 258:97–108

Brito LO, Arantes R, Magnotti C, Derner R, Pchara F, Olivera A (2014) Water quality and growth of Pacific white shrimp *Litopenaeus vannamei* (Boone) in co-culture with green seaweed *Ulva lactuca* (Linnaeus) in intensive system. Aquacult Int 22:497–508

Cahill PL, Hurd CL, Lokman M (2010) Keeping the water clean — seaweed biofiltration outperforms traditional bacterial biofilms in recirculating aquaculture. Aquaculture 306:153–159

Castro R, Zarra I, Lamas J (2004) Water-soluble seaweed extracts modulate the respiratory burst activity of turbot phagocytes. Aquaculture 229:67–78

Copertino MDS, Tormena T, Seeliger U (2009) Biofiltering efficiency, uptake and assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Chlorophyceae) cultivated in shrimp aquaculture waste water. J Appl Phycol 21:31–45

Cuzon G, Lawrence A, Gaxiola G, Rosas C, Guillaume J (2004) Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture 235:513–551

Cruz-Suárez LE, León A, Peña-Rodríguez A, Rodríguez-Peña G, Moll B, Ricque-Marie D (2010) Shrimp/*Ulva* co-culture: a sustainable alternative to diminish the need for artificial feed and improve shrimp quality. Aquaculture 301:64–68

Esparza-Leal HM, Xavier JAA, Jr WW (2016) Performance of *Litopenaeus vannamei*, postlarvae reared in indoor nursery tanks under biofloc conditions at different salinities and zero-water exchange. Aquacult Int 24:1–13

Furtado PS, Campos BR, Serra FP, Klosterhoff M, Romano LA, Wasielesky W Jr (2015) Effects of nitrate toxicity in the Pacific white shrimp, *Litopenaeus vannamei*, reared with biofloc technology (BFT). Aquacult Int 23:315–327

Gao G, Zhong ZH, Zhou XH, Xu JT (2016) Changes in morphological plasticity of *Ulva prolifera* under different environmental conditions: a laboratory experiment. Harmful Algae 59:51–58

Ge HX, Li J, Chang ZQ, Chen P, Shen MM, Zhao FZ (2016a) Effect of microalgae with semicontinuous harvesting on water quality and zootechnical performance of white shrimp reared in the zero water exchange system. Aquacult Eng 72-73:70–76

Ge HX, Li J, Chen P, Chang ZQ, Shen MM, Zhao FZ (2016b) Cultivation of *Platymonas helgolandica* in rearing water enhances the growth performance and resistance of *Litopenaeus vannamei* against *Vibrio parahaemolyticus* infection. Aquacult Int 25:1279–1290

Guerdat TC, Losordo TM, Classen JJ, Osborne JA, DeLong DP (2010) An evaluation of commercially available biological filters for recirculating aquaculture systems. Aquacult Eng 42:38–49

Han JE, Mohney LL, Tang KFJ, Pantoja CR, Lightner DV (2015) Plasmid mediated tetracycline resistance of *Vibrio parahaemolyticus* associated with acute hepatopancreatic necrosis disease (AHPND) in shrimps. Aquacult Rep 2:17–21

Holl CM, Glazer CT, Moss SM (2011) Nitrogen stable isotopes in recirculating aquaculture for super-intensive shrimp production: tracing the effects of water filtration on microbial nitrogen cycling. Aquaculture 311:146–154

Jiao L, Li X, Li T, Jiang P, Zhang L, Wu M (2009) Characterization and anti-tumor activity of alkali-extracted polysaccharide from *Enteromorpha intestinalis*. Int Immunopharmacol 9:324–329

Khoi LV, Fotedar R (2011) Integration of western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) and green seaweed (*Ulva lactuca* Linnaeus, 1753) in a closed recirculating aquaculture system. Aquaculture 322-323:201–209

Kim JK, Cho ML, Karnjanapratum S, Shin IS, You SG (2011) In vitro and in vivo immunomodulatory activity of sulfated polysaccharides from *Enteromorpha prolifera*. Int J Biol Macromol 49:1051–1058

Kuhn DD, Drahos DD, Marsh L, Flick GJ Jr (2010a) Evaluation of nitrifying bacteria product to improve nitrification efficacy in recirculating aquaculture systems. Aquacult Eng 43:78–82

Kuhn DD, Smith SA, Boardman GD, Angier MW, Marsh L, Flick GJ Jr (2010b) Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: impacts on survival, growth, antennae length, and pathology. Aquaculture 309:109–114

Li H, Zhang Y, Chen J, Zheng X, Liu F, Jiao N (2018a) Nitrogen uptake and assimilation preferences of the main green tide alga *Ulva prolifera* in the Yellow Sea, China. J Appl Phycol. https://doi.org/10.1007/s10811-018-1575-2

Li Y, Huang Z, Qiao L, Gao Y, Guan H, Hwan H (2015) Purification and characterization of a novel enzyme produced by *Catenovulum* sp. LP and its application in the pre-treatment to *Ulva prolifera* for bioethanol production. Process Biochem 50:799–806

Li W, Wang K, Jiang N, Liu X, Wan M, Chang X, Liu D, Qi H, Liu S (2018b) Antioxidant and antihyperlipidemic activities of purified polysaccharides from *Ulva pertusa*. J Appl Phycol 30:2619–2627

Liu D, Keesing JK, Xing Q, Shi P (2009) World's largest macroalgal bloom caused by expansion of seaweed aquaculture in China. Mar Poll Bull 58:888–895

Mantri VA, Singh RP, Bijo AJ, Kumari P, Reddy CRK, Jha B (2011) Differential response of varying salinity and temperature on zoospore induction, regeneration and daily growth rate in *Ulva fasciata* (Chlorophyta, Ulvales). J Appl Phycol 23:243–250

Murphy C, Hotchkiss S, Worthington J, McKeown SR (2014) The potential of seaweed as a source of drugs for use in cancer chemotherapy. J Appl Phycol 26:2211–2264

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231:361–391

Pintar A, Besson M, Gallezot P, Gibert J, Martin D (2004) Toxicity to *Daphnia magna* and *Vibrio fischeri* of Kraft bleach plant effluents treated by catalytic wet-air oxidation. Water Res 38:289–300

Pungrasmi W, Phinitthanaphak P, Powtongsook S (2016) Nitrogen removal from a recirculating aquaculture system using a pumice bottom substrate nitrification-denitrification tank. Ecol Eng 95:357–363

Qiu X, Neori A, Kim JK, Yarish C, Shpigel M, Guttman L, Ben Ezra D, Odintsov V, Davis DA (2018) Evaluation of green seaweed *Ulva* sp. as a replacement of fish meal in plant-based practical diets for Pacific white shrimp, *Litopenaeus vannamei*. J Appl Phycol 30:1305–1316

Samocha TM, Fricher J, Ali AM, Shpigel M, Neori A (2015) Growth and nutrient uptake of the macroalga *Gracilaria tikvahiae* cultured with the shrimp *Litopenaeus vannamei* in an integrated multi-trophic aquaculture (IMTA) system. Aquaculture 446:263–271

Shao LL, Xu J, Shi MJ, Wang XL, Li YT, Kong LM, Hider RC, Zhao T (2017) Preparation, antioxidant and antimicrobial evaluation of

hydroxamated degraded polysaccharides from *Enteromorpha prolifera*. Food Chem 237:481–487

Shi MJ, Wei XY, Xu J, Chen BJ, Zhao DY, Cui S, Zhou T (2017) Carboxymethylated degraded polysaccharides from *Enteromorpha prolifera*: preparation and in vitro antioxidant activity. Food Chem 215:76–83

Sun KM, Li RX, Li YM, Xiao J, Wang ZL, Tang XX, Pang M (2015) Responses of *Ulva prolifera* to short-term nutrient enrichment under light and dark conditions. Estuar Coast Shelf Sci 163:56–62

Teng ZL, Qian L, Zhou Y (2013) Hypolipidemic activity of the polysaccharides from *Enteromorpha prolifera*. Int J Biol Macromol 62: 254–256

van Rijn J (2013) Waste treatment in recirculating aquaculture systems. Aquac Eng 53:49–56

Vinatea L, Gálvez AO, Browdy CL, Stokes A, Venero J, Haveman J, Lewis BL, Lawson A, Shuler A, Leffler JW (2010) Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: interaction of water quality variables. Aquacult Eng 42:17–24

Wang H, Liu CF, Qin CX (2007a) Using a macroalgae *Ulva pertusa* biofilter in a recirculating system for production of juvenile sea cucumber *Apostichopus japonicus*. Aquacult Eng 36:217–224

Wang H, Liu CF, Qin CX, Cao SQ, Ding J (2007b) Using a macroalgae *Ulva pertusa* biofilter in a recirculating system for production of juvenile sea cucumber *Apostichopus japonicas*. Aquacult Eng 36: 217–224

Wang P, Wu CW, Zhou ZZ (2010) Ecological effects of *Ulva lactuca* on *Sebastiscus marmoratus* aquculture. South China Fish Sci 6:63–67

Wik TEI, Lindén BT, Wramner PI (2009) Integrated dynamic aquaculture and wastewater treatment modelling for recirculating aquaculture systems. Aquaculture 287:361–370

Xu WJ, Pan LQ (2012) Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquaculture 356-357:147–152

Yabe T, Ishii Y, Amano Y, Koga T, Hayashi S, Nohara S, Tatsumoto H (2009) Green tide formed by free-floating *Ulva* spp. at Yatsu tidal flat. Japan Limnol 10:239–245

Zhang Z, Wang X, Yu S (2011) Synthesized oversulfated and acetylated derivatives of polysaccharide extracted from *Enteromorpha linza* and their potential antioxidant activity. Int J Biol Macromol 49: 1012–1015

Zhao J, Jiang P, Liu ZY, Wang JF, Cui YL, Qin S (2011) Genetic variation of Ulva (*Enteromorpha*) *prolifera* (Ulvales, Chlorophyta) – the causative species of the green tides in the Yellow Sea. China J Appl Phycol 23:227–233

Zhu SM, Deng YL, Ruan YJ, Guo XS, Shi MM, Shen JZ (2015) Biological denitrification using poly (butylene succinate) as carbon source and biofilm carrier for recirculating aquaculture system effluent treatment. Bioresour Technol 192:603–610