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Coculture of the Pacific white shrimp *Litopenaeus vannamei* and the macroalga *Ulva linza* enhances their growth rates and functional properties

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ABSTRACT

Integrated multi-trophic aquaculture (IMTA) has been proposed as a potential solution to supply aquatic food in an environmentally friendly way. However, little is known regarding the impacts of IMTA on the growth rates and food quality of both animals and plants, as well as the seasonal effects. In this study, we conducted field mesocosm experiments using monoculture and coculture systems with the shrimp *Litopenaeus vannamei* and the macroalga *Ulva linza* for four weeks in different seasons (autumn, spring and summer) to address the research gap. To evaluate the environmental pollution caused by shrimp culture, there was no water exchange for culture systems including shrimps. Compared to monoculture of *L. vannamei*, coculture with *U. linza* significantly reduced dissolved inorganic nitrogen (DIN, 98.5–99.0%) and dissolved inorganic phosphorus (DIP, 98.2–98.8%) but enhanced dissolved oxygen (DO, 56.2–68.7%) and pH (10.7–18.6%) by the end of culture. Compared to monoculture, coculture stimulated the growth rates of *L. vannamei* by 38.1–58.8% and *U. linza* by 241.9–290.4% in all seasons, and increased the lipid content of *L. vannamei* by 24.2% in autumn and by 37.9% in summer and the contents of protein and ash of *U. linza* by 23.8–29.0% and by 27.6–68.6%, respectively, in all seasons. Coculture enhanced the content of most amino acids in *U. linza* and the content of total fatty acids (FA) and polyunsaturated fatty acids in *L. vannamei* in all seasons in comparison with monoculture. In addition, coculture lifted swelling capacity by 28.9–40.5%, water holding capacity by 39.8–43.3% and oil holding capacity by 31.4–32.4% for *U. linza* in autumn and summer. Apart from relieving eutrophication, deoxygenation and acidification, IMTA increased growth rates of both shrimp and *Ulva* and improved functional properties of *U. linza*, suggesting a green and productive aquaculture mode.

1. Introduction

To deal with the rising human demand for seafood, aquaculture has been intensively developed and become one of the fastest growing sectors of food production (FAO, 2020). The Pacific white shrimp, termed as *Litopenaeus vannamei*, is the most highly consumed crustacean species in the world, accounting for about half of the crustacean production (FAO, 2020). *L. vannamei* is an extreme healthy source of protein and minerals, particularly essential amino acids (Panini et al., 2017). In addition, shrimps contain high levels of polyunsaturated fatty acids (PUFA), such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (Hulefeld et al., 2018) that are deemed essential for human health, particularly for individuals during pregnancy and infant (Jiang et al., 2016).

On the other hand, intensive aquaculture is causing severe environmental pollution. Due to high feed conversion ratios (1.3–1.7 for commonly farmed species) (Naylor et al., 2021), feed residues produced as uneaten feed, faeces and excretion products are retained in the water or as organic sediment in the pond bottom, resulting in around 60–80% of nitrogen and phosphorus in feeds released into the aquatic environment (Chatvijitkul et al., 2017). The accumulation and drainage of nutrients can lead to water quality deterioration and the formation of harmful algae blooms (Bohnes et al., 2019). In addition, aquaculture effluent usually has the feature of low dissolved oxygen (DO) and pH, and the direct discharge would result in acid and hypoxia environment, threatening the survival of vulnerable aquatic organisms (Pollock et al., 2007). Wastewater treatment combined with biomass production is deemed as an ideal solution to deal with environmental pollution (Zheng et al., 2021). Therefore, integrated multi-trophic aquaculture (IMTA), in

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Abbreviations

DHA	docosahexaenoic acid
DIN	dissolved inorganic nitrogen
DO	dissolved oxygen
DIP	dissolved inorganic phosphorus
EAA	essential amino acids
EPA	eicosapentaenoic acid
FA	fatty acids
IMTA	Integrated multi-trophic aquaculture
MUFA	monounsaturated fatty acids
NEAA	non-essential amino acids
OHC	oil holding capacity
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
SGR	specific growth rate
SWC	swelling capacity
WHC	water holding capacity

which species from two or more trophic levels grow in one farm and where the waste of one feeds another, has been proposed as a solution to mitigate aquaculture waste release and improve water quality (Shpigel et al., 1993; Chopin et al., 2001). Seaweeds can 'purify' the farm effluents by acting as biofilters and thus are often referred to as extractive organisms (Chopin et al., 2001). Furthermore, IMTA can also increase farm revenues by providing additional commercial crops (principally seaweeds) and by reducing the discharge of effluents, suggesting an environmentally friendly and cost effective aquaculture system (Chopin et al., 2001; Koesling et al., 2021).

Among various seaweeds, *Ulva* species have been considered as an ideal biofilter to deal with eutrophication caused by aquaculture due to their strong capacity in nitrogen and phosphorus uptake and robust resilient to environmental changes (Al-Hafedh et al., 2015; Gao et al., 2018a). Additionally, *Ulva* has wide application in food, animal feed, biofuel, medicine, etc. (Bolton et al., 2016; Cadillo-Benalcazar et al., 2020; Gao et al., 2020). Studies have been conducted to investigate the feasibility of integrated shrimps and *Ulva* (Brito et al., 2014; Ge et al., 2019). However, these studies either did not set up monoculture or focused on the analysis of single trophic level (Brito et al., 2014; Ge et al., 2019). Therefore, our understanding of the effects of IMTA on the growth rates, chemical composition and functional properties of both shrimp and *Ulva* are still very scarce, which is very essential for the economic assessment of IMTA. In addition, most studies were conducted in one season and little is known in regard to how IMTA would work in different seasons. Macroalgae are usually sensitive to high temperature and cultivation is difficult to conduct in summer (Friedlander, 2008). In this study, we used a high-temperature resistant green macroalga *Ulva linza* that is also a bloom-forming species as the extractive organism to deal with the summer challenge (Gao et al., 2019). Based on the previous studies (Brito et al., 2014; Ge et al., 2019), we hypothesized that apart from reducing environmental pollution, IMTA could enhance the growth rates and affect chemical composition of both shrimp and *Ulva*, and the effect may be regulated by season. In this study, we carried out outdoor culture in three seasons, monitored the changes of nutrients, DO and pH, and investigated the effects of IMTA on growth rates and chemical composition of both *L. vannamei* and *U. linza* to test this hypothesis. To investigate the seasonal effects, we chose a site in a coastal farm in Lianyungang (119.46°E, 34.68°N) which has clear four seasons in a year with the temperature ranging 3.8–29.9 °C (Table S1). Our study makes solid contribution to assessing the impacts of IMTA on food quantity and quality of both animals and plants and examining both environmental and economic benefits of IMTA.

2. Materials and methods

2.1. Sample preparation and experimental setup

Ulva linza was collected from the coastal water of Lianyungang (119.49°E, 34.70°N), Jiangsu province, China and the healthy thalli (~5 cm in length) were selected for the experiment. *Litopenaeus vannamei* was purchased from Rizhao Xinhui aquaculture breeding Co., Ltd in Shandong province, China and cultured at a stocking density of 800 shrimp m^{-3} in a nursery tank until the shrimps reached a mean weight of 3.26 ± 0.02 g.

Cultures were conducted in 9 enclosures (1.2 m length × 1.2 m width × 1.0 m depth) locating in a coastal farm in Lianyungang (119.46°E, 34.68°N). The skeletons of the enclosures were made from bamboo poles, and the walls were made of water-proof polyethylene cloth (Yanxin, Shandong Yanxin Environmental Protection Technology Co., Ltd, China). Three culture systems, shrimp only, *Ulva* only and shrimp + *Ulva*, were set up and each employed three enclosures respectively. Nine enclosures were placed in a linear arrangement with equal spacing (1.2 m) between them based on systematic design (Fig. S1) that is acceptable for field experiments (Hurlbert, 1984). The aim of this study was to assess the advantages of coculture compared to monoculture and thus control ponds with only water were not set up. The stocking density of shrimp was 200 shrimp m^{-3} and the initial weight of shrimp was 3.26 ± 0.01 g. The stocking density of *U. linza* was about 600 g fresh weight m^{-3}. The biomass ratio of shrimp and *U. linza* was determined based on a preliminary experiment. In *Ulva* only and shrimp + *Ulva* systems, thalli were clamped in a horizontal nylon net suspended in the water at a depth of 0.4 m. Density of *U. linza* was maintained by reduced algal biomass to the initial weight every week. This manipulation aimed to prevent the decrease of growth due to increased biomass density (Bruhn et al., 2011). To evaluate the environmental pollution caused by shrimp culture, there was no water exchange for culture systems including shrimps; while daily water exchange for seaweed only system was conducted to supply fresh nutrients and maintain algal growth. The seawater in the enclosures was exchanged 25% daily with the untreated surface seawater from the coastal farm during the experimental period. The initial seawater in the enclosures was also from the coastal farm. Water exchange was commonly used for tank and pond culture of seaweeds (Hiraoka and Oka, 2008; Bruhn et al., 2011) and thus the *Ulva* only and shrimp + *Ulva* systems in this study represents traditional monoculture and new coculture respectively. To evaluate the effects of *Ulva* on the enhancement of dissolved oxygen and pH, no aeration was conducted in all culture systems.

Shrimps were fed with commercial pellets (Weitong, Huaian, China) twice daily (06:30 and 18:30 h) and the daily feeding rate was 5% of shrimp body weight based on previous studies (Gao et al., 2012; Ge et al., 2019). To set flexible feeding rates based on temperature changes would improve utilization efficiency of feeds, which will be managed in future studies after obtaining specific models. The feed pellets contain a crude protein content of 38.0%, crude lipid content of 4.0%, fiber content of 5.0%, and ash content of 18.0%. Newly bought commercial pellets with the same mode form the same manufacturer were used for each season. The profiles of amino acids and fatty acids were the same (statistically insignificant) among different batches (Tables S2 and S3). The cultivation experiments were carried out for four weeks in each season except winter from 2018 to 2019 (Table S1) because *L. vannamei* could not survive winter in outdoor environments, although *U. linza* could grow year-round.

2.2. Environmental conditions monitoring

The seawater samples in each enclosure were collected at 15:00 weekly to measure dissolved inorganic nitrogen and dissolved inorganic phosphorus. Dissolved inorganic nitrogen (DIN) is the sum of nitrate,

nitrite and ammonia, which was measured with the cadmium–copper reduction method as described by Wood et al. (1967), the Griess-Ilosvay method as described by Benschneider and Robinson (1952), the indophenol blue method as described by Aminot et al. (1997), respectively. Dissolved inorganic phosphorus (DIP) in seawater phosphate was determined by the phosphomolybdenum blue colorimetry method (Murphy and Riley, 1962). Dissolved oxygen (DO), pH, temperature and salinity of seawater were monitored at 15:30 weekly using a multi-parameter water quality meter (Hach HQ40d, Hach Company, Loveland, Colorado, USA).

2.3. Measurement of growth

The specific growth rate (SGR) for both the shrimp and the alga was estimated as follows: SGR (% d^{-1}) = ($\ln Wt_2 - \ln Wt_1$)/t × 100; thermal growth coefficient (TGC) of shrimp = ($Wt_2^{1/3} - Wt_1^{1/3}$)/(T × t) × 100, where Wt_1 and Wt_2 are the initial and final weight after t days of culture, respectively, and T is average temperature during culture. Final weight of shrimp was measured after 28 days culture and final weight of *Ulva* was measured every week before reducing biomass to the initial density. Survival (%) = (final number of live shrimps/initial number of live shrimps) × 100. Feed conversion ratio (FCR) = total weight of feed offered/total shrimp weight gained.

2.4. Chemical composition and functional properties

After 28-day culture, the shrimp and the alga were collected and transport to laboratory in insulation boxes (4 °C) within half an hour where they were carefully rinsed in filtered (0.2 μm) natural seawater to remove any sediment, epiphytes and small grazers. These samples were then stored at −20 °C prior to analysis. For shrimp, only shrimp meat was used for the following analysis of chemical composition and functional properties. Dry mass of samples was recorded after being oven dried at 100 °C until consistent mass (for 24 h). Moisture is determined by the difference fresh mass and dry mass. Total protein was determined by the Kjeldahl method. The protein content was calculated using a nitrogen conversion factor of 6.25 and 5.45 for the shrimp and the alga, respectively (Audelo-Naranjo et al., 2011; Gao et al., 2017a). Total nitrogen was measured by an Elemental Analyzer (Vario Max CN, Elementar Analysensysteme, Hanau, Germany). Lipid was extracted according to the Folch gravimetric method with some modifications (Gao et al., 2017a). Dietary fiber was determined following the AOAC method (AOAC, Official Method 985.29 and Official Method 991.43, 2006).

To measure amino acid, the samples were dried in an oven at 105 °C until constant weight. The dry samples were grinded and about 50 mg powders were placed in a 15 ml tube. Ten ml 6N HCl were added and hydrolysis was conducted at 110 °C for 22 h. The hydrolyzed sample was filtered (pore size 0.45 μm) and then dried under nitrogen. It was dissolved in 10 ml of 0.2 M sodium citrate buffer (pH 2.2), filtered using a syringe filter (pore size 0.2 μm), and then diluted 40 times with ultrapure water. Samples (20 μL) were injected into an Amino Acid Analyzer (LA8080, Hitachi High-Technologies, Japan). Amino acid standard solution (Aladdin, China) was also injected for the system calibration and amino acid quantification. Content of amino acid was expressed as mg g^{-1} dry weight (DW). To measure fatty acid, about 50 mg DW thalli were grinded and the powder was placed in a 10 ml centrifuge tube. Then 2 ml of H_2SO_4–CH_3OH were added, and the solution was mixed with a multi-tube vortex mixer (DMT-2500, China) for 10 min and heated in water bath at 80 °C for 1 h. Afterwards 1 ml of deionized water and 2 ml of isooctane were added and the mixture was centrifuged at 1700 *g* for 5 min. The supernatant layer containing FAME was collected, filtered through a filter membrane (0.22 μm, Leigu, China) and analyzed with a gas chromatography mass spectrometer (Shimadzu, GCMS-QP2010SE, Japan). Chromatograph peaks were identified based on the retention time of a Supelco 37 component FAME mix (Sigma-Aldrich). Fatty acids were quantified by using an internal standard, nonadecanoic acidmethyl ester (C 19:0, Sigma-Aldrich, St. Louis, MO, USA) and expressed as mg g^{-1} DW.

Swelling capacity (SWC), water holding capacity (WHC) and oil holding capacity (OHC) of *U. linza* were measured because they represent important functional properties of seaweeds for human health (Gao et al., 2018b), which was introduced in details in section 3.5. Swelling capacity of *U. linza* was estimated according to Gao et al. (2018b). *Ulva* powders (0.5 g) were placed into a 10 ml of measuring cylinder. Ten ml of distilled water was added and the volume was recorded (V_0). The mixture was immediately vigorously and then left to stand for 18 h at 37 °C. The volume was recorded (V_1) and swelling capacity was expressed as ml of swollen volume (V_1–V_0) per gram of sample. Water holding capacity of *U. linza* was analyzed by the centrifugation method (Gao et al., 2018b). *Ulva* powder (0.5 g) was placed into a pre-weighed centrifuge tube. Five ml of distilled water was added and the mixture was stirred vigorously. Afterwards, the mixture was left to stand for 1 h at 37 °C. The dispersion was centrifuged for 25 min at 3000 *g* and the supernatant was removed. The bound water by sample was determined by dehydration in an oven for 25 min at 50 °C. The water holding capacity was expressed as grams of water bound per gram of the sample. Oil holding capacity of *U. linza* was measured according to Gao et al. (2018b). *Ulva* powder (0.5 g) was placed in a 15 ml centrifuge tube and then 5 ml of food grade corn oil was added. The mixture was stirred and left at 37 °C for 1 h, followed by centrifugation at 3000*g* for 25 min. The oil supernatant was then transferred to a 10 ml measuring cylinder and measured. The OHC of *U. linza* was expressed as grams of oil held by 1 g of sample. The density of the corn oil was 0.92 g ml^{-1}.

2.5. Statistical analysis

Data were analyzed using the software SPSS v.21. Results were expressed as means of replicates ± standard deviation. The data under every treatment conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) and the variances could be considered equal (Levene's test, $P > 0.05$). Three-way univariate analysis of variance (ANOVA) was conducted to assess the effects of culture mode, season and period on DIN, DIP, DO, and pH. Two-way univariate ANOVA was conducted to assess the effects of culture mode and season on specific growth rate and functional properties of the shrimp and seaweed. Two-way multivariate ANOVA was conducted to analyze the effects of culture mode and season on chemical composition and the profiles of amino acids and fatty acids in the shrimp and seaweed. Least Significant Difference (LSD) was conducted for post hoc investigation. A confidence interval of 95% was set for all tests.

3. Results and discussion

3.1. Changes of environmental factors

The changes of DIN and DIP in different culture systems were monitored (Fig. 1). Culture mode, season and period interacted on DIN and DIP and each had a significant effect (Table S4). In each season, DIN and DIP in monoculture for shrimp increased with culture time and reached a very high concentration (591.00–748.57 μM L^{-1} for DIN, 26.61–37.51 μM L^{-1} for DIP) after four-week culture. In contrast, nutrients decreased after one-week culture and retained in low levels until the end of culture (6.77–8.99 μM L^{-1} for DIN, 0.45–0.47 μM L^{-1} for DIP) in coculture. The differences of nutrient between coculture and monoculture for *Ulva* were not significant.

The variation of DO and pH with culture period in different culture systems was also observed (Fig. 2). Each factor (culture mode, season and period) affected DO and culture mode interacted with culture season or period (Table S5). In autumn (Fig. 2a), DO in shrimp monoculture decreased (from 6.65 to 4.05 mg L^{-1}) with culture period although the difference between weeks 0 and 1 was not statistically significant; DO in

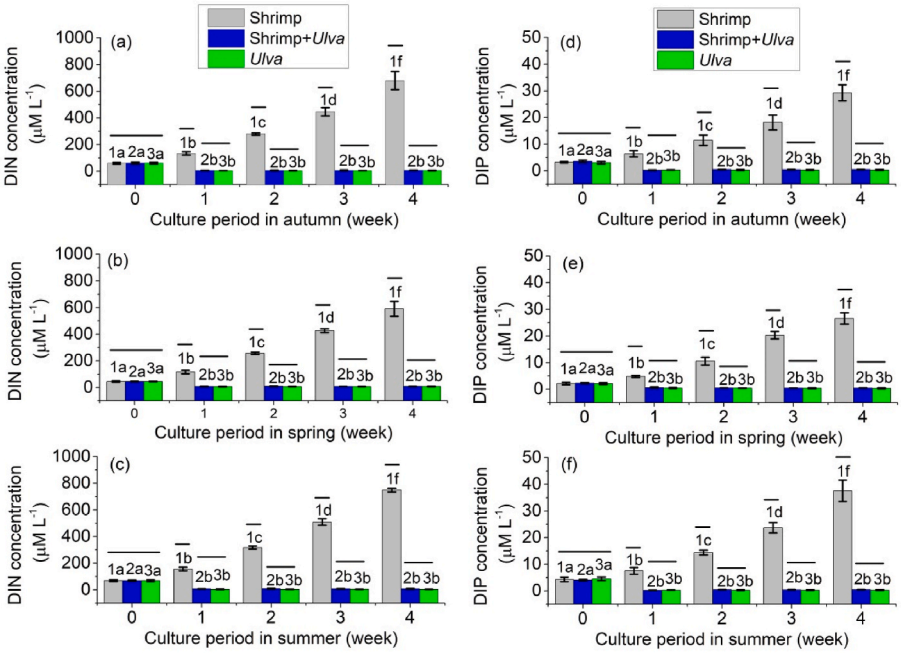

Fig. 1. Changes of dissolved inorganic nitrogen (DIN, a-c) and dissolved inorganic phosphorus (DIP, d-f) with culture period in different culture modes and seasons. The error bars indicate the standard deviations (n = 3). Different letters above the error bars represent significant differences ($P < 0.05$) among culture periods and the numbers of 1, 2, and 3 represent the comparisons in the modes of shrimp, shrimp + *Ulva*, and *Ulva*, respectively. Horizontal short bars represent significant differences ($P < 0.05$) among culture modes.

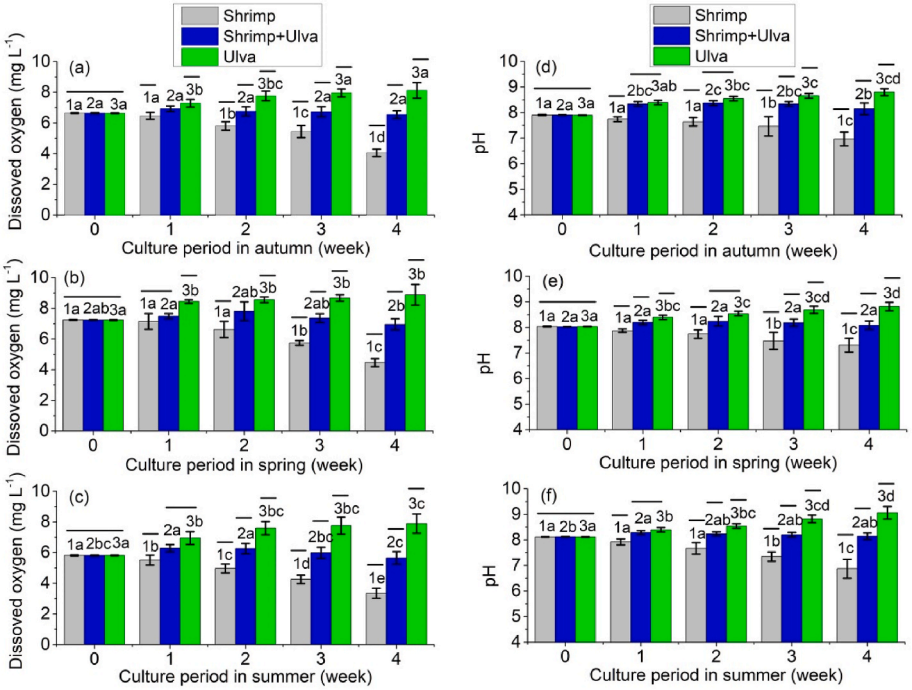

Fig. 2. Changes of dissolved oxygen (DO, a-c) and pH (d–f) with culture period in different culture modes and seasons. The error bars indicate the standard deviations (n = 3). Different letters above the error bars represent significant differences ($P < 0.05$) among culture periods and the numbers of 1, 2, and 3 represent the comparisons in the modes of shrimp, shrimp + *Ulva*, and *Ulva*, respectively. Horizontal short bars represent significant differences ($P < 0.05$) among culture modes.

coculture maintained stable during the whole culture period; while DO in *Ulva* monoculture showed an rising trend (from 6.64 to 8.13 mg L^{-1}) with culture period. The different patterns in culture modes with culture period resulted in significant differences in DO among culture modes since week 1: the lowest in shrimp monoculture and the highest in *Ulva* monoculture. In spring (Fig. 2b), DO in shrimp monoculture also decreased (from 7.25 to 4.46 mg L^{-1}) with culture period; DO in coculture maintained relatively stable; DO in *Ulva* monoculture increased (from 7.24 to 8.88 mg L^{-1}) with culture period although the increase among weeks 1–4 was not statistically significant. The case changed a little in summer (Fig. 2c); while DO in the shrimp and the alga monocultures still demonstrated decreasing and increasing patterns respectively, DO increased by week 1 and then gradually decreased with culture period in coculture mode. In terms of pH (Fig. 2 d-f), culture mode interacted with season or culture period, and culture mode and period had a significant effect (Table S5). Similar to DO, pH in the shrimp and the alga monoculture showed a decreasing and increasing trend respectively regardless of season, with the lowest and highest pH

being 6.87 and 9.07 respectively. In coculture, pH increased first and then decreased with culture period in autumn and summer although the variation ranges (7.91–8.37 for autumn and 8.12–8.29 for summer) were small. It is worth noting that all environmental parameters were measured at daytime (15:00–15:30). The patterns may be different if they were measured at night. The harvest of farmed shrimps and seaweeds and the assessment of aquaculture effluent are commonly conducted during daytime. Therefore, the data in the present study could provide meaningful references.

The findings in this study indicate the noticeable effect of *U. linza* on remitting eutrophication, deoxygenation and acidification caused by shrimp culture although further study is needed to confirm if this culture mode remains work when it is conducted in a commercial culture scale. The DIN, DIP, pH levels in the coculture systems conform to the Water Drainage Standard for Mariculture in China (SC/T 9103-2007), in which the first-class drainage standard for DIN and DIP are below 35.71 and 1.61 μM L^{-1}, and in the range of 7.00–8.50, respectively. Although DO is not defined in the Water Drainage Standard, hypoxia could significant damage metabolisms of marine animals and thus lead to the decreased growth and even death (Penn et al., 2018). The strong capacities of *U. linza* in nutrient uptake and enhancing DO and pH could be attributed to its fast growth and involved high nutrient assimilation and photosynthetic rates (Gao et al., 2018a). Furthermore, many seaweed species, such as *Laminaria* and *Pyropia*, tend to decay at higher temperatures and thus cannot be used as biofilter in summer (Friedlander, 2008). The robust acclimation to environments enables *U. linza* to be qualified as the extractive organism for bioremediation in summer.

3.2. Growth performance of shrimp and seaweed

The specific growth rate of *L. vannamei* ranged from 0.52 to 2.95% (Fig. 3a). Culture mode and season interacted on SGR of shrimp (Table S6). Coculture enhanced SGR of shrimp in all seasons but the increased extent changed with season. The largest extent of increase occurred in summer (58%), followed by autumn (54%) and spring (38%). A similar pattern was found in TGC (Tables 1 and S6). Irrespective of culture mode, shrimp had the highest SGR in summer, followed by autumn, while the differences in TGC between autumn and summer disappeared. Culture mode and season affected survival of shrimp (Table S6). Shrimp had higher survival rates in coculture mode than in monoculture mode and the highest survival rate occurred in summer (Table 1). Culture mode and season also changed FCR (Table S6); shrimp had higher FCR in monoculture and the lowest FCR was also observed in summer (Table 1). The highest growth rate of *L. vannamei* is lower than that (3.29–3.80%) in Ge et al. (2019)'s study but higher than that (1.70–1.97%) in Fourooghifard et al. (2017)'s study. The higher growth rate in Ge et al. (2019) could be attributed to the indoor culture where the environment was stable and thus facilitated the growth of shrimp. In this study, the growth rates and survival rates of *L. vannamei* in spring were much lower than those in summer and autumn. This could be attributed to the low temperatures in spring (Table S1), as *L. vannamei* tends to enter a state of torpor when temperature is below 20 °C (Walker et al., 2011). The effect of such a low temperature could not even be eliminated by using TGC since TGC model is applicable only within the normal temperature range of the given species (Jobling, 2003). Therefore, it is not effective to conduct *L. vannamei* culture in cold seasons and to develop psychrotolerant trains is essential to achieve year-round outdoor culture. Different effects of coculture with seaweed on growth of shrimp have been reported. Coculture with the red seaweed *Gracilaria corticata* increased growth of *L. vannamei,* which could be attributed to decreased ammonia that is poisonous to shrimp (Fourooghifard et al., 2017). In contrast, the growth of *L. vannamei* in coculture with the Philippines seaweed *Kappaphycus alvarezii* did not show a significant increase compared to monoculture (Lombardi et al., 2006). In the present study, coculture increased growth and survival of shrimp in all seasons although the increased extent changed with season. It has been shown that high ammonia can inhibit growth of shrimp (Cobo et al., 2014) and therefore the increased growth of shrimp could be attributed to decreased ammonia levels in coculture mode.

The specific growth rate of *U. linza* varied from 2.43 to 19.36% (Fig. 3b). Culture mode and season affected SGR of *U. linza* and they both had an interactive effect (Table S6). Coculture dramatically increased SGR of thalli by 278.57%, 290.42% and 241.85% in autumn, spring and summer, respectively. For coculture, the highest SGR of *U. linza* occurred in autumn and the lowest was found in spring while the difference between autumn and summer or between summer and spring was not significant for monoculture. The optimal growth temperature for *U. linza* in the Yellow Sea has been demonstrated to be 20 °C (Cui et al., 2015), and our finding also showed that the highest growth rate occurred in autumn (18.3–21.4 °C). However, difference in growth among seasons for monoculture was not as large as for coculture. It is

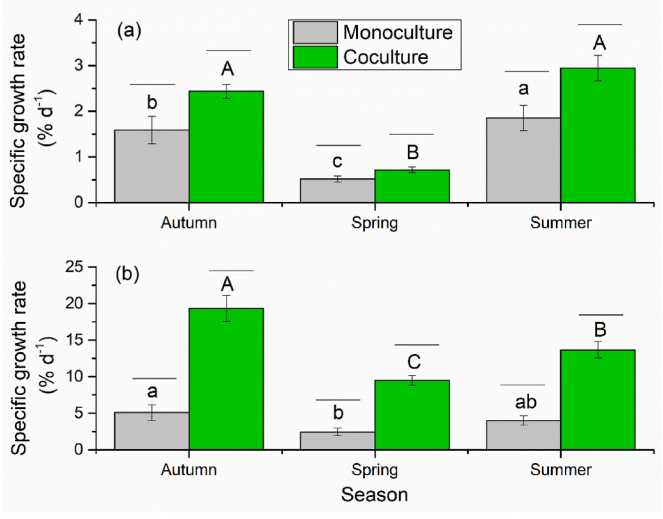

Fig. 3. Specific growth rates of *L. vannamei* (a) and *U. linza* (b) in monoculture and coculture in different seasons. The error bars indicate the standard deviations (n = 3). Different letters (lower case for monoculture and capital for coculture) above the error bars represent significant differences ($P < 0.05$) among seasons. Horizontal short bars represent significant differences ($P < 0.05$) between culture modes.

Table 1
Growth performance of shrimp in different seasons during 28 days of culture.

Parameters	Initial weight (g)	Final weight (g)	TGC [$g^{1/3}$(°C × d)$^{-1}$]	Survival (%)	FCR
Autumn					
Monoculture	3.26 ± 0.01	5.09 ± 0.44^{A*}	0.041 ± 0.008^{A*}	91.09 ± 2.26^{A*}	2.01 ± 0.12^{B*}
Coculture	3.26 ± 0.01	6.45 ± 0.27^{b}	0.066 ± 0.005^{a}	95.83 ± 1.04^{b}	1.72 ± 0.10^{b}
Spring					
Monoculture	3.26 ± 0.01	3.77 ± 0.08^{B}	0.019 ± 0.003^{B*}	84.49 ± 1.75^{B*}	2.78 ± 0.22^{A}
Coculture	3.26 ± 0.01	3.98 ± 0.08^{c}	0.027 ± 0.003^{b}	87.85 ± 1.39^{c}	2.55 ± 0.23^{a}
Summer					
Monoculture	3.26 ± 0.01	5.49 ± 0.44^{A*}	0.039 ± 0.006^{A*}	91.44 ± 1.91^{A*}	1.80 ± 0.11^{B*}
Coculture	3.26 ± 0.01	7.45 ± 0.59^{a}	0.065 ± 0.007^{a}	96.99 ± 1.22^{a}	1.51 ± 0.11^{b}

Each value represents mean ± S.D. (n = 3). Different superscript letters (capital for monoculture and lower case for coculture) represent significant differences ($P < 0.05$) among seasons. Asterisks represent significant differences ($P < 0.05$) among culture modes.

very likely that the lower nutrient levels in monoculture confine the effect of season.

3.3. Bulk biochemical composition

The content of moisture in shrimp ranged from 68.48 to 74.37% (Fig. 4a). Neither culture mode nor season affected moisture in shrimp (Table S7). Protein content in shrimp varied from 15.35 to 21.61% (Fig. 4b). Culture mode did not affect protein content while season had a significant effect (Table S7). The highest protein content was found in summer and the lowest in spring. The synthesis of protein in shrimp usually increases with temperature (Ocampo et al., 2000), which explains the seasonal pattern in the present study. Lipid content in shrimp varied from 1.35 to 2.24% (Fig. 4c). Both culture mode and season affected lipid content in shrimp (Table S7). Coculture increased lipid content in all seasons though the increase in spring was not significant. The highest lipid content occurred in spring and the lowest in summer regardless of culture mode. Ammonia stress can significantly induce shrimp's response, such as increased oxygen consumption (Racotta and Hernández-Herrera, 2000). Lipids have the advantage of providing more energy than carbohydrates and proteins. Therefore, shrimp may consume lipids to deal with environmental disturbance and stress (Racotta and Hernández-Herrera, 2000). This could explain the lower lipid content in monoculture. Meanwhile, coculture reduced nitrogen in seawater, which could lead to the lower nitrogen content in biofloc in seawater. It has reported that biofloc can be ingested by shrimp (Xu and Pan, 2012) and lower nitrogen in biofloc can induce more lipids in shrimp (Xu and Pan, 2012). This may explain the higher lipid content of shrimp in coculture. The highest lipid content was found in spring, which is consistent with the finding in the brown shrimp, *Crangon crangon* (Mika et al., 2014). This may be related to the change of temperature and the cycle of reproduction (Campos et al., 2010). Ash content in shrimp varied from 2.24 to 2.62% (Fig. 4d) and only culture mode affected it (Table S7). Coculture reduced it in autumn and summer, and did not affect it in spring. Seaweed in coculture could uptake minerals in seawater (Brito et al., 2014) which may reduce the ash content in shrimp directly or via biofloc.

In terms of *U. linza*, the protein content ranged from 13.33 to 21.48% (Fig. 4e). Both culture mode and season affected protein content (Table S8). Coculture increased protein content in each season. For each culture mode, the highest protein content was found in summer and the lowest in spring. The high nitrogen levels in seawater could stimulate the protein synthesis in seaweeds since nitrogen is the primary component for protein, which was also found in *U. ohnoi* (Angell et al., 2014) and *U. rigida* (Gao et al., 2017a). The seasonal pattern of protein content could be due mainly to the change of temperature since higher temperatures also simulates the protein content in *U. fasciata* (Mohsen et al., 1973) and *U. rigida* (Gao et al., 2017a). The lipid content in *U. linza* varied from 1.53 to 2.49% (Fig. 4f). Both culture method and season affected lipid content (Table S8). Coculture reduced lipid content although the effect was not statistically significant in each season. The lowest lipid content occurred in summer and the highest in autumn. Contrary to shrimp, *Ulva* in monoculture had higher lipid content. This may be due to the nitrogen limitation-induced lipid synthesis since monoculture had much lower nitrogen levels in seawater compared to coculture. A similar result was also reported in *U. rigida* (Gordillo et al., 2001). Nitrogen limitation can commonly induce lipid synthesis in algae (Jiang et al., 2016). But opposite findings were also reported in *U. rigida* (Gao et al., 2017a) and *U. fenestrata* (Toth et al., 2020). The differential findings may be attributed to the different physiological status of plants. Contrary to shrimp, the highest lipid was found in autumn. Gao et al. (2017) reported that an increase from 14 to 18 °C enhanced lipid content in *U. rigida*. In addition, it was demonstrated that the largest lipid yield in *U. fasciata* was at 20 °C, followed by 15 °C, and 25 °C (Mohsen et al., 1973). The previous findings combined with the present study indicate that the optimal temperature for lipid biosynthesis in *Ulva* may be around 20 °C.

The dietary fiber content in *U. linza* varied 36.08–50.04% (Fig. 4g).

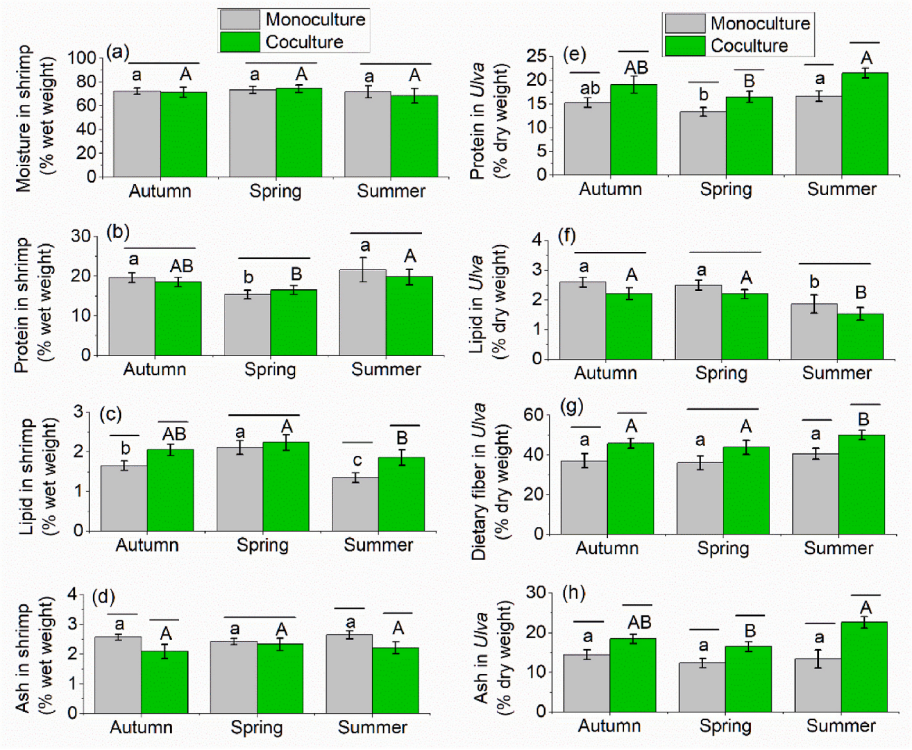

Fig. 4. Chemical composition of *L. vannamei* (a–d) and *U. linza* (e–h) in monoculture and coculture in different seasons. The error bars indicate the standard deviations (n = 3). Different letters (lower case for monoculture and capital for coculture) above the error bars represent significant differences ($P < 0.05$) among seasons. Horizontal short bars represent significant differences ($P < 0.05$) between culture modes.

Both culture mode and season affected the dietary fiber content in *U. linza* (Table S8). Coculture increased it in all seasons although the increase in spring was not significant. For monoculture the difference among seasons was not significant while the highest dietary fiber content occurred in summer for coculture. The release of CO_2 by respiration of shrimp could supply more carbon sources for photosynthesis of *U. linza*, which may contribute to the increased carbohydrate in coculture. The ash content in *U. linza* was in the range of 12.36–22.61% (Fig. 4h). Culture mode and season interacted on ash content (Table S8). Coculture increased the ash content in *U. linza* in each season. This could be due to the increased minerals deriving from feeds in coculture system since *Ulva* species have a high capacity to absorb minerals (Qiu et al., 2018). The highest ash occurred in summer for coculture while the difference among seasons was not significant for monoculture.

3.4. Amino acids and fatty acids

For shrimp (Tables 2 and S9), coculture decreased glutamic acid (Glu), serine (Ser), alanine (Ala) and essential amino acids (EAA). Season affected histidine (His) and total amino acids, with the highest values in summer, followed by autumn and spring. No interactive effects of culture mode and season were found. Crustaceans have developed several mechanisms to address the high ammonia levels in the environment and body. They are (1) formation of glutamine and glutamic acid, (2) formation of alanine and serine, (3) synthesis of purines and (4) conversion to urea. Therefore, increased Glu, Ser and Ala in monoculture in the present study indicate shrimp's response to high ammonia stress. Increases of hemolymph glutamic acid, glutamine, alanine and urea were also observed in *P. monodon* (Chen and Chen, 2000) following 24 h exposure to high ammonia levels.

For *Ulva* (Tables 2 and S9), coculture increased the contents of most amino acids except for aspartic acid (Asp), His, cysteine (Cys) and proline (Pro). Season also affected the contents most amino acids except for His, Cys, tryptophane (Trp), isoleucine (Ile), and the ratio of essential to non-essential amino acids (EAA/NEAA). Generally, *Ulva* in summer had the highest amino acids content, followed by autumn and spring. Culture system and season interacted on the contents Glu, arginine (Arg), total amino acids and non-essential amino acids (NEAA). The stimulating effect of coculture on these amino acids was more significant in autumn and summer than in spring. In plants, glutamic acid is the substrate for all nitrogen based organic compounds, representing the most energy efficient way to store excess nitrogen, and arginine synthesis can also effectively eliminate excess nitrogen (Angell et al., 2014). In summer and autumn, DIN in seawater was higher than that in spring. Therefore, more Glu and Arg could be synthesized.

For shrimp (Tables 3 and S10), coculture and season interacted on the contents of C18, C18:1(n-7), C18:3(n-3) and monounsaturated fatty acids (MUFA). Coculture increased the contents of most fatty acids except for C14, C16:1(n-7), C18:1(n-9), C18:3(n-3), and the ratio of total Omega-6 fatty acids to total Omega-3 fatty acids (n-6/n-3). Season affected the content of each fatty acid. Generally, the lowest content was found in summer. It is interesting that lower contents of saturated fatty acids (SFA) and MUFA but more polyunsaturated fatty acids (PUFA) were found in spring compared to autumn. It seems that cells tend to synthesize more PUFA in spring but consume SFA and MUFA to deal with low temperature, which is also found in the Green Tiger Shrimp *Penaeus semisulcatus* (Kumlu et al., 2019). Since PUFA is helpful for human health (Hulefeld et al., 2018), the increased content of PUFA in coculture suggests coculture with *U. linza* could enhance food quality and functional properties of *L. vannamei*.

For *Ulva* (Tables 3 and S10), culture mode and season interacted on the contents of C16, C16:1(n-7), C17:1(n-6), C18:0, C18:1(n-9), C18:2 (n-6), C20:3(n-6), C20:4(n-6), C20:5(n-3), C22:4(n-6), C22:5(n-3), total fatty acids (FA), SFA, PUFA, and total Omega-6 fatty acids (FA(n-6)). For instance, coculture decreased the contents of C16, C16:1(n-7), C20:4(n-6), C20:5(n-3), C22:5(n-3), and SFA in summer but did not affect them in autumn or spring. *Ulva* tend to reproduce and discharge swarmers under unfavourable conditions (Gao et al., 2017b). The high temperature in summer combined with nutrient limitation (*Ulva*-monoculture) could induce the occurrence of reproduction events. Reproductive cells have higher lipid content compared to vegetative cells, to support the movement, settlement, and germination of swarmers (Steinhoff et al., 2011). This may one of the reasons that the contents of many fatty acids in *Ulva* cultured separately was higher than that in *Ulva*-coculture mode in summer rather than in autumn or spring. It is worth noting that the ingestion of *U. linza* by *L. vannamei* was not found in this study and the profile of amino acids and fatty acids in artificial feed was the same (statistically insignificant) in different seasons. Therefore, the variation of biochemical composition in *L. vannamei* in different culture modes and seasons may be mainly caused by the changing environmental conditions.

3.5. Functional properties

The swelling capacity (SWC) of *U. linza* ranged from 7.24 ± 0.67 to 13.94 ± 0.68 ml g^{-1} DW (Fig. 5a). Both culture mode and season affected swelling capacity and they had an interactive effect (Table S11). Coculture increased swelling capacity in autumn and summer but did not affect it in spring. The highest SWC was found in summer and the lowest SWC in spring. The highest swelling capacity of *U. linza* in the present study was slightly higher than that reported in *U. lactuca* (13.0 ± 0.70 ml g^{-1} DW) by Wong and Cheung (2000) and in *U. rigida* (11.24 ± 0.17 ml g^{-1} DW) by Gao et al. (2018b), which may be due to the species differences. More importantly, the swelling capacity of seaweed is mainly determined by the content of protein and dietary fiber as both of them play a role in hydration properties (Yaich et al., 2011; Gao et al., 2018b). In the present study, coculture increased SWC compared to monoculture, which could be related to the higher content of protein and dietary fiber in coculture systems. The SWC of seaweed can enhance satiety and thus reduce calorie intake. Accordingly, seaweed with strong SWC can be applied in an adjunctive therapy for obesity (Dettmar et al., 2011). The increased SWC in coculture indicates that seaweeds grown in coculture with shrimp can play a more significant role in treating obesity.

The range for WHC was 5.02 ± 0.67–10.61 ± 0.89 g g^{-1} DW (Fig. 5b). Culture mode and season also affected WHC (Table S11). Coculture increased WHC in autumn and summer but did not affect it in spring. The highest WHC was found in summer while there was no significant difference between autumn and spring. The WHC in this study was comparable to that reported in *U. lactuca* (9.71 ± 0.11 g g^{-1} DW) by Wong and Cheung (2000) and in *U. rigida* (5.22 ± 0.10 to 7.24 ± 0.04 g g^{-1} DW) by Gao et al. (2018b). Furthermore, the highest WHC in *U. linza* in this study was higher than some commercial dietary fiber-rich supplements (6.60–9.00 g g^{-1} DW) (Goñi and Martin-Carrón, 1998). The highest WHC was found in summer and coculture also increased WHC compared to monoculture, which could be related to higher protein and fiber content in summer and coculture (Gao et al., 2018b). The water-holding capacity can amend the viscosity and texture of formulated food. Water absorption can lead to the increased viscosity and hence slower rates of intestinal absorption, which is able to decrease postprandial glycaemia and blood cholesterol (Willett et al., 2002).

The OHC of thalli varied from 1.54 ± 0.07 to 2.31 ± 0.14 g g^{-1} DW (Fig. 5c). Similar to SWC and WHC, coculture increased OHC in autumn and summer but did not affect it in spring (Table S11). OHC in monoculture did not change with season but it was lower in spring compared to autumn and summer for coculture. The OHC in this study was slightly higher than the value reported in *U. lactuca* (around 1.60 g g^{-1} DW at treatment temperature of 40 °C) by Yaich et al. (2011) and in *U. rigida* (1.46 ± 0.08 to 1.84 ± 0.07 g g^{-1} DW) by Gao et al. (2018b). Wong and Cheung (2000) demonstrated a high correlation between OHC and total amount of protein and dietary fiber. Coculture increased content of protein and dietary fiber in the present study, which may result in the

Table 2
Amino acid composition (mg g^{-1} DW) of *L. vannamei* and *U. linza* grown in different systems and seasons. Values are means of three replicates ± standard deviation. Different capital and lowercase letters represent significant differences (LSD, $P < 0.05$) among seasons in monoculture or coculture, respectively. Asterisks represent significant differences (LSD, $P < 0.05$) between monoculture and coculture in each season.

	Autumn		Spring		Summer		Autumn		Spring		Summer	
Amino acids	Shrimp-monoculture	Shrimp-coculture	Shrimp-monoculture	Shrimp-coculture	Shrimp-monoculture	Shrimp-coculture	*Ulva*-monoculture	*Ulva*-coculture	*Ulva*-monoculture	*Ulva*-coculture	*Ulva*-monoculture	*Ulva*-coculture
Asp	62.91 ± 4.60	63.75 ± 5.23	59.01 ± 2.31	60.12 ± 4.40	64.41 ± 4.06	65.16 ± 5.40	10.70 ± 0.75^{AB}	10.56 ± 01.04^{b}	9.31 ± 1.08^{B}	9.21 ± 1.13^{b}	11.91 ± 0.60^{A}	12.88 ± 1.35^{a}
Glu	84.86 ± 3.61*	75.71 ± 3.14	82.53 ± 3.34	81.42 ± 3.05	87.04 ± 4.47*	76.82 ± 3.34	13.48 ± 1.10*	22.52 ± 2.42^{a}	12.22 ± 1.05*	15.20 ± 0.86^{b}	14.28 ± 1.17*	20.64 ± 1.18^{a}
Ser	23.32 ± 1.69*	17.24 ± 3.04^{b}	22.86 ± 1.62	20.42 ± 2.22^{ab}	25.05 ± 2.41	21.26 ± 2.25^{a}	6.57 ± 10.85	8.20 ± 10.09^{a}	5.87 ± 0.78	6.47 ± 1.49^{b}	6.45 ± 0.39*	9.58 ± 0.82^{a}
His	19.30 ± 2.29	21.44 ± 3.46^{a}	17.59 ± 1.03	16.52 ± 0.98^{b}	21.41 ± 2.23	21.89 ± 3.12^{a}	3.60 ± 0.48^{AB}	3.89 ± 0.70	2.95 ± 0.33^{B}	3.80 ± 0.31	4.11 ± 0.61^{A}	4.33 ± 0.62
Gly	31.36 ± 3.10	30.4 ± 2.93	31.02 ± 1.63	30.42 ± 1.93	33.70 ± 2.94	39.39 ± 3.12	6.38 ± 0.36^{B}*	7.54 ± 0.46^{b}	5.97 ± 0.30^{B}*	6.99 ± 0.19^{b}	7.08 ± 0.29^{A}*	8.31 ± 0.56^{a}
Thr	29.32 ± 2.05	30.01 ± 2.60	28.64 ± 1.64	28.37 ± 1.14	30.78 ± 2.62	29.08 ± 2.65	6.82 ± 0.57^{AB}*	8.63 ± 0.54^{ab}	6.18 ± 0.55^{B}*	8.04 ± 0.62^{b}	7.48 ± 0.41^{A}*	9.26 ± 0.39^{a}
Arg	44.75 ± 2.69	42.94 ± 3.71	43.69 ± 2.50	41.29 ± 3.03	47.60 ± 1.91	43.64 ± 3.30	13.44 ± 0.82^{A}*	18.23 ± 1.29^{b}	10.91 ± 1.47^{B}*	14.73 ± 1.00^{c}	13.37 ± 1.16^{A}*	21.26 ± 0.94^{a}
Ala	34.47 ± 2.72*	27.37 ± 4.95	32.86 ± 1.67	29.45 ± 1.97	37.00 ± 2.81	31.83 ± 2.97	12.39 ± 1.17^{AB}*	15.52 ± 1.21	10.59 ± 1.21^{B}*	14.63 ± 1.25	13.25 ± 0.97^{A}*	16.31 ± 0.90
Tyr	28.06 ± 3.88	30.25 ± 3.83	26.24 ± 2.05	28.49 ± 1.66	30.73 ± 4.24	32.33 ± 4.01	13.21 ± 1.19^{A}*	16.53 ± 1.18^{ab}	11.06 ± 1.21^{B}*	15.24 ± 0.97^{b}	14.34 ± 0.89^{A}*	17.65 ± 1.06^{a}
Cys	7.72 ± 1.06	8.75 ± 2.06	6.81 ± 0.91	8.46 ± 1.18	9.42 ± 1.41	9.24 ± 2.04	1.85 ± 0.79	1.93 ± 0.81	1.51 ± 0.17	1.40 ± 0.29	2.09 ± 0.34	2.24 ± 0.36
Val	25.64 ± 2.94	23.11 ± 2.90	24.31 ± 2.91	23.28 ± 1.53	28.00 ± 2.92	24.49 ± 2.84	9.62 ± 0.71^{AB}*	12.64 ± 1.04^{a}	8.68 ± 1.09^{B}*	10.99 ± 0.36^{b}	10.55 ± 1.09^{A}*	13.59 ± 1.01^{a}
Met	15.29 ± 1.61^{ab}	14.94 ± 1.68	14.06 ± 1.43^{b}	14.26 ± 1.07	17.00 ± 1.65^{a}	15.56 ± 1.33	3.41 ± 0.43*	4.62 ± 0.45	3.09 ± 0.27*	4.49 ± 0.48	3.72 ± 0.28*	5.17 ± 0.42
Trp	4.62 ± 0.62	4.74 ± 0.34	4.21 ± 0.66	4.57 ± 0.50	5.22 ± 0.86	4.92 ± 0.34	4.66 ± 0.94	5.67 ± 0.87^{ab}	4.01 ± 0.39	4.79 ± 0.53^{b}	4.75 ± 0.43*	5.79 ± 0.43^{a}
Phe	24.98 ± 3.78	23.75 ± 2.89	22.94 ± 2.83	22.09 ± 2.52	26.70 ± 3.32	26.23 ± 1.96	9.59 ± 0.66^{AB}	10.77 ± 1.00	8.81 ± 0.62^{B}	10.25 ± 0.98	10.56 ± 0.72^{A}	11.34 ± 0.97
Ile	44.39 ± 1.61	43.16 ± 3.15	42.88 ± 2.40	42.75 ± 2.59	47.20 ± 2.11	45.32 ± 2.80	6.34 ± 1.20*	8.83 ± 1.11	5.67 ± 0.55*	8.27 ± 0.61	6.87 ± 0.67*	9.55 ± 1.27
Leu	57.37 ± 0.02	58.45 ± 4.49	54.77 ± 3.66	57.52 ± 4.38	59.34 ± 4.00	61.57 ± 4.38	11.34 ± 1.06^{AB}*	13.20 ± 1.00^{ab}	10.35 ± 0.89^{B}*	12.88 ± 0.61^{b}	12.34 ± 1.06^{A}*	14.68 ± 0.89^{a}
Lys	38.45 ± 4.30	36.82 ± 2.94	36.59 ± 3.46	35.46 ± 2.56	40.08 ± 3.38	38.01 ± 2.97	6.63 ± 0.93*	9.82 ± 0.84^{ab}	5.88 ± 0.54*	8.68 ± 0.61^{b}	6.86 ± 0.63*	10.52 ± 0.63^{a}
Pro	27.25 ± 2.13^{ab}	26.95 ± 3.22	24.36 ± 3.10^{b}	23.85 ± 2.139	28.86 ± 1.56^{a}	26.32 ± 2.09	6.20 ± 0.91	6.97 ± 0.77^{ab}	6.08 ± 0.34	6.04 ± 0.37^{b}	6.84 ± 0.54	7.36 ± 0.55^{a}
Total	604.07 ± 19.71^{ab}	579.77 ± 19.97	575.38 ± 15.66^{b}	568.73 ± 33.72	639.54 ± 24.11^{a}	603.07 ± 16.42	146.24 ± 2.63^{B}*	186.08 ± 2.56^{b}	129.14 ± 1.66^{C}*	162.08 ± 4.58^{c}	156.85 ± 1.45^{A}*	200.65 ± 1.34^{a}
EAA[a]	299.50 ± 14.15	265.54 ± 33.40	285.48 ± 14.05	281.53 ± 6.71	318.11 ± 13.59*	275.82 ± 32.95	70.80 ± 2.18 ^{B}*	90.63 ± 1.44^{b}	62.53 ± 0.71^{C}*	82.12 ± 2.57^{c}	75.85 ± 2.29^{A}*	99.71 ± 0.98^{a}
NEAA[b]	304.57 ± 5.95	314.23 ± 44.85	289.89 ± 6.44	287.20 ± 37.56	321.43 ± 10.76	327.24 ± 47.01	75.45 ± 0.62 ^{B}*	95.45 ± 3.35^{b}	66.61 ± 0.99^{C}*	79.96 ± 2.07^{c}	81.00 ± 1.48^{A}*	100.94 ± 0.97^{a}
EAA/NEAA	0.98 ± 0.03	0.86 ± 0.25	0.99 ± 0.05	0.99 ± 0.15	0.99 ± 0.01	0.87 ± 0.24	0.94 ± 0.02	0.95 ± 0.05^{b}	0.94 ± 0.01*	1.03 ± 0.01^{a}	0.93 ± 0.04*	0.99 ± 0.01^{b}

[a] EAA, essential amino acids: His, Thr, Arg, Val, Met, Phe, Ile, Leu, and Lys.
[b] NEAA, non-essential amino acids: Asp, Glu, Ser, Gly, Ala, Tyr, Cys, Trp, and Pro.

Table 3
Fatty acid composition (mg g^{-1} DW) of *L. vannamei* and *U. linza* grown in different systems and seasons. Values are means of three replicates ± standard deviation. Different capital and lowercase letters represent significant differences (LSD, $P < 0.05$) among seasons in monoculture or coculture, respectively. Asterisks represent significant differences (LSD, $P < 0.05$) between monoculture and coculture in each season.

	Autumn		Spring		Summer		Autumn		Spring		Summer	
Fatty acids	Shrimp-monoculture	Shrimp-coculture	Shrimp-monoculture	Shrimp-coculture	Shrimp-monoculture	Shrimp-coculture	*Ulva*-monoculture	*Ulva*-coculture	*Ulva*-monoculture	*Ulva*-coculture	*Ulva*-monoculture	*Ulva*-coculture
C14:0	0.25 ± 0.02^{A}	0.28 ± 0.02^{a}	0.25 ± 0.03^{AB}	0.23 ± 0.02^{b}	0.21 ± 0.02^{B}	0.24 ± 0.01^{ab}	0.22 ± 0.02	0.21 ± 0.02^{a}	0.19 ± 0.01	0.18 ± 0.02^{a}	0.21 ± 0.02*	0.15 ± 0.01^{b}
C16:0	9.57 ± 0.46^{A}*	11.01 ± 0.68^{a}	9.05 ± 0.76^{A}	9.16 ± 0.44^{b}	7.84 ± 0.39^{B}*	8.88 ± 0.64^{b}	7.69 ± 0.32^{AB}	7.51 ± 0.46^{a}	7.14 ± 0.54^{B}	7.42 ± 0.43^{a}	7.94 ± 0.50^{A}*	5.43 ± 0.16^{b}
C16:1(n-7)	0.62 ± 0.06^{A}	0.69 ± 0.07^{a}	0.51 ± 0.05^{B}	0.48 ± 0.04^{c}	0.50 ± 0.05^{B}	0.59 ± 0.05^{b}	0.28 ± 0.03^{A}	0.28 ± 0.02^{a}	0.28 ± 0.03^{AB}	0.27 ± 0.01^{a}	0.32 ± 0.02^{B}*	0.21 ± 0.02^{b}
C16:2(n-4)	0.24 ± 0.03^{B}	0.26 ± 0.02^{b}	0.30 ± 0.03^{A}*	0.36 ± 0.03^{a}	0.20 ± 0.02^{B}	0.21 ± 0.02^{c}	–	–	–	–	–	–
C16:3(n-4)	0.44 ± 0.03^{B}*	0.52 ± 0.04^{b}	0.54 ± 0.03^{A}*	0.62 ± 0.04^{a}	0.37 ± 0.04^{C}*	0.45 ± 0.03^{c}	–	–	–	–	–	–
C17:1(n-6)	–	–	–	–	–	–	0.09 ± 0.00^{AB}	0.09 ± 0.01^{a}	0.09 ± 0.01^{A}	0.07 ± 0.01^{b}*	0.08 ± 0.01^{B}	0.08 ± 0.00^{a}
C18:0	5.56 ± 0.56^{A}*	6.99 ± 0.45^{a}	5.06 ± 0.37^{AB}	5.03 ± 0.26^{b}	4.79 ± 0.29^{B}*	5.83 ± 0.34^{c}	0.16 ± 0.00^{A}*	0.14 ± 0.00^{a}	0.13 ± 0.01^{B}	0.13 ± 0.01^{b}	0.14 ± 0.01^{B}*	0.12 ± 0.00^{c}
C18:1(n-9)	5.72 ± 0.46^{A}	6.24 ± 0.49^{a}	5.57 ± 0.45^{A}	5.55 ± 0.41^{ab}	4.78 ± 0.34^{B}	5.46 ± 0.36^{b}	0.26 ± 0.02	0.27 ± 0.02^{a}	0.30 ± 0.02	0.21 ± 0.03^{b}*	0.28 ± 0.02	0.17 ± 0.02^{b}*
C18:1(n-7)	2.19 ± 0.16*	3.55 ± 0.20a	2.12 ± 0.38	2.31 ± 0.24^{c}	1.80 ± 0.12*	2.90 ± 0.18^{b}	4.12 ± 0.38*	3.38 ± 0.29^{a}	4.15 ± 0.27*	3.37 ± 0.19^{a}	3.99 ± 0.23*	2.75 ± 0.32^{b}
C18:2(n-6)	6.52 ± 0.43^{B}*	7.88 ± 0.40^{b}	7.93 ± 0.52^{A}*	9.56 ± 0.68^{a}	5.44 ± 0.38^{C}*	6.40 ± 0.51^{c}	1.96 ± 0.05	2.03 ± 0.26^{a}	1.98 ± 0.13*	1.23 ± 0.11^{c}	1.85 ± 0.09*	1.49 ± 0.12^{b}
C18:3(n-6)	0.74 ± 0.06^{B}	0.81 ± 0.05^{b}	0.89 ± 0.05^{A}*	1.06 ± 0.08^{a}	0.60 ± 0.04^{C}	0.68 ± 0.04^{c}	0.07 ± 0.01	0.06 ± 0.01^{a}	0.07 ± 0.01	0.06 ± 0.01^{a}	0.06 ± 0.01*	0.05 ± 0.01^{b}
C18:3(n-3)	0.38 ± 0.03^{B}	0.41 ± 0.03^{b}	0.68 ± 0.05^{A}*	0.60 ± 0.06^{a}	0.31 ± 0.02^{B}*	0.41 ± 0.04^{b}	4.46 ± 0.12	4.51 ± 0.37^{a}	4.44 ± 0.30*	3.83 ± 0.30^{b}	3.98 ± 0.24*	3.44 ± 0.32^{b}
C20:0	0.59 ± 0.03^{B}*	0.68 ± 0.05^{b}	0.70 ± 0.02^{A}*	0.83 ± 0.05^{a}	0.49 ± 0.03^{C}*	0.58 ± 0.04c	0.04 ± 0.01^{A}	0.03 ± 0.01	0.04 ± 0.00^{A}*	0.02 ± 0.00	0.03 ± 0.01^{B}	0.02 ± 0.00
C20:2(n-6)	0.68 ± 0.06^{B}	0.75 ± 0.07^{b}	0.83 ± 0.06^{A}	0.90 ± 0.05^{a}	0.57 ± 0.04^{C}	0.65 ± 0.05^{c}	–	–	–	–	–	–
C22:0	0.46 ± 0.04^{B}	0.50 ± 0.04^{b}	0.56 ± 0.04^{A}	0.62 ± 0.05^{a}	0.38 ± 0.02^{C}	0.41 ± 0.04^{c}	0.37 ± 0.02^{A}	0.36 ± 0.04^{a}	0.27 ± 0.02^{B}	0.23 ± 0.02^{b}	0.30 ± 0.03^{B}*	0.23 ± 0.04^{b}
C20:3(n-6)	–	–	–	–	–	–	0.09 ± 0.00^{A}*	0.07 ± 0.00^{a}	0.07 ± 0.00^{B}	0.07 ± 0.01^{a}	0.08 ± 0.01^{B}*	0.05 ± 0.00^{b}
C20:4(n-6)	0.12 ± 0.01^{B}	0.13 ± 0.01^{b}	0.15 ± 0.01^{A}*	0.17 ± 0.01^{a}	0.10 ± 0.01^{C}	0.11 ± 0.00^{c}	0.13 ± 0.02*	0.09 ± 0.00^{b}	0.12 ± 0.01	0.13 ± 0.02^{a}	0.12 ± 0.02*	0.08 ± 0.01^{b}
C20:4(n-3)	0.45 ± 0.07^{AB}*	0.68 ± 0.09^{b}	0.56 ± 0.06^{A}*	0.81 ± 0.05^{a}	0.38 ± 0.03^{B}*	0.58 ± 0.04^{b}	–	–	–	–	–	–
C20:5(n-3)	9.67 ± 0.56^{B}*	11.42 ± 0.84^{b}	16.61 ± 0.66^{A}	16.55 ± 0.89^{a}	7.77 ± 0.46^{C}*	9.37 ± 0.84^{c}	0.29 ± 0.01^{A}	0.30 ± 0.03^{a}	0.27 ± 0.02^{AB}*	0.23 ± 0.03^{b}	0.24 ± 0.02^{B}*	0.18 ± 0.02^{c}
C22:4(n-6)	–	–	–	–	–	–	0.04 ± 0.00	0.04 ± 0.00^{a}	0.04 ± 0.00	0.03 ± 0.00^{a}	0.04 ± 0.00*	0.02 ± 0.01^{b}
C22:5(n-6)	0.14 ± 0.01^{B}*	0.16 ± 0.01^{b}	0.16 ± 0.01^{A}*	0.19 ± 0.01^{a}	0.11 ± 0.01^{C}*	0.14 ± 0.01^{c}	–	–	–	–	–	–
C22:5(n-3)	0.55 ± 0.05^{B}*	0.73 ± 0.03^{b}	0.75 ± 0.06^{A}*	0.91 ± 0.06^{a}	0.46 ± 0.04^{C}*	0.60 ± 0.03^{c}	0.08 ± 0.00	0.08 ± 0.01^{a}	0.08 ± 0.01	0.08 ± 0.00^{a}	0.08 ± 0.01*	0.06 ± 0.00^{b}
C22:6(n-3)	7.73 ± 0.51^{B}*	9.86 ± 0.73^{b}	10.38 ± 0.62^{A}*	12.90 ± 0.74^{a}	6.44 ± 0.38^{C}*	8.09 ± 0.61^{c}	0.05 ± 0.00	0.05 ± 0.00^{a}	0.05 ± 0.00*	0.03 ± 0.00^{b}	0.04 ± 0.00	0.03 ± 0.01^{b}
Total FA	52.63 ± 3.50^{B}*	63.53 ± 4.04^{b}	63.60 ± 2.05^{A}*	68.83 ± 1.66^{a}	43.54 ± 2.66^{C}*	52.58 ± 2.88^{c}	20.38 ± 0.93	19.49 ± 1.44^{a}	19.70 ± 0.69*	17.60 ± 0.84^{b}	19.75 ± 1.12*	14.56 ± 0.81^{c}
SFA[a]	16.43 ± 1.10^{A}*	19.46 ± 1.23^{a}	15.61 ± 1.12^{A}	15.86 ± 0.57^{b}	13.72 ± 0.75^{B}*	15.94 ± 1.05^{b}	8.48 ± 0.33^{AB}	8.25 ± 0.47^{a}	7.76 ± 0.52^{B}	7.99 ± 0.42^{a}	8.60 ± 0.53^{A}*	5.94 ± 0.19^{b}
MUFA[b]	8.53 ± 0.68^{A}*	10.48 ± 0.75^{a}	8.20 ± 0.67^{A}	8.34 ± 0.44^{b}	7.07 ± 0.51^{B}*	8.95 ± 0.58^{b}	4.76 ± 0.42*	4.01 ± 0.29^{a}	4.82 ± 0.25*	3.92 ± 0.20^{a}	4.67 ± 0.24*	3.20 ± 0.32^{b}
PUFA[c]	27.66 ± 1.72^{B}*	33.59 ± 2.13^{b}	39.79 ± 1.16^{A}*	44.63 ± 1.94^{a}	22.75 ± 1.41^{C}*	27.69 ± 1.60^{c}	7.15 ± 0.18^{A}	7.23 ± 0.62^{a}	7.12 ± 0.20^{AB}*	5.69 ± 0.22^{b}	6.48 ± 0.35^{B}*	5.41 ± 0.44^{b}
FA(n-6)[d]	8.19 ± 0.57^{B}*	9.73 ± 0.51^{b}	9.96 ± 0.66^{A}*	11.89 ± 0.83^{a}	6.82 ± 0.48^{C}*	7.98 ± 0.48^{c}	2.28 ± 0.05	2.30 ± 0.26^{a}	2.28 ± 0.12*	1.52 ± 0.10^{b}	2.14 ± 0.10*	1.69 ± 0.13^{b}
FA(n-3)[e]	18.79 ± 1.10^{B}*	23.09 ± 1.56^{b}	28.99 ± 0.69^{A}*	31.77 ± 1.19^{a}	15.36 ± 0.88^{C}*	19.06 ± 1.41^{c}	4.87 ± 0.14	4.94 ± 0.40^{a}	4.84 ± 0.32*	4.17 ± 0.32^{b}	4.34 ± 0.26*	3.72 ± 0.31^{b}
n-6/n-3[f]	0.44 ± 0.01^{A}	0.42 ± 0.01^{a}	0.34 ± 0.02^{B}	0.37 ± 0.02^{b}	0.44 ± 0.01^{A}	0.42 ± 0.04^{a}	0.47 ± 0.01	0.46 ± 0.03^{a}	0.47 ± 0.06*	0.37 ± 0.05^{b}	0.49 ± 0.01	0.46 ± 0.01^{a}

The symbol "-" means these fatty acids were undetectable for shrimp or *Ulva*.

[a] SFA, saturated fatty acids.

[b] MUFA, monounsaturated fatty acids.

[c] PUFA, polyunsaturated fatty acids.

[d] FA(n-6), total Omega-6 fatty acids.

[e] FA(n-6), total Omega-3 fatty acids.

[f] n-6/n-3, the ratio of total Omega-6 fatty acids to total Omega-3 fatty acids.

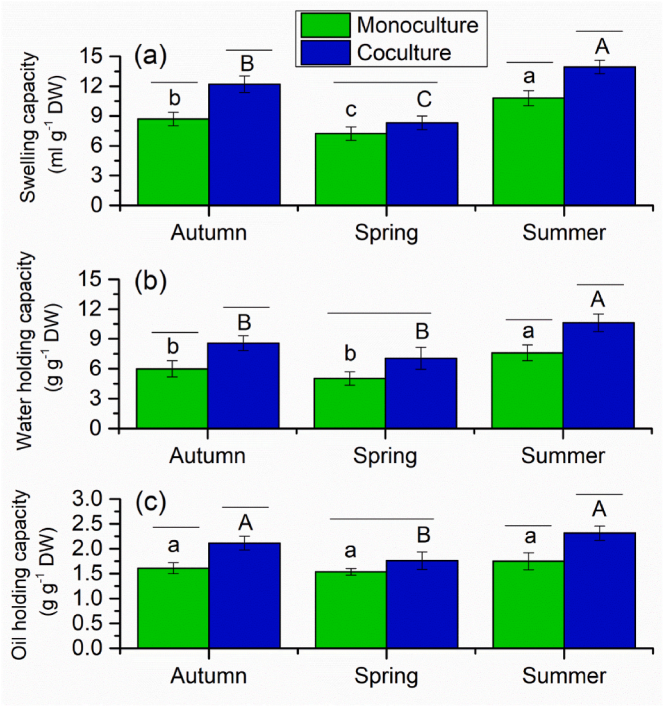

Fig. 5. Swelling capacity (a), water holding capacity (b) and oil holding capacity (c) of *U. linza* in different culture modes and seasons. The error bars indicate the standard deviations (n = 3). Different letters (lower case for monoculture and capital for coculture) above the error bars represent significant differences ($P < 0.05$) among seasons. Horizontal short bars represent significant differences between culture modes.

higher OHC compared to monoculture. Seaweeds are considered as a valuable functional food due to their high OHC because OHC can reduce blood lipid level, obesity and coronary heart disease risk (Hong et al., 2007; Gao et al., 2018b). The findings in this study indicate that *U. linza* could be consumed as a functional food particularly for those grown in coculture with shrimp.

4. Conclusions

By setting up monoculture and coculture systems, our study assessed the effects of coculture system with *U. linza* and *L. vannamei* on growth rates and functional properties of both the shrimp and the alga in different seasons for the first time. *U. linza* showed its strong capacities in remitting eutrophication, acidification and deoxygenation caused by shrimp culture in all seasons. Coculture immensely stimulated SGR of the alga. The shrimp also had higher final weight, SGR, TGC and survival in coculture compared to monoculture. Coculture enhanced total FA in shrimp and total AA in *U. linza*. Furthermore, bioremediating *U. linza* in coculture systems had higher SWC, WHC and OHC. These findings suggest the double benefits of this culture mode in terms of bioremediation of environment and increased growth rates. Due to the limit of manpower and finance, the culture size and period in this study is smaller and shorter than expected, and microbial evaluation (like microbial community in water and shrimp gut) was not conducted either. The amount of feed and its impact on the water quality will change as the shrimps become larger. Future research should be conducted on a larger size and over a longer period of time with microbial evaluation to assess the production and bioremediation efficiency for developing this model on a commercial scale, and to further explain potential mechanisms.

CRediT authorship contribution statement

Guang Gao: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Lin Gao:** Investigation, Visualization, Writing – review & editing. **Qianqian Fu:** Investigation, Methodology, Formal analysis. **Xinshu Li:** Methodology, Investigation. **Juntian Xu:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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