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The Potential of Kelp *Saccharina japonica* in Shielding Pacific Oyster *Crassostrea gigas* From Elevated Seawater pCO_2 Stress

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Ocean acidification (OA) caused by elevated atmospheric CO_2 concentration is predicted to have negative impacts on marine bivalves in aquaculture. However, to date, most of our knowledge is derived from short-term laboratory-based experiments, which are difficult to scale to real-world production. Therefore, field experiments, such as this study, are critical for improving ecological relevance. Due to the ability of seaweed to absorb dissolved carbon dioxide from the surrounding seawater through photosynthesis, seaweed has gained theoretical attention as a potential partner of bivalves in integrated aquaculture to help mitigate the adverse effects of OA. Consequently, this study investigates the impact of elevated pCO_2 on the physiological responses of the Pacific oyster *Crassostrea gigas* in the presence and absence of kelp (*Saccharina japonica*) using *in situ* mesocosms. For 30 days, mesocosms were exposed to six treatments, consisting of two pCO_2 treatments (500 and 900 μatm) combined with three biotic treatments (oyster alone, kelp alone, and integrated kelp and oyster aquaculture). Results showed that the clearance rate (CR) and scope for growth (SfG) of *C. gigas* were significantly reduced by elevated pCO_2, whereas respiration rates (MO_2) and ammonium excretion rates (ER) were significantly increased. However, food absorption efficiency (AE) was not significantly affected by elevated pCO_2. The presence of *S. japonica* changed the daytime pH_{NBS} of experimental units by ~0.16 units in the elevated pCO_2 treatment. As a consequence, CR and SfG significantly increased and MO_2 and ER decreased compared to *C. gigas* exposed to elevated pCO_2 without *S. japonica.* These findings indicate that the presence of *S. japonica* in integrated aquaculture may help shield *C. gigas* from the negative effects of elevated seawater pCO_2.

Keywords: *Saccharina japonica*, *Crassostrea gigas*, elevated seawater pCO_2, mesocosm, physiological responses

INTRODUCTION

Ocean acidification (OA) caused by elevated concentrations of atmospheric CO_2 is one of the most serious environmental issues facing the world in the 21st century. Multiple studies have indicated that this more acidic oceanic environment will have negative consequences for marine life, such as decreasing calcification rates and impairing feeding, respiration, and physiological energetics (Gazeau et al., 2007; Rastrick et al., 2018a; Jiang et al., 2021). Damage to marine organisms by OA is likely to alter coastal biodiversity, ecosystem functioning and services, and global marine harvests (Cooley et al., 2009; Barry et al., 2011). Calcifying marine organisms such as molluscs, crustaceans, corals, and planktonic calcifies are predicted to be particularly sensitive due to the additional costs associated with calcification and maintenance of calcified structures (Fabry et al., 2008; Hofmann et al., 2010; Findlay et al., 2011; Rastrick et al., 2014). Marine bivalves are calcifying ecosystem engineers, which are important in ecosystem functioning and aquaculture (Cranford et al., 2012). In accordance with their ecological and commercial relevance, the interest in OA research on bivalves is growing steadily and has increased particularly in recent years (e.g., Berge et al., 2006; Cummings et al., 2011; Li et al., 2017; Zhao et al., 2017; Rastrick et al., 2018a; Tan and Zheng, 2019). Evidence from several previous studies suggests that elevated seawater pCO_2 affects calcification (e.g., Ries et al., 2009; Melzner et al., 2011), growth (e.g., Thomsen et al., 2010; Kroeker et al., 2013), burrowing behavior (e.g., Clements et al., 2015; Peng et al., 2017), energetics (e.g., Wang et al., 2015; Zhao et al., 2017; Rastrick et al., 2018a) and immune response (e.g., Bibby et al., 2008; Zha et al., 2017) in bivalves such as oysters, mussels, and clams. However, much of the published information on bivalves comes from short-term laboratory-based manipulative experiments, which reduces the generalizability of results to real-world situations. With the increasing demand for improving the ecological validity of OA studies, it is necessary to scale up the methodology from laboratory- to field-level (Riebesell et al., 2008; Rastrick et al., 2018b).

The Pacific oyster, *Crassostrea gigas*, is one of the most important ecologically and economically cultured bivalves worldwide, with a global mariculture production of 643 thousand tons in 2018 (FAO, 2020). The effects of OA on the Pacific oyster have been well documented. Previous studies show that early developmental stages of oysters are more susceptible to increased seawater pCO_2 (Kurihara et al., 2007; Gazeau et al., 2011). (Barton et al. 2012; 2015) reported that larval production and mid-stage growth (~120 to ~150 mm) of *C. gigas* showed a significantly negative correlation to naturally elevated carbon dioxide levels and clearly linked increased CO_2 to the cause of severe loss of production at the Whiskey Creek Shellfish Hatchery on the Oregon coast. The calcification rates of *C. gigas* have been predicted to decline by 10% following the IPCC IS92a scenario (~740 ppmv in 2,100) (Gazeau et al., 2007). Negative impacts of elevated pCO_2 to *C. gigas* acid–base status and immune response have also been reported by Lannig et al. (2010) and Wang et al. (2016). Despite increased understanding of the potential impacts of OA on Pacific oysters, studies regarding the physiological effects of OA on *C. gigas* under natural systems are scarce. Furthermore, there is still a lack of effective ways for the oyster aquaculture industry to help mitigate the potential negative impacts of OA.

Previous studies have postulated that Integrated Multi-Trophic Aquaculture (IMTA) may be a possible and sustainable solution to help mitigate some of the effects of climate change on production (Clements and Chopin, 2016; Tan and Zheng, 2020). The term "IMTA" refers to the combined aquaculture of various organisms at different trophic levels with complementary ecosystem functions in which the wastes and by-products from one species serve as the food source for one or a number of other species (Chopin, 2013). The seaweed aquaculture sector may be one of the key components of IMTA systems (Chopin, 2014). Seaweed within IMTA is traditionally regarded as a source of nutrient removal from the seawater of the nearby bivalve farms and plays a role in the bioremediation and ecological regulation of the aquaculture environment. Seaweed also has the ability to add oxygen and absorb dissolved carbon dioxide from the surrounding seawater through photosynthesis, producing biomass and contributing to carbon sequestration. It has been reported that ~0.34 million t carbon is removed from the coastal ecosystems each year by seaweed harvesting (Tang et al., 2011). The sea–air CO_2 flux is enhanced by seaweed aquaculture (Jiang et al., 2013). Bay scale dissolved inorganic carbon (DIC) budget research showed that the annual DIC uptake by seaweed *Saccharina japonica* and *Gracilaria lemaneiformis* was about 1.0×10^5 t in Sanggou Bay, which drove the bay as a net DIC sink (Jiang et al., 2015). By sequestering carbon dioxide dissolved in seawater, seaweed plays a potential role in reducing OA and its biological effects on a local scale. An interesting study on the effect of dissolved CO_2 levels (0, 100, 200, 250, and 300 ppm) on the dissolution rate of the outer shells of dead mollusks and spines of sea urchins in the presence and absence of seaweed, *Chaetomorpha antennina*, showed that the weight loss of the samples was curbed when seaweed was introduced (Kaladharan et al., 2019). In the coastal regions of southern Korea, an innovative research approach called the Coastal CO_2 Removal Belt (CCRB) has established a pilot farm of perennial brown alga, *Eklonia calva*, which sequesters ~10 t of CO_2 per ha per year (Chung et al., 2013). Jiang et al. (2014) carried out a mesocosm experiment to monitor the function of seaweed *G. lemaneiformis* in eliminating dissolved inorganic carbon released from the calcification and respiration processes of the scallop *Chlamys farreri*. The results showed that seaweed and bivalve integrated aquaculture practice can not only effectively balance the seawater carbonate system but also buffer acidification. However, although the ecological and economic benefits of IMTA have been highlighted, the role of seaweed within IMTA in mitigating the impacts of OA on bivalves (e.g., physiological activities, energy allocation and growth, etc.) needs further investigation, especially within natural systems.

The goal of this research was to examine: (1) whether the physiological processes and net energy balance of *C. gigas* were

affected after medium-term exposure (30 days) to elevated pCO_2 and (2) whether the presence of *S. japonica* could help mitigate these biological effects of elevated pCO_2 on *C. gigas* within a natural system.

MATERIALS AND METHODS

Animal Collection and Acclimatization

Experimental oysters (56.37 ± 8.20 mm shell length, 29.67 ± 3.91 g wet weight) and kelps (70–80 cm length) were collected on 7 May 2017 from a large-scale commercial aquaculture area (37° 03′52.17″N, 122°33′11.54″E) in Sanggou Bay, Yellow Sea, China. All specimens were transported on ice to a small semi-enclosed harbor (37°02′14.71″N, 122°33′02.09″E; area 7900 m^2; mean depth 1.5 m). The oysters were cleaned and maintained in flowing seawater in *in situ* mesocosms at ambient conditions (temperature ~19.0°C, salinity ~32, and seawater pH_{NBS} ~8.10) for one week before the start of the experiment. One hundred and thirty healthy oysters were selected and individually labeled with a plastic-laminated number on the upper valve.

Experimental Setup and Procedure

Experiments were conducted using *in situ* mesocosms with six treatments: three biotic treatments (namely "oyster alone", "kelp alone," and "integrated aquaculture"), crossed with two pCO_2 treatments (ambient, ~500 µatm and elevated, ~900 µatm). Each of the six treatments was replicated three times, for a total of 18 mesocosms. The mesocosms were suspended from a floating platform. Each sealed mesocosm was made of translucent polyethylene and was cylinder-shaped (0.5 m × 1.2 m, diameter × height) (**Figure 1**). Inlet and outlet valves allow for the control of seawater flow through each mesocosm. The top of each mesocosm was immersed to a depth of ~30 cm with the inlet and outlet pipes (Φ ~3 cm) extending ~50 cm above the water surface. The inlet pipe extended to the bottom of each mesocosm, allowing water mixing and preventing gradients from forming within the mesocosm.

Elevated pCO_2 treatment was controlled by enriching natural seawater with CO_2 (from a CO_2 gas cylinder) in a submerged 1,000 L reservoir tank suspended from the same floating platform as the mesocosms (modified from Rastrick et al., 2014; Jiang et al., 2019; Jiang et al., 2021). The flow of pure CO_2 to the reservoir tank was controlled *via* a pH controller (Aqua Medic GmbH, Germany) by switching on or off a valve when pH readings in the tank deviated from the predetermined set-points by ±0.05 pH units. The acidified seawater was then pumped to each elevated pCO_2 mesocosm *via* flow rate controllers (60 L h^{-1}). As the CO_2-adjusted seawater was pumped out of the reservoir tank, it was replaced with natural ambient seawater using an automatic pump triggered by changes in volume within the tank. This gave a seawater residence time in the reservoir tank of just under an hour. Pumps in the reservoir tank prevented the formation of CO_2 or temperature gradients, and the ambient temperature was maintained due to the submersion of the reservoir tank and the mesocosms.

Each "oyster alone" treatment contained 8 oysters hugged along a rope tied at the top of the mesocosm to simulate farm conditions. The kelp alone treatment contained 1 blade with holdfasts (70–80 cm length) that were tied at the top of the mesocosm to keep the orientation vertical as in the farm environment. An integrated aquaculture treatment contained both oysters (n = 8) and kelp (n = 1) hung in the same way. The oyster number and the ratio of oyster and kelp were determined based on our modeling research which was published in Lin et al. (2020). The experiment started on the 14 May 2017 (experiment day 1) and continued until 12 June 2017.

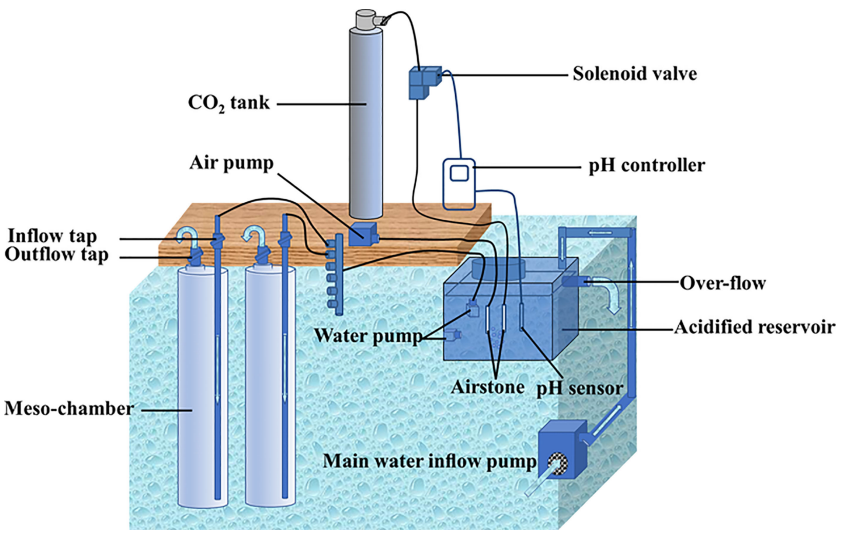

FIGURE 1 | Schematic diagram of the pH controlling and mesocosm system.

Monitoring of the Key Parameters of Seawater

During the 30-day experiment, seawater temperature, salinity, and dissolved oxygen concentration (DO) in each mesocosm were measured three times a day. The pH in the reservoir tank was also measured three times a day, and the pH in the inflow and outflow of each mesocosm was measured only at 12:00 daily. Seawater temperature, salinity, and dissolved oxygen concentration (DO) were measured using a Vernier's LabQuest® 2 (Vernier Software & Technology, USA) equipped with the corresponding probes. The pH level was measured using a commercial combination electrode (Ross type, Orion) calibrated daily using a 3-point calibration procedure on the U.S. National Bureau of Standards (NBS) scale with a precision of ± 0.001 pH units. The concentration of chlorophyll-a in the ambient seawater was continuously monitored using the ACLW-RS chlorophyll turbidity temperature sensor (ALEC Electronics Co., Ltd., Japan) with a precision of ±0.1 μg L^{-1}. Total alkalinity (A_T) was analysed weekly *via* a Metrohm 848 Titrino plus automatic titrator (Metrohm, USA) on 100 ml of GF/F filtered seawater with an accuracy of ±5 mmol L^{-1}. Carbonate system parameters, such as total dissolved inorganic carbon, aqueous partial pressure of CO_2 (pCO_2), the $CaCO_3$ saturation state for calcite (Ω_{cal}), and aragonite (Ω_{arag}), were calculated from pH_{NBS} and A_T by the CO2SYS Package (Pierrot et al., 2006) using the dissociation constants of Mehrbach et al. (1973) and refit by Dickson and Millero (1987). No mortality of oysters was observed in both ambient and elevated pCO_2 treatments throughout the experiment.

Physiological Measurements

The physiological measurements of oysters under different treatments were performed on 12 June 2017 (experiment day 30) by flow-through system after Jiang et al. (2017). Feeding experiments were performed by transferring 9 labeled individual oysters (3 randomly selected from each of the 3 replicate mesocosms per treatment) to individual flow-through chambers which were continuously supplied with the same water as supplied to the respective treatment mesocosms. The flow rates of each chamber were adjusted to 180–200 ml min^{-1} and determined by simultaneously measuring the volume of water collected from each outflow. Three identical chambers without oysters served as the control. The oysters were left undisturbed in chambers for 1 h to resume feeding before sampling water from the outlet of the chambers. The particle number in each chamber was measured by PAMAS S4031GO particle counter equipped with a tube of 50 μm aperture and set to count all particles >2 μm diameter in a 0.5 ml sub-sample. Three replicate counts are made on each sample and the mean calculated. This measurement was repeated at 60 min intervals over a period of 3 h. Clearance rate (CR) is then calculated as follows:

$$CR\left(L\,h^{-1}\right) = \text{Flow rate}\left(\text{in } L\,h^{-1}\right) \times (C_1 - C_0)/C_1$$

where C_1 is the particulate concentration in control chamber (particle number ml^{-1}), and C_0 is the particulate concentration from each experimental chamber (particle number ml^{-1}).

The absorption efficiency (AE) was determined using the following equation (Conover, 1966):

$$AE(\%) = (F - E)/[(1 - E) \times F]$$

Where F represents the ash-free dry weight: dry weight ratio of food, and E represents the ash-free dry weight: dry weight ratio of the feces. Approximately 4 L of food samples were taken from the seawater being supplied to each treatment, and 4 sub-samples were filtered with pre-ashed and weighted glass fiber filters of 1.2 μm (Whatman GF/C 47mm). These filters were rinsed with isotonic ammonium formate (3%) to remove salts and dried to a constant weight (~48 h) at 65°C and weighed (W_{60}), then ashed at 450°C for 6 h to get the ash-free dry weight $W_{450.}$ The particulate organic matter concentration (POM, mg L^{-1}) is calculated by POM = (W_{60} − W_{450})/v. Each oyster was placed in the individual feces collector at the respective treatment conditions for ~24 h after the CR measurements. The feces collector (Φ ~10 cm) was made of the casing pipe equipped with an 80 μm mesh screen (after, Rastrick et al., 2018a). Feces were collected by a pipette and filtered with the organic content of the feces measured as described above.

The respiration rate (MO_2) of individual oysters was measured in ~200 ml stop-flow glass respirometers held in a water bath at the same ambient temperature as the mesocosms and supplied with the same amount of water. Twelve respirometers were run simultaneously, with three chambers without oysters being used as controls. The inflow of each respirometer came from corresponding treatments, and constant flow rates were maintained by a multichannel peristaltic pump. Thirty minutes were allowed for the oysters to recover from handling stress, open and resume pumping, then the flow of water was stopped and MO_2 was measured over the next 90 min. The oxygen concentration in each respirometer was not allowed to drop below 70% saturation throughout the experiment. The rate of decline in oxygen saturation in each respirometer was measured by a calibrated optical oxygen probe connected to a Vernier's LabQuest® 2 (Vernier Software & Technology, USA). The initial and final oxygen concentrations in each respirometer were measured. The respiration rate (MO_2) was then calculated using the following equation:

$$\dot{M}O_2\left(\mu\text{mol } O_2 h^{-1}\right) = \left[C_{(t0)} - C_{(t1)}\right] \times V_r \times 60/(t_1 - t_0)$$

Where t_0 and t_1 represent the initial and finish times (min) of the measurement period, C_t represents the concentration of oxygen in the water (μmol O_2 L^{-1}) at time t; V_r represents the volume of the respirometer minus the animal.

After MO_2 measurement, the ammonia excretion rate (ER) of the same group of oysters was measured. Water samples were taken from each respirometer, filtered by 0.45 μm cellulose acetate membrane filters, and then analysed for ammonia

using the phenol-hypochlorite method. ER was calculated using the following equation:

$$ER\left(\mu mol\ NH_4 - N\ h^{-1}\right) = \left[C_{test} - C_{control}\right] \times \left(V_r / 1000\right) \times 1/t$$

Where C_{test} and $C_{control}$ represent the ammonia concentration in the sample and control, respectively, V_r represents the volume of the respirometer minus the animal; t is the exposure time.

After all the physiological measurements, the oyster tissues were dissected from the shell and dried to a constant weight at 65°C. The CR, MO_2, and ER were then corrected to a 'standard body size' of 1 g of dry weight mass-specific rates using the following equation: $Y_s = (W_s/W_e)^b \times Y_e$, where Y_s is the physiological rate for an animal of standard weight, W_s is the standard weight (1 g), W_e is the observed weight of the animal (g), Y_e is the uncorrected (measured) physiological rate, and b is the weight exponent for the physiological rate function (b = 0.67). Each physiological rate was then converted into energy equivalents (J h^{-1}) and used to calculate the energy available for scope for growth (SfG). Values for MO_2 were transformed into J h^{-1} using a conversion factor of 0.456 J μmol^{-1} O_2 (Gnaiger, 1983), ER was converted to J h^{-1} using the conversion factor: 1 µmol NH_4-N h^{-1} = 0.349 J h^{-1}. SfG (J h^{-1} g^{-1}) was calculated by SfG = A_b − R − U, where A_b is the total absorbed energy (J h^{-1} g^{-1}), R is the energy lost in respiration (J h^{-1} g^{-1}), and U is the energy lost in ammonia excretion (J h^{-1} g^{-1}). A_b = CR (L g^{-1} h^{-1}) × (mg POM L^{-1}) × AE (%). The POM concentration was converted to joules using a conversion factor of 23 J mg^{-1} ash-free dry weight (Widdows and Johnson, 1988).

Specific Growth Rate

The wet weight-specific growth rate (SGR, % d^{-1}) of each oyster was measured as follows: SGR = $(\ln(W_t/W_0)/t) \times 100\%$, where W_t and W_0 are the wet weight (g) of the oyster at time t and at time 0, respectively, and t is the sampling time (d).

Data Analysis

Statistical analyses were conducted using the software SPSS 22.0 for Windows. The general linear mixed model (GLMM) was used to test the effects of culture treatments (oyster alone and integrated aquaculture) or pCO_2 treatments (ambient, ~500 µatm and elevated, ~900 µatm) on physiological parameters with a mesocosm as a random variable nested within fixed factors (culture or pCO_2 treatments). This design considers that 3 replicate mesocosms in each treatment were supplied with the same seawater, and therefore, mesocosms may not be considered true replicates. Differences between culture or pCO_2 treatments were further tested by *F*-tests with a significance threshold of $\alpha = 0.05$. All data are represented as means ± SD.

RESULTS

Seawater Carbonate Chemistry

The daily variations in temperature, salinity, and pH during the experiment are shown in **Figure 2**. The ambient temperature and salinity were 19.08 ± 1.27°C and 32.86 ± 0.23, respectively.

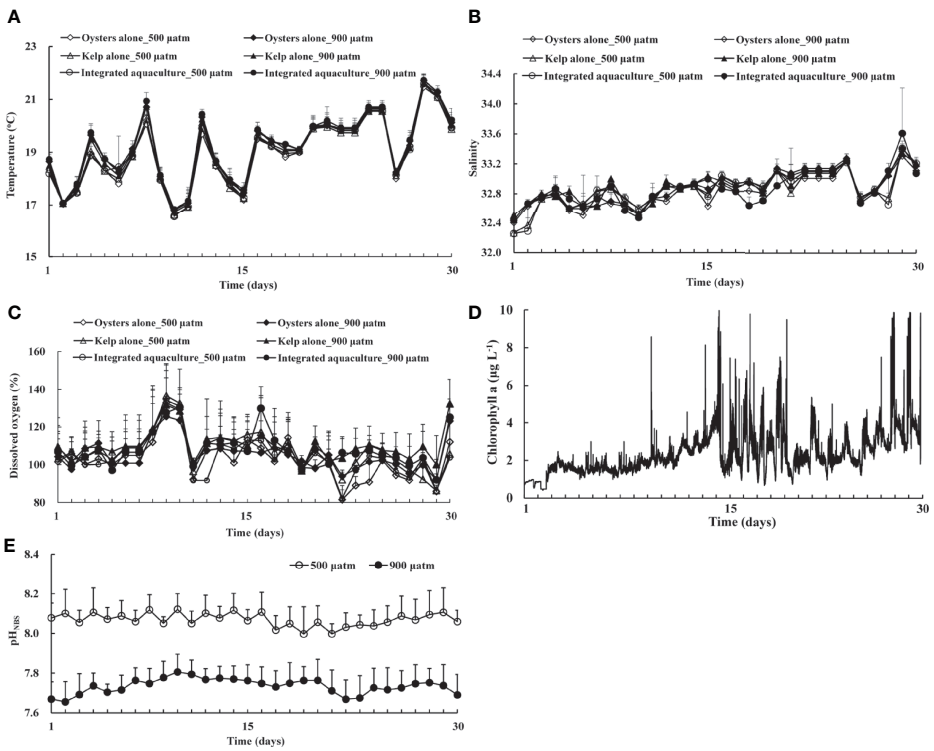

FIGURE 2 | Daily variations of environmental conditions during the 30-day exposure from 14 May to 12 June 2017. **(A)** Temperature; **(B)** salinity; **(C)** dissolved oxygen; **(D)** chlorophyll a in the ambient seawater; and **(E)** pH_{NBS} in the inflow of experimental units across the treatments. Means ± SD are shown.

The controlled injection of CO_2 separates the treatments successfully (pH_{NBS} ~7.83 in elevated pCO_2 treatments and 8.07 ± 0.03 in the ambient treatment) and keeps them relatively stable during the 30 days of exposure. Seawater chemistry variables over the 30-day experimental period for each pCO_2 treatment are summarized in **Table 1**. There was no significant difference in temperature, salinity, chlorophyll a, DIC, Ω_{cal}, Ω_{arag}, and HCO_3^- between treatments (P >0.05). The saturation percentage of dissolved oxygen in the exposure containers consistently exceeded 100% and was similar across treatments during the experiment. There was a significant difference between pH_{NBS} and pCO_2 between treatments (P <0.05).

The average change in daytime pH_{NBS} between the inflow and outflow of the mesocosms for the different treatments during the 30 days of exposure is shown in **Figure 3**. Results showed that the seaweed alone treatment increased the daytime pH_{NBS} by ~0.08 units, while oyster alone decreased ~0.05 units compared to the inflow under ambient conditions. The magnitude of change in daytime pH_{NBS} was significantly greater in kelp alone treatment under elevated pCO_2 condition than the ambient pCO_2 condition. The kelp alone treatment increased daytime pH_{NBS} by ~0.11 units, while the oyster and kelp integrated aquaculture treatment increased the daytime pH_{NBS} by ~0.06 units.

Clearance Rate and Absorption Efficiency

A significant effect of elevated pCO_2 on the clearance rate of oysters was observed. After 30 days of exposure, the clearance rate of oysters was significantly reduced in the elevated pCO_2 treatments and in the absence of kelp, as compared with ambient pCO_2 treatments (P <0.05) (**Figure 4A**). The presence of kelp in elevated pCO_2 treatment increased the clearance rate of oysters compared with oysters alone treated at the same elevated pCO_2 level (P <0.01). Additionally, no significant difference in CR was found between oysters incubated with seaweed at elevated pCO_2 and oysters incubated at ambient pCO_2 levels (P >0.05).

Absorption efficiency varied between 41 and 72% and was not affected significantly by elevated pCO_2 during the experiment. The absorption efficiency was lower in the oyster alone treatment at elevated pCO_2 treatment but showed no significant difference from the integrated aquaculture treatment (P >0.05) (**Figure 4B**).

TABLE 1 | Seawater chemistry variables over the 30 days experimental period for each pCO_2 treatment.

	Ambient pCO_2	Elevated pCO_2
Measured		
Temperature (°C)	19.00 ± 1.26	19.12 ± 1.25
Salinity	32.83 ± 0.24	32.87 ± 0.23
Chlorophyll a	2.69 ± 1.30	2.89 ± 1.09
pH_{NBS}	8.07 ± 0.03[a]	7.83 ± 0.04[b]
A_T (μmol kg^{-1})	2,201 ± 42	2,150 ± 76
Calculated		
pCO_2 (μatm)	514 ± 18[a]	927 ± 57[b]
DIC (μmol kg^{-1})	2,017 ± 43	2,066 ± 78
Ω_{cal}	3.27 ± 0.08	1.97 ± 0.07
Ω_{arag}	2.12 ± 0.05	1.28 ± 0.05
HCO_3^- (μmol kg^{-1})	1,865 ± 38	1,946 ± 72

A_T, total alkalinity; pCO_2, aqueous partial pressure of CO_2; DIC, dissolved inorganic carbon; Ω_{cal}, the $CaCO_3$ saturation state for aragonite; Ω_{arag}, the $CaCO_3$ saturation state for aragonite. Means ± SD are presented. Different letters indicate significant variation between treatments (P < 0.05).

Respiration Rate and Ammonia Excretion Rate

After 30 days of exposure, the respiration rate of oysters was significantly higher in the absence of kelp at elevated pCO_2 compared with oysters in ambient pCO_2 treatments (P <0.05). The presence of kelp in elevated pCO_2 treatment lowered the respiration rate and ammonia excretion rate of oysters compared to ambient conditions, but no significant difference was found (P >0.05). Compared with all other treatments, the respiration rate and ammonia excretion rate were significantly increased in the oyster alone treatment (P <0.05) (**Figure 5**).

Scope for Growth and Specific Growth Rate

Elevated pCO_2 exposure significantly reduced the SfG of *C. gigas* when unintegrated with kelp (**Figure 6A**). Compared to other treatments, SfG was negative (–9.45 J g^{-1} h^{-1}) in the elevated pCO_2 treatment in the absence of kelp (P <0.01). However, in the elevated pCO_2 treatment in the presence of kelp, SfG (35.82 J g^{-1} h^{-1}) was not significantly different to that of oysters in ambient pCO_2 treatments (P >0.05). Moreover, after 30 days of exposure, the wet weight-specific growth rates of *C. gigas* were also observed to be dramatically reduced in the oyster alone treatment compared with the integrated aquaculture treatment at elevated pCO_2 levels (**Figure 6B**).

DISCUSSION

The field mesocosms used in this study followed fluctuations in the natural environment, with CO_2 controlled to give two natural but distinct treatments, maintaining a difference of about 0.25 pH units over time (**Figure 2**). Using this novel approach, our results indicate that medium-term elevated pCO_2 exposure reduces the CR of Pacific oyster, *C. gigas*, while increasing the MO_2 and ER, reducing energy available for growth. Importantly, the presence of kelp, *S. japonica*, in the mesocosms mitigates this negative physiological impact of OA on oysters.

CR is generally considered the most sensitive parameter for the feeding activity and energy acquisition of bivalves. In this study, elevated pCO_2 induced a reduction in CR, limiting the capacity of energy acquisition from food sources. Zhao et al. (2017) and Xu et al. (2016) also demonstrated reductions of CR by the blood clam *Tegillarca granosa* and Manila clam *R. philippinarum* under similar acidified conditions. The gills are an important organ serving both a filter-feeding and respiration function in bivalves. The accumulation of GABA in the gill under elevated pCO_2 conditions may be a potential explanation for the suppression of feeding activity (Jiang et al., 2019). However, Liu and He (2012) reported that CR of pearl oyster *Pinctada fucata* increased at extreme pH reductions of up to 0.7 units, and Sui et al. (2016) found that the physiological energetics of thick shell

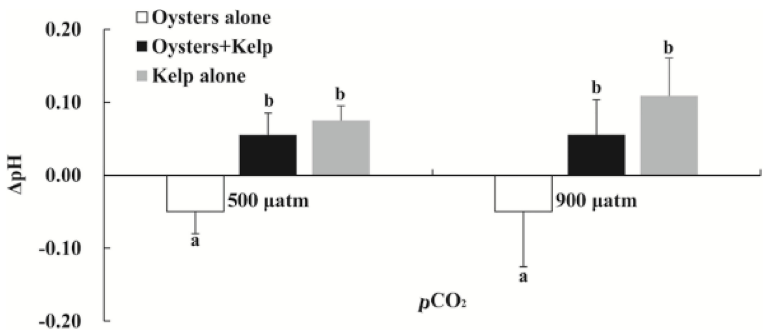

FIGURE 3 | Average change in daytime pH_{NBS} in the inflow and outflow of experimental units across the treatments during the 30 days. Means ± SD are presented. Different letters indicate significant variation between culture treatments ($P < 0.05$).

mussel *Mytilus coruscus* in terms of feeding, absorption and ultimately the energy available for growth remained unchanged by low pH conditions, perhaps due to the species-specific responses or the different experimental methods.

Here, AE was also not affected by acidified conditions, indicating the stable function of the digestive system, at least under the specific conditions and exposure time of this study. However, changes in CR, combined with increased MO_2 and ER in response to elevated pCO_2, resulted in a significant deficit in energy supply for *C. gigas* and ultimately less energy available for growth. For the mariculture industry, the significant reduction in energy available for growth (SfG) and actual growth in *C. gigas* under elevated pCO_2 represents a potential challenge to oyster farming operations, which could lead to economic loss. Particularly during the season of this study, when spat is first introduced to the farm environment and when most growth occurs.

The wide variety of species and exposure-dependent responses reported for bivalves to elevated pCO_2 (Navarro et al., 2013; Sui et al., 2016; Wang et al., 2016) may, in part, be due to differences in methodologies (e.g., measurement of CR *via* static or flow-through systems), which makes it difficult to compare results between studies. Most studies to date are also laboratory-based experiments, making it difficult to scale responses to the ecosystem level. Given the complexity of natural environments (such as food source and availability, daily fluctuation of environmental factors, etc.), particularly in coastal areas where bivalve aquaculture occurs, some recent studies, as here, have used field mesocosms (Jiang et al., 2019; Jiang et al., 2021) to advance the use of field experiments and improve the ecological relevance of data collected (e.g., Rastrick et al., 2018b).

Our results indicated that the presence of *S. japonica* under farm conditions helped to mitigate the negative effects of elevated pCO_2 on *C. gigas*, at least at the mesocosm scale. Being highly effective autotrophic, *S. japonica* use dissolved CO_2 in the seawater as the primary carbon source for photosynthesis. The rate of *S. japonica* photosynthetic carbon fixation was reported to 30 µmol g^{-1} h^{-1} (Han, 2013; Xu et al., 2016). Consequently, it may benefit from a future elevation in pCO_2. For example, Xu et al. (2018) reported that projected pCO_2 (1,000 µatm in 2,100 under RCP 8.5) may increase the growth of *S. japonica*. The increased photosynthesis rates of *S. japonica* improve the growing conditions for nearby *C. gigas* by removing sufficient amounts of CO_2, reducing local seawater acidification, increasing pH, and providing dissolved oxygen at the local scale. Giving the CO_2 released from the calcification and respiration processes of bivalves into account (Chauvaud et al., 2003; Mistri and Munari, 2013), *S. japonica* may also alleviate the potential self-

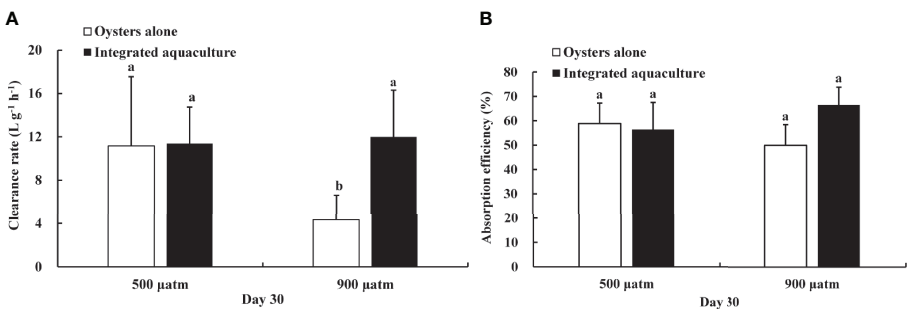

FIGURE 4 | Clearance rate **(A)** and absorption efficiency **(B)** of *C. gigas* exposed to different treatments for 30 days. Means ± SD are shown. Different letters indicate significant variation between treatments ($P < 0.05$).

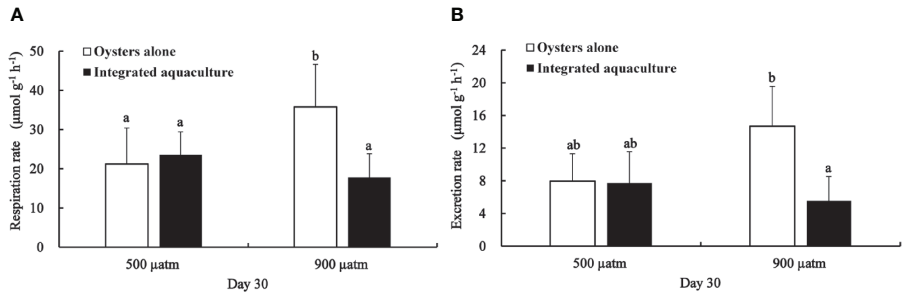

FIGURE 5 | Respiration rate **(A)** and ammonia excretion rate **(B)** of *C. gigas* exposed to different treatments for 30 days. Means ± SD are shown. Different letters indicate significant variation between treatments ($P < 0.05$).

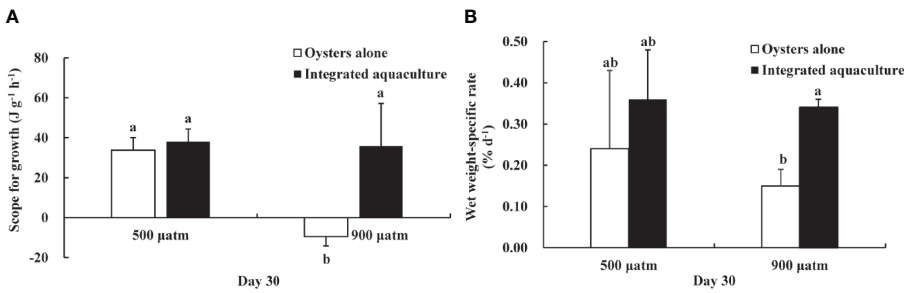

FIGURE 6 | Scope for growth **(A)** and wet weight-specific rate **(B)** of *C. gigas* exposed to different treatments for 30 days. Means ± SD are shown. Different letters indicate significant variation between treatments ($P < 0.05$).

acidification risk caused by *C. gigas* itself in smaller or more enclosed aquaculture settings. Here, *S. japonica* increased the mean daytime pH of the seawater by ~0.11 units in the elevated pCO_2 treatment (**Figure 3**). Such biogenic pH fluctuations are important to the function of bivalves. Wahl et al. (2018) demonstrated that the blue mussel *M. edulis* can take advantage of these fluctuations by shifting most of their costly physiological activities to the daytime when the surrounding chemical conditions are more favorable. Microprofiling studies have shown that kelp *E. radiata* can modify the chemical environment in a micro-zone called the diffusive boundary layer (DBL), and DBL microenvironments at the blade surface may create potential refuges from OA for calcifying epifauna (Noisette and Hurd, 2018).

By using a novel *in situ* field mesocosm approach, this study demonstrates that elevated pCO_2 can negatively affect the physiological energetics of *C. gigas*. Exposure to elevated pCO_2 significantly reduced CR while increasing MO_2 and ER, thereby reducing the energy allocated for reproduction and growth, presumably affecting production. However, similar energetic responses between *C. gigas* incubated at ambient CO_2 levels and those incubated at elevated CO_2 levels in the presence of *S. japonica* indicate that in integrated aquaculture systems, *S. japonica* may benefit the production of *C. gigas* by, in part, mitigating the negative physiological impact of elevated pCO_2. However, further *in situ* investigations are needed to elucidate the mutual benefit mechanisms and quantify context-dependency, particularly at larger farm scales.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZJ: Conceptualization, methodology, writing—original draft, writing—review & editing, and funding acquisition. WJ: Investigation, data curation, formal analysis, writing—original draft, and writing—review & editing. SR: Conceptualization, methodology, investigation, and writing–review & editing. XW, JinF, MD, and YG: Investigation and formal analysis. ØS and JiaF: Supervision, resources, funding acquisition, and writing—review & editing. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.862172/full#supplementary-material

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