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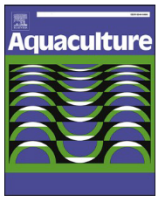

Integrated multi-trophic aquaculture mitigates the effects of ocean acidification: Seaweeds raise system pH and improve growth of juvenile abalone

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ABSTRACT

Integrated multi-trophic aquaculture (IMTA) has the potential to enhance growth, reduce nutrient loads, and mitigate environmental conditions compared to traditional single-species culture techniques. The goal of this project was to develop a land-based system for the integrated culture of seaweeds and shellfish, to test the efficacy of integrated versus non-integrated designs, and to assess the potential for IMTA to mitigate the effects of climate change from ocean acidification on shellfish growth and physiology. We utilized the red abalone (*Haliotis rufescens*) and the red seaweed dulse (*Devaleraea mollis*) as our study species and designed integrated tanks at three different recirculation rates (0%, 30%, and 65% recirculation per hour) to test how an integrated design would affect growth rates of the abalone and seaweeds, modify nutrient levels, and change water chemistry. We specifically hypothesized that IMTA designs would raise seawater pH to benefit calcifying species. Our results indicated that juvenile abalone grew significantly faster in weight (22% increase) and shell area (11% increase) in 6 months in tanks with the highest recirculation rates (65%). The 65% recirculation treatment also exhibited a significant increase in mean seawater pH (0.2 pH units higher) due to the biological activity of the seaweed in the connected tanks. We found a significant positive relationship between the mean pH of seawater in the tanks and juvenile abalone growth rates across all treatments. There were no significant differences in the growth of dulse among treatments, but dulse growth did vary seasonally. Seawater phosphate and nitrate concentrations were depleted in the highest recirculation rate treatment, but ammonium concentrations were elevated, likely due to the abalone effluent. Overall, our results indicate that there are benefits to IMTA culture of seaweeds and abalone in terms of improving growth in land-based systems, which will reduce the time to market and buffer commercial abalone operations against the effects of ocean acidification during vulnerable early life stages.

1. Introduction

A paradox currently exists concerning the sustainable management of marine resources. Global ocean fisheries are showing clear signs of stress as a result of over-fishing, habitat degradation, and climate change, yet the demand for marine products is increasing. Aquaculture is helping to alleviate the problem of overfishing. However, aquaculture operations, may also be negatively affected by environmental stressors associated with climate change in the coming decades, such as increasing temperatures, ocean acidification, and hypoxia (Costa-Pierce, 2002; Tan and Zheng, 2020). Creative strategies are therefore needed to develop techniques and infrastructure that can buffer aquaculture operations from the deleterious effects of a changing marine environment that could harm their economic outlook. One potential solution is integrated multi-trophic aquaculture (IMTA), both in land- and field-based operations. Linking seaweed and shellfish culturing activities has the potential to capitalize on the biological buffering capacity of seaweeds to raise seawater pH, thereby promoting growth and shell calcification, which are generally negatively impacted by ocean acidification conditions in shelled organisms. (Barrington et al., 2009). This project involved the design of integrated land-based systems, testing optimal recirculation rates to maximize the pH buffering (for shellfish) and

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nutrient subsidy (for seaweeds) benefits of integrated culture.

Most research and application to date on IMTA has focused on offshore systems due to the emphasis on integration with large-scale fish farms (see review by Troell et al., 2009), including all previous work on seaweed-oyster IMTA. Past work on land-based studies of seaweed-abalone IMTA also supports the potential benefits of integrated culture (e.g., Troell et al., 2006; Nobre et al., 2010). Although offshore IMTA systems have been shown to be effective in scrubbing finfish/shellfish-derived nutrients from the system (Neori et al., 2004; Ray et al., 2015), the open nature of offshore systems could limit the ability of the seaweeds to buffer the pH of waters within which the shellfish grow (Ray et al., 2015). We hypothesized that land-based IMTA systems will be simultaneously effective in pH buffering and nutrient scrubbing/subsidies.

In integrated aquaculture systems with seaweeds and shellfish, seaweeds are grown in seawater fertilized by effluent from cultured shellfish, with the seaweeds absorbing CO_2 from the water feeding the shellfish tanks. As such, the seaweeds provide a double buffer for shellfish aquaculture by lowering the concentrations of both nutrients and CO_2 and possibly ameliorating the effects of eutrophication and ocean acidification. Integrated culture with seaweeds has an additional benefit in that a new product can be harvested and sold to support the demand of a rapidly expanding seaweed market (e.g., for human consumption, biofuel production, chemical additives, etc.) or used as additional feed in aquaculture operations (e.g. for abalone). The high nitrogen (ammonium) concentration of the shellfish effluent has been shown to increase seaweed growth rates, pigmentation, and protein content (Demetropoulos and Langdon, 2004a, 2004b), which in turn can provide a higher quality food for cultured species, such as abalone, thereby increasing growth rates, taste, and disease resistance, while decreasing food consumption (Naidoo et al., 2006).

Understanding how IMTA systems may improve shellfish aquaculture is critical and timely, because many studies have raised alarms that marine calcifying organisms will fare poorly with increased acidification (Kroeker et al., 2010, 2013). Some organisms appear to be highly susceptible to changes in carbonate chemistry (e.g., molluscs, corals, echinoderms, calcified seaweeds) (Kroeker et al., 2013). In particular, exposure of early life stages to low pH water may have disproportionate effects on calcifying species, such as abalone, due to the sensitivities of larval development and juvenile performance to changing environmental conditions (Byrne et al., 2011; Kim et al., 2013; Boch et al., 2017). Impairment of early development can have long lasting carry-over effects on fitness that may affect growth, physiological performance, disease resistance, and other traits important to maintaining a high product quality for the aquaculture industry. We focused this project on red abalone, *Haliotis rufescens*, testing the capacity for IMTA to buffer early growth and development in land-based hatcheries. Early life stages of red abalone are susceptible to low pH and undersaturated waters, exhibiting reduced growth in treatments mimicking seasonal upwelling (Kim et al., 2013). Similar negative impacts of low pH have been observed in other abalone species (Crim et al., 2011; Wessel et al., 2018; Auzoux-Bordenave et al., 2020; Avignon et al., 2020).

Additionally, abalone aquaculture has replaced commercial harvest in California, due to severe overfishing and habitat destruction (Barrett, 1963; Shaw, 1997; McBride, 1998; Rogers-Bennet et al., 2002; Friedman et al., 2014). While the abalone aquaculture industry is sustainable and relatively immune to issues regarding the environmental impacts of other types of marine farming (e.g., eutrophication or habitat modification), there is concern that climate change may threaten land- and field-based operations in the future (Avignon et al., 2020; Tan and Zheng, 2020). Specifically, as calcifying organisms, farmed abalone may be extremely vulnerable to the physiological challenges posed by ocean acidification, especially at the scale of larvae, spat and juveniles (Crim et al., 2011; Barton et al., 2012; Hettinger et al., 2012; Kim et al., 2013; Wessel et al., 2018; Auzoux-Bordenave et al., 2020). Research predicts that current trends in declining pH will result in under-saturation of calcium carbonate necessary for the biomineralization of skeletal and protective structures of many economically important marine calcifying species (Turley et al., 2006). Likewise, climate change has been implicated as the ultimate driver of changes in upwelling timing and frequency (Mendelssohn and Schwing, 2002), which acts to deliver low pH corrosive waters from the deep sea to nearshore habitats. During bouts of strong upwelling, the U.S. West Coast currently endures exposures lasting days to weeks of corrosive waters that can drop as low as pH 7.4, exceeding predicted levels by 2100 (Hofmann et al., 2011; Gruber et al., 2012; Chan et al., 2017).

Many fleshy seaweed species are harvested for commercial purposes to be used as product additives, food for cultured abalone, and increasingly for human consumption (Trevelyan et al., 1998). Algae can change seawater pH through biological processes of photosynthesis and respiration (Anthony et al. 2011). Carbon dioxide (CO_2) and bicarbonate (HCO_3^-) are substrates for carbon fixation during photosynthesis (Raven et al., 2002). Because some species of seaweeds are carbon limited and exhibit enhanced growth with elevated pCO_2 (Gao et al., 1993; Kroeker et al., 2013; Kram et al., 2015), including seaweeds in an integrated aquaculture design may act to scrub CO_2 and HCO_3^- and thus buffer shellfish from the negative effects of reduced pH. Recent research indicates that biological activity by tropical corals and algae can cause changes of up to 0.3–0.4 pH units in a single day (Anthony et al., 2008; Hofmann et al., 2011). Similar variability in pH has been observed on diel and seasonal cycles in kelp forests off San Diego (Frieder et al., 2012), in the nearshore of Monterey Bay (Booth et al., 2012), and in upwelling regions in California (Hofmann et al., 2011) in response to biological activity and environmental forcing.

The overall goal of this project was to test the ability of IMTA to provide physiological, economical, and environmental benefits for seaweed and shellfish culture compared to non-integrated operations. We developed integrated systems for land-based seaweed tumble-culture paired with abalone culture. Specifically, our goals were: (1) to measure the capacity for seaweeds to raise pH through photosynthesis, thereby buffering abalone from the negative effects of ocean acidification on growth and shell calcification; (2) to culture juvenile abalone and red algae separately and together (i.e., with linked recirculation in modular two-stage tanks), in order to test the potential benefits of integrated culture on shellfish growth; and (3) to determine the effects of aquaculture integration on algal growth and nutrient uptake. We predicted that seaweeds will increase seawater pH, thereby buffering water chemistry from potential corrosive effects and aragonite undersaturation. We also predicted that abalone growth will be elevated in integrated culture with seaweeds compared to non-integrated culture, that the integration of abalone culture with seaweed tumble-culture will increase seaweed physiological performance due to the positive effects of shellfish nutrients and excreted metabolic waste products, and that the performance benefits of integration will be greatest in tanks with the highest rates of recirculation (i.e., lowest turnover and exchange) because this will maximize the pH buffering capacity of the seaweeds and the nutritional supplement provided by the shellfish. These positive impacts of IMTA may have economic benefits for commercial seaweed and shellfish operations.

2. Materials and methods

2.1. Seaweed species selection criteria

For the seaweed tumble-culture integration, we used the red algae dulse (*Devaleraea mollis*), which is the most utilized red seaweed in aquaculture systems in California and does not require seeding for propagation. This species was chosen because of (1) its ability to be grown in tumble-culture, (2) rapid growth rates in culture (doubling time is 1–2 weeks), (3) palatability to abalone, and (4) demonstrated enhancement of protein content under elevated nutrient conditions (Carmona et al., 2001; Langdon et al., 2004). In addition, dulse is

predicted to increase its growth rate in response to elevated pCO_2 (Koch et al., 2013), and thus is a promising candidate to mitigate the effects of ocean acidification on shellfish. Dulse utilizes HCO_3^- or CO_2 for photosynthesis and is carbon-limited at current oceanic levels of dissolved inorganic carbon (DIC).

2.2. Recirculation experiments to test pH buffering capacity of shellfish-seaweed integration

The research project occurred at the Aquaculture Facility at Moss Landing Marine Laboratories (MLML)/San Jose State University. There were two stages to the project: (1) developing the integrated culture system for shellfish and seaweeds; and (2) conducting experiments testing the seawater pH buffering capacity of seaweeds in tumble-culture and its effects on shellfish growth. Integrated recirculation tanks consisted of two side-by-side 300-US gallon (1135 L) conical bottom tanks (Fig. A.1) that were insulated with two layers of double bubble reflective foil insulation to help stabilize water temperature. Twenty-four tanks, configured into twelve experimental replicate pairs, were used to test the effects of shellfish-seaweed integration on pH stability and pH buffering capacity and the response of algae and abalone. Incoming water to the MLML seawater facility was pumped in continuously from intakes at 20-m depth, situated at the head of the Monterey Submarine Canyon. Water was then passed through a series of sand filters and the flow rates into individual tanks was precisely controlled by valves and flow meters. Water temperatures and chemical properties reflected ambient levels offshore in the Monterey Bay and fluctuated seasonally and daily based on tidal influence.

After initial testing of the pH and temperature responses across a range of recirculation rates, we learned that temperatures were similar in tanks receiving full flow through seawater (0% recirculation) up to flow rates resulting in 80% recirculation per hour (Fig. A.2), and that pH responses reflected biological activity of photosynthesis and respiration in treatments over 40% recirculation per hour (Fig. A.3). Based on the pilot study, we chose 3 recirculation treatments (0%, 30%, and 65% of the water recirculated per hour; Fig. 1) to test how the rate of water turnover and exchange will affect the degree of pH buffering and the strength of the nutrient subsidy for the algae. The 0% recirculation treatment consisted of full flow-through at 4.0 gal per minute (gpm; equivalent to 15.2 L/min) into separate seaweed and abalone tanks with their own inflow and outflow (Fig. 1), which is the same flow rate used in the commercial land-based culture of *Devaleraea mollis* and red abalone hatchery at our facility. The 30% and 65% recirculation treatments consist of linked seaweed and abalone tanks (using an Iwaki WMD-30RLXT-115 magnetic drive seawater pump at 4 gpm) with different incoming water flow rates and different residence times of recirculated water, connecting the potential nutrient subsidy of abalone effluent with the seaweed and the potential pH buffering capacity of the seaweed with the water flowing to the shellfish tank. The 30% recirculation treatments received 2.8 gpm (10.6 L/min) of new incoming water, with 1.2 gpm (4.5 L/min) returned from the abalone tank to the seaweed tank and 2.8 gpm flowing out down the drain (Fig. 5). The 65% recirculation treatment had the highest residence time and received 1.4 gpm (5.3 L/min) of new incoming water, with 2.6 gpm (9.8 L/min) recirculated between the abalone and seaweed tanks, and 1.4 gpm flowing down the drain (Fig. 1). A high recirculation rate (65% recirculation per hour), and thus low turnover with incoming seawater, was predicted to maximize the buffering capacity for the shellfish and nutrient subsidy for the seaweed, while maintaining similar water temperatures to the other treatments. Water flow was controlled with PRM Filtration Rotameter flow meters (0–10 gpm, ± 0.1) that were monitored and adjusted daily to ensure recirculation rates were consistent. This design ensured that all tanks across all treatments received a consistent combined inflow of 4 gpm (new incoming seawater + recirculated seawater). We had $n = 4$ replicate tank pairs for each level of response and ran the culturing experiment for 203 days.

Juvenile abalone (6 month in age and 20 mm in shell diameter) provided by the Monterey Abalone Company were used for the growth experiments testing the IMTA design. Abalone were held in the abalone tank of the paired array inside an inner plastic barrel/cage, where they were fed 1 kg of dulse twice per week (2 kg total weekly feeding dose per tank). The mesh cages were stocked with 700 juvenile abalone and were designed to ensure adequate water flow, easy cleaning and feeding, and assisted in concentrating the seaweed feed and abalone (Fig. A.1). The enclosures which held the abalone were scrubbed biweekly with a brush to remove diatom growth. The seaweed side of the tank for each replicate was inoculated with 4.5 kg of dulse at the start of the experiment, provided by Monterey Bay Seaweeds, and the seaweed was vegetatively propagated as free-floating rosettes. The rosettes were continuously re-suspended using air-lines that line the bottom of the tank (i.e., tumble-culture) and as the rosettes grew, they fragmented creating new rosettes. Dulse biomass in the seaweed tanks were reset to 4.5 kg every two weeks. The seaweed tanks were cleaned weekly and deep cleaned biweekly with a brush. The sides of the wall near the surface as well as the standpipe and mesh were scrubbed weekly. The seaweed tank was fully drained every two weeks during seaweed growth measurements and the tank was scrubbed with a brush and sprayed down with a hose.

2.3. Water chemistry monitoring and environmental analyses

To measure natural variability in seawater pH and the capacity of seaweeds to stabilize and buffer those natural fluctuations, we used Campbell Scientific ISFET pH probes (CS525) and transmitters (CR1000) to provide a continuous high-resolution record of pH from the outflowing water from the seaweed tanks to the abalone tanks. Twelve pH probes were deployed (one for each pair of tanks) and networked to a central server where data were processed and stored. The probes were installed through a water-tight port in the pipe connecting the seaweed tank to the abalone tank, just ahead of the pump. Recirculation between the tanks for the 30% and 65% recirculation treatments homogenized that water fairly rapidly. These probes were field calibrated every 6-weeks using a two-point calibration method and an offset check. Each probe was initially calibrated in the shade at ambient temperature (~17 °C) in Fischer Scientific 7.00 and 10.00 pH buffer solutions (CAS#7778-77-0 and #7732-18-5, respectively). The sensor was then placed in a TRIS buffer solution in synthetic seawater (Nemzer and Dickson, 2005) and the offsets were used to set the calibration to seawater on the total pH scale. The offset was rechecked between calibrations for reassurance in pH measurements. Each probe was immersed in new TRIS buffer and temperature-adjusted measurements were taken for 15 min, averaged, and compared to the calculated temperature-adjusted TRIS value for accuracy. Once the sensors were calibrated and deployed, a pH measurement was taken from each tank pair every 5 min for the duration of the project. pH data were quality control checked using Quality Assurance for Real-time pH data standards developed by the US Integrated Ocean Observing System (IOOS, 2019). Data were further quality checked to remove drift or outliers.

Water samples were taken to measure nutrient concentrations approximately every 2 weeks from May–September 2019 in the abalone tanks and analyzed on a Lachat Quikchem 8000 flow-injection analyzer at MLML. We determined concentrations of dissolved inorganic and organic nutrients, including: Phosphate (PO_3^{-4}), nitrite (NO_2^-), nitrate (NO_3^-) + nitrite (NO_2^-), and ammonium (NH_4^+). Values reported are the amount of P or N in mg/L of water.

2.4. Growth responses of abalone and seaweeds to integrated culture

The experiments were initiated in March 2019 with a sample size of $n = 700$ juvenile abalone in each replicate tank. The abalone were randomly allocated to each treatment. A sub-sample of $n = 100$ abalone per tank were haphazardly measured for weight (nearest 0.1 g) on a digital balance, and shell length (nearest 1 mm) and width (nearest 1

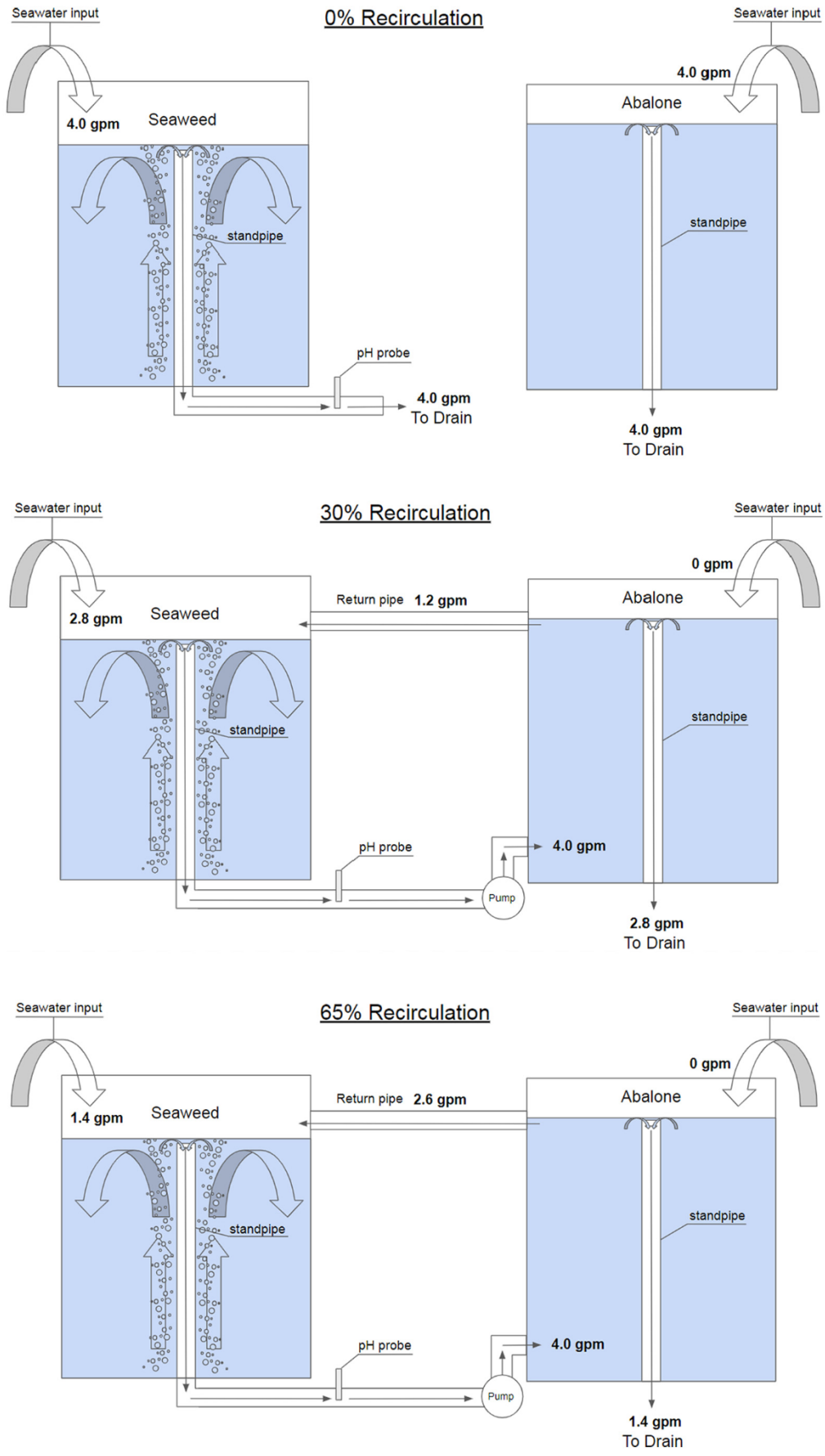

Fig. 1. Schematic diagram of the IMTA tank design for the three recirculation treatments, detailing the plumbing, airflow, and flow rates for each treatment.

mm) using calipers. At intervals ranging from 1 to 3 months (see Fig. 3), the abalone were reweighed and measured to provide estimates of growth over that time period. Initially a subsample of $n = 50$ abalone was measured, while the last two time points had $n = 100$ abalone measured per replicate tank. Growth was calculated as the change in weight per day or the change in shell area per day. Shell area was calculated using the formula for an oval and the length and width of the abalone shell.

Growth of dulse on the seaweed side of the array was measured every 2 weeks. All of the seaweed was removed from each tank using nets and spun using a salad spinner to remove water. The wet weight of dulse was then obtained using a digital balance to the nearest 0.1 kg and the change in weight was calculated, given the starting weight of 4.5 kg. After the seaweed growth was determined, each tank was reset with a starting weight of 4.5 kg of dulse at the start of each two-week period. IMTA tanks were outdoors with a photoperiod ranging from 11.5 h (March and September) to 14.5 h (June) in the middle of the experiment.

2.5. Statistical analysis

For statistical analysis, the mean weight, change in weight, shell area, or change in shell area in each tank were used as independent replicates. The change in weight and shell area over the full duration of the experiment were tested with an analysis of variance (ANOVA) using the recirculation treatments (0%, 30% and 65% recirculation) as fixed factor ($n = 4$ paired tanks per recirculation treatment). Post-hoc comparisons were made using Tukey HSD. We tested model assumptions of normality of residuals and homogeneity of variance and the data was deemed to meet statistical assumptions. We also compared the trajectories of abalone weight and shell area over the duration of the experiment using repeated measures ANOVA, with the factors of treatment, time, and treatment x time. We calculated the mean pH and the standard deviation in pH (i.e., pH variability) in each treatment using the time series of pH from each treatment tank over the duration of the experiment. We then used linear regression to test for a significant association between the mean change in weight or mean change in shell area and mean pH or the standard deviation in pH experienced by the abalone. To test for differences in growth of dulse and water nutrient levels among treatments, we used a linear mixed model (LMM) with the factors of treatment, time (2-week interval), treatment x time, and a random effect for tank to control for potential autocorrelation in seaweed growth or nutrient responses due to tank effects. For all tests, we used a significance value of $\alpha = 0.05$. All statistical analyses were conducted in JMP v.16.

3. Results

After approximately 6 months in the recirculation treatment conditions, we detected a significant difference in the change in abalone weight (ANOVA, $F_{2,9} = 6.35$, $P = 0.019$; Fig. 2A, Table 1) and change in shell area (ANOVA, $F_{2,9} = 6.78$, $P = 0.016$; Fig. 2B, Table 1). Abalone grew significantly more in weight and shell area in the 65% recirculation treatment with no difference between the other two treatments (Tukey HSD). Juvenile abalone grew 22% more in weight and 11.4% more in shell area in the 65% recirculation treatment compared to the 0% recirculation treatment (i.e., the non-IMTA treatment). While not significantly different, abalone also grew 4.9% more in weight in the 30% recirculation treatment than the 0% recirculation treatment, but were 3% smaller in shell area. Using the full time-course of the experiment to test for differences in abalone growth trajectories, we found a significant effect of recirculation treatment on abalone weight (RM-ANOVA, Treatment, $F_{2,9} = 9.86$, $P = 0.0054$; Fig. 3A) and shell area (RM-ANOVA, Treatment, $F_{2,9} = 8.51$, $P = 0.0084$; Fig. 3B), abalone were consistently heavier in weight and larger in shell area in the 65% recirculation treatment over time. There was equivocal evidence that abalone weight (RM-ANOVA, Treatment x Time, Wilks Lambda, $F_{6,14} =$ 2.55, $P = 0.069$) and shell area (RM-ANOVA, Treatment x Time, Wilks Lambda, $F_{6,14} = 1.89$, $P = 0.15$) trajectories were diverging over time among the recirculation treatments, although the largest differences in weight and shell area occurred at the last time point, nearly 200 days from the start of the experiment. Mortality rates were low in all treatments (<0.5%) with 3 dead abalone in the 0% treatment, 12 in the 35% treatment, and 7 in the 65% treatment, out of a starting number of 2800 per treatment.

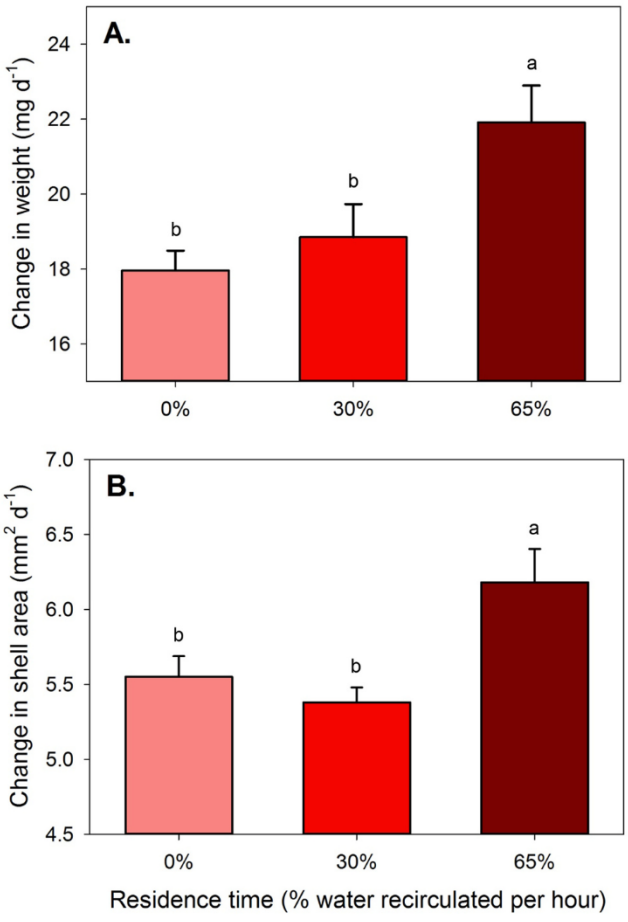

Fig. 2. Change in abalone weight (A) and shell area (B) in the three recirculation treatments over the 6-month duration of the experiment. Bars are means of 4 replicate tanks in each treatment ± standard error. Letters above bars denote significance of a Tukey HSD post-hoc test. Treatments that do not share the same letter are significantly different at $P < 0.05$.

In comparing the differences in the pH environment of the recirculation treatments (Table 1), the pH was significantly higher in the 65% recirculation treatment compared to the other treatments (ANOVA, $F_{2,8} = 7.02$, $P = 0.017$; Fig. 4A); on average the pH was 0.2 units higher in the 65% recirculation treatment. There was no significant difference in the pH variability among treatments (ANOVA, $F_{2,8} = 2.29$, $P = 0.16$; Fig. 4B); however, the trend was for the standard deviation in pH to be higher in the 65% recirculation treatment (Table 1). In using the growth responses and the pH data from the individual tanks, we found a significant positive association between the change in weight and shell area and the pH environment experienced by the abalone. The change in weight increased as the mean pH of the tanks increased (linear regression, $F_{1,9} = 14.46$, $r^2 = 0.62$, $P = 0.0042$; Fig. 5A), with the pH being highest and growth in weight being highest in the 65% recirculation replicates. The change in abalone weight also increased with the variability in pH (linear regression, $F_{1,9} = 9.71$, $r^2 = 0.52$, $P = 0.012$; Fig. 5B), as the pH standard deviation also tended to be highest in the 65% recirculation treatment. Similarly, the change in shell area

Table 1
Treatment pH values and abalone growth during the IMTA experiment. Shown are means ±1 standard error of growth for each recirculation treatment.

Treatment	pH	pH standard deviation	Change in weight (mg day^{-1})	Change in shell area (mm^2 day^{-1})
0% recirculation	8.00 ± 0.088	0.15 ± 0.016	17.9 ± 0.52	5.5 ± 0.14
30% recirculation	8.06 ± 0.090	0.17 ± 0.016	18.9 ± 0.88	5.4 ± 0.10
65% recirculation	8.23 ± 0.081	0.20 ± 0.041	21.9 ± 0.98	6.2 ± 0.22

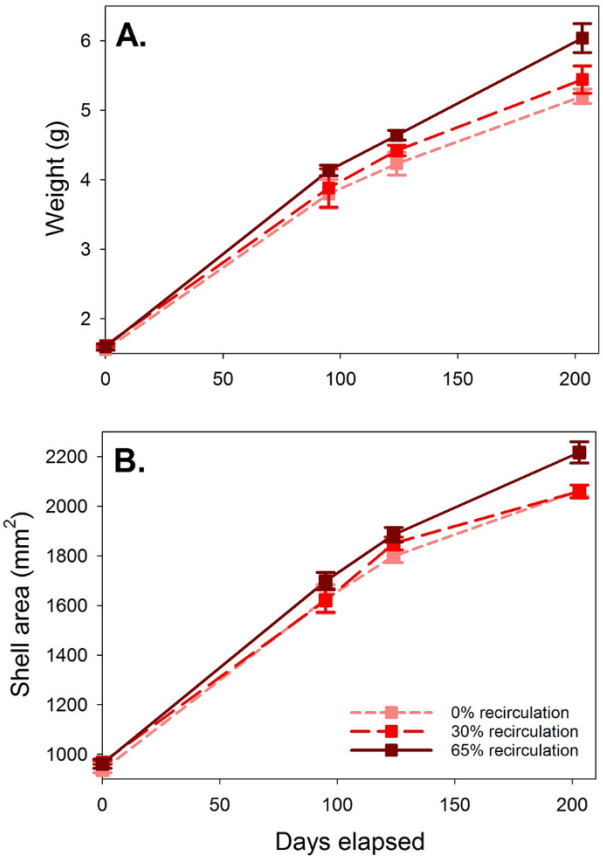

Fig. 3. Time series showing mean abalone weight (A) and shell area (B) over time in the three recirculation treatments. Points are means of 4 replicate tanks in each treatment ± standard error.

increased as the mean pH of the tanks increased (linear regression, $F_{1,9} = 15.42$, $r^2 = 0.63$, $P = 0.0035$; Fig. 5C), with the pH being highest and growth in shell area being highest in the 65% recirculation treatments. The change in abalone shell area also increased with the variability in pH (linear regression, $F_{1,9} = 17.86$, $r^2 = 0.66$, $P = 0.0022$; Fig. 5D).

We did not detect any significant differences in the growth of dulse as a function of the recirculation treatments. Seaweed growth, however, did vary significantly over time, with higher growth in the summer than spring months (LMM, Treatment: $F_{2,9} = 3.39$, $P = 0.08$; Time: $F_{8,72} = 11.09$, $P < 0.0001$; Treatment x Time: $F_{12,72} = 0.39$, $P = 0.98$; Fig. 6). The photoperiod was approximately 2 h longer during the summer (14+ hrs of daylight) vs. the spring (12 h of daylight). The growth pattern among treatments was also consistent over time and we did not detect a significant treatment x time interaction. Despite similar seaweed growth responses, we did detect differences in nutrient concentrations among the recirculation treatments and as a function of time (Table 2). Phosphate concentrations were generally low and differed among sampling dates, but showed a consistent pattern in being highest in the 0% recirculation treatment and lowest in the 65% recirculation treatment (LMM Treatment: $F_{2,106} = 4.89$, $P = 0.039$; Time: $F_{12,106} = 17.99$, $P < 0.0001$; Treatment x Time: $F_{24,106} = 0.89$, $P = 0.60$; Fig. 7A). Nitrate concentrations differed among sampling dates (generally being higher in the spring months) and exhibited a consistent pattern in being elevated in the 0% recirculation treatment and depleted in the 65% recirculation treatment (LMM, Treatment: $F_{2,106} = 5.92$, $P = 0.019$; Time: $F_{12,106} = 5.05$, $P < 0.0001$; Treatment x Time: $F_{24,106} = 0.65$, $P = 0.87$; Fig. 7B). In contrast, ammonium concentrations differed among sampling dates and was highest in the later part of the experiment in summer, and ammonium levels were most elevated in the 65% recirculation treatment and lowest in the 0% recirculation treatment (Two-way ANOVA, Treatment: $F_{2,106} = 4.08$, $P = 0.049$; Time: $F_{12,106} = 3.62$, $P = 0.0002$; Treatment x Time: $F_{24,106} = 0.96$, $P = 0.52$; Fig. 7C).

4. Discussion

Our results indicate that IMTA designs for the land-based co-culture of seaweeds and shellfish can have positive benefits for the growth of juvenile abalone compared to non-integrated designs. Growth in weight and shell area was highest in the 65% recirculation treatment, which had both the highest residence time of water in the system and the highest mean pH (although being somewhat more variable). By photosynthesizing during the day, seaweeds absorb CO_2 from the water, thereby raising seawater pH. The elevated residence time of water in the 65% recirculation treatment allowed more time for these biological processes to raise seawater pH. A similar response was observed in experimental studies that manipulated water residence time in eelgrass mesocosms (Kaldy et al., 2022). In that study, the longer the residence time in the experimental system, the higher the pH increased over ambient conditions. Elevated pH, in turn, appeared to positively benefit abalone growth. The higher standard deviation in this treatment may be due to the dominance of seaweed respiration increasing CO_2 and lowering pH and night (Wahl et al., 2018), compared to the 0% and 30% recirculation treatments. Despite the higher pH variability of the 65% recirculation treatment, abalone growth was not compromised. This species has evolved in a highly dynamic coastal environment characterized by strong pH fluctuations (Hofmann et al., 2011; Frieder et al., 2012) due to processes such as upwelling, internal waves, and tidal action. The 30% recirculation treatment had an intermediate pH and growth response, likely because the biological processes raising pH could not keep pace with the rate of incoming fresh seawater that acted to reduce pH. The 0% recirculation treatment reflected offshore seawater chemistry conditions with full flow-through and no connection between the seaweed and abalone tanks. We also found a strong positive correlation between the pH environment in individual tanks and the growth response of the abalone, with elevated growth in the tanks with the highest mean pH. The preliminary trials, ranging between 0%–100% recirculation, confirmed the results in the recirculation treatments, as we observed that the pH shifted from a tidal signal with high frequency pH variability at 0% recirculation, to a diurnal signal reflective of photosynthesis and respiration at treatments above 40–50% recirculation. Water temperatures were similar across all three treatments (and did not become elevated until 80% recirculation in our preliminary trials) further emphasizing that the differences observed in abalone growth are most likely attributed to the differences in pH generated by the linked culture to seaweeds and the choice of flow rates and residence time in the IMTA system.

Prior studies have shown that low pH/high CO_2 exposure can be detrimental for abalone. The related northern abalone, *H. kamtschatkana*, exhibits a 40% reduction in shell growth and a 40% increase in the frequency of shell abnormalities at CO_2 levels of 800 ppm

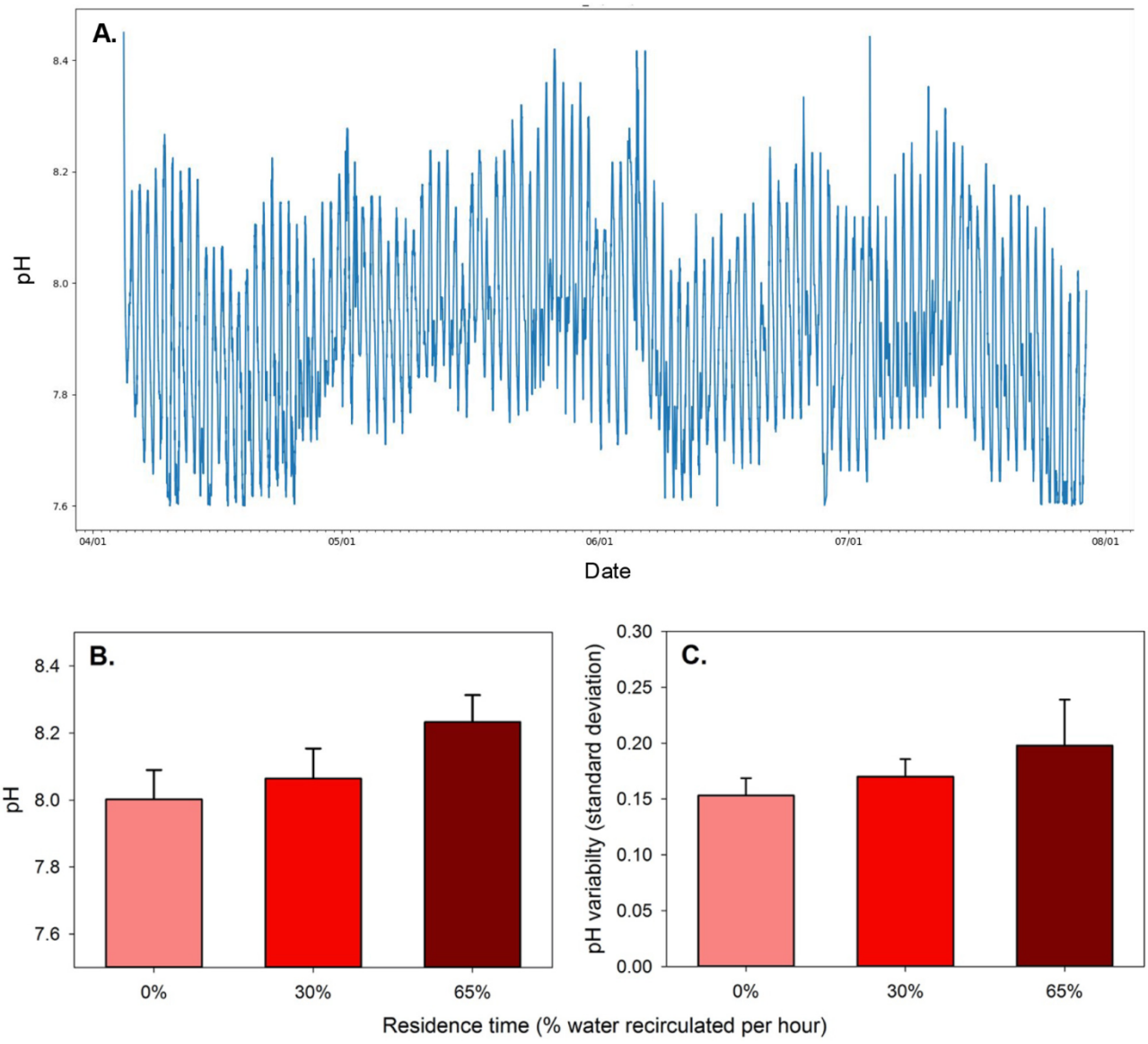

Fig. 4. (A) Example pH time series from one of the treatment tanks (30% recirculation). pH environmental characteristics as a function of residence time treatment. (B) Mean pH in each experimental treatment and (C) standard deviation in pH. Bars are averages ± standard error.

(pH of 7.9) (Crim et al., 2011). Higher CO_2 levels (1800 ppm) result in almost complete shell deformity or the lack of a shell altogether. Similar disruptions to shell formation were documented in larval European abalone (*H. tuberculata*) at CO_2 levels of 1000 ppm (pH 7.7) and 1400 ppm (pH 7.6), in addition to decreased survival and growth (Wessel et al., 2018). Both juvenile and adult European abalone produce thinner, weaker periostracum layers of the shell with visible corrosion on both the outer surface and inner nacre layers (CO_2 levels of 1000 ppm and 1400 ppm, respectively) (Auzoux-Bordenave et al., 2020; Avignon et al., 2020). In our experiments, abalone likely demonstrated increased growth in tanks with the highest pH (65% recirculation treatment) because elevated pH values during the day when seaweeds were photosynthesizing created an improved environment for both growth and calcium carbonate shell construction, which is energetically intensive and influenced by both seawater pH and aragonite or calcite saturation states (reviewed in Kroeker et al., 2013; Melzner et al., 2020).

As found in this study, seaweeds and seagrasses may enhance the calcification rates of bivalve species, with daytime increases in calcification rates occurring when macrophytes are actively photosynthesizing. In the mussel, *Mytilus edulis*, calcification rates increased significantly when grown in the presence of a brown alga (*Fucus vesiculosus*) and seagrass (*Zostera marina*) both in field and laboratory experiments, which, similar to this study, increased the overall mean pH of the water by 0.3 units (Wahl et al., 2018). Shell and muscle tissue growth rates of hard clams (*Mercenaria mercenaria*), eastern oysters (*Crassostrea virginica*), and bay scallops (*Argopecten irradians*) also showed significant increases when grown with green algae (*Ulva*) (Young and Gobler, 2018). Interestingly, when these same species were grown under experimentally manipulated diel cycles (by artificially bubbling CO_2 into treatments on a diurnal cycle), they did not show improved growth under fluctuating verses static low pH conditions (Clark and Gobler, 2016), suggesting that the actual presence of seaweeds as a food source yielded enhanced growth. Other studies that experimentally manipulated pH values to reflect diurnal conditions produced by seaweeds under ambient versus both fluctuating and static ocean acidification conditions found similar amelioration of responses to low pH. For example, semidiurnal fluctuations of low pH water can benefit larvae of mussels (*M. californianus* and *M. galloprovincialis*) by preventing developmental delays and impaired shell and tissue growth that are otherwise observed in animals exposed to static low pH water

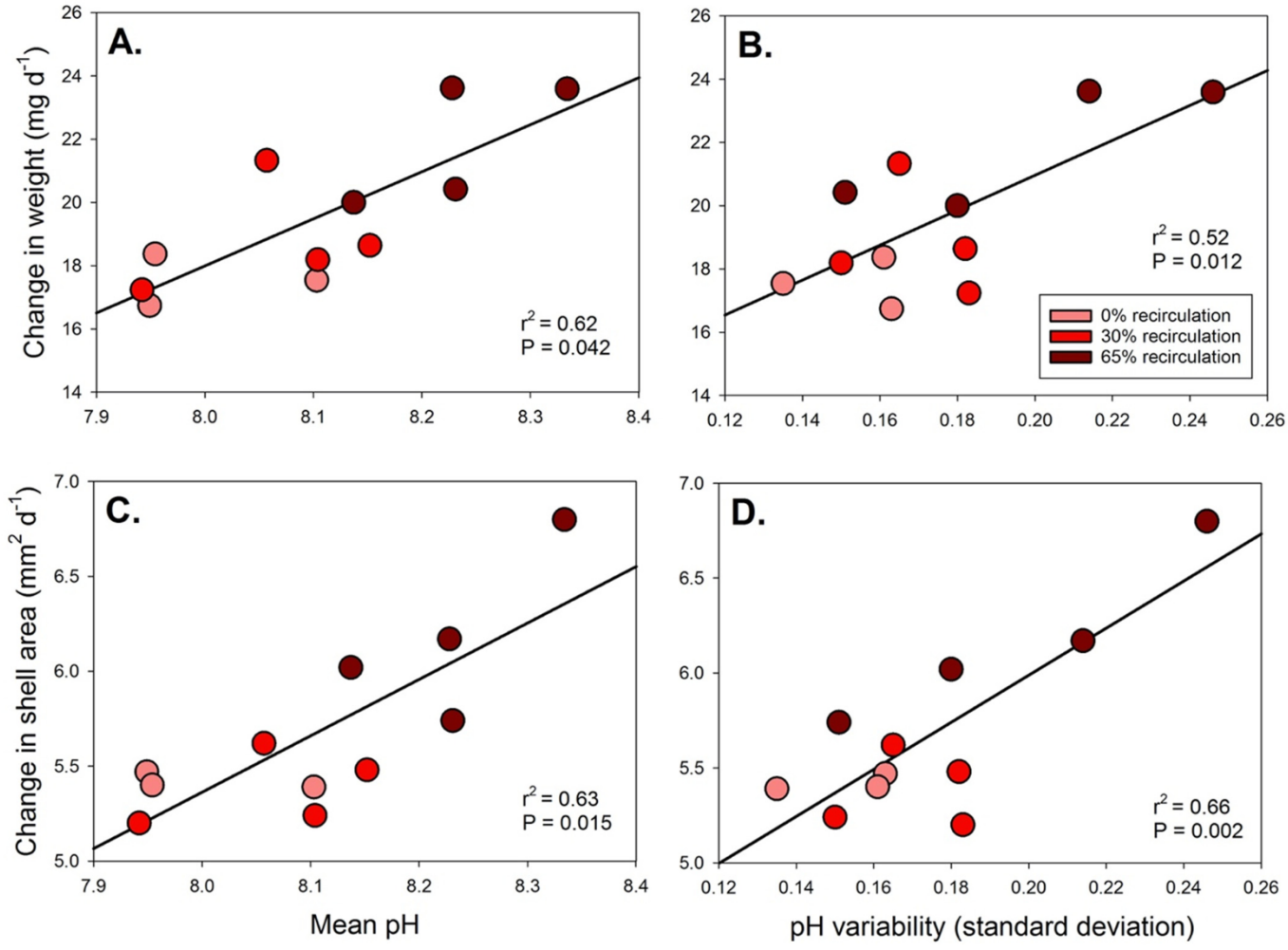

Fig. 5. Relationship between mean pH and pH variability and abalone growth in the three recirculation treatments. (A) Change in weight and (C) change in shell area vs. mean pH experienced in each tank. (B) Change in weight and (D) change in shell area vs. the standard deviation in pH in each tank. Note: pH sensor failure required the exclusion of 2 tanks.

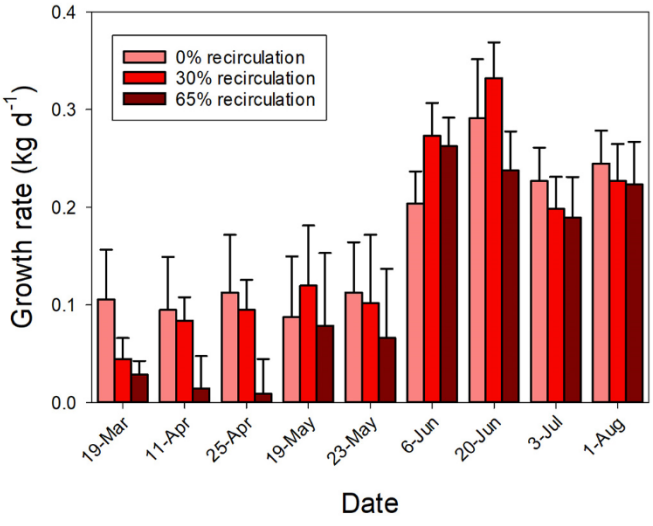

Fig. 6. Growth rate of dulse (*Devaleraea mollis*) in the IMTA treatments. Shown are the mean daily growth rates (kg d^{-1}) ± standard error of new production in each treatment per 2-week time period.

Table 2
Nutrient concentrations for phosphate, nitrate, and ammonium measured in the IMTA tanks during the experiment. Shown are means ±1 standard error for each recirculation treatment.

Treatment	PO_4^{-3} (mg/L)	NO_3^- (mg/L)	NH_4^+ (mg/L)
0% recirculation	0.014 ± 0.0032	0.074 ± 0.018	0.022 ± 0.0034
30% recirculation	0.012 ± 0.0028	0.053 ± 0.014	0.033 ± 0.0088
65% recirculation	0.0095 ± 0.0021	0.028 ± 0.0086	0.042 ± 0.011

(Frieder et al., 2012; Kapsenberg et al., 2018). The barnacle, *Balanus improvises,* showed increased variation in growth under fluctuating low pH, indicating the potential for adaptability to low pH environments over evolutionary time (Eriander et al., 2016). Taken together, these results suggest that co-culturing shellfish and seaweeds provides key benefits to shellfish growth and calcification that can be applied generally to shellfish aquaculture.

We did not observe significant differences in the growth of the red seaweed dulse as a function of the recirculation treatments. However, the trends suggested that seaweed growth was lowest in the 65% recirculation treatment during the spring when dulse growth was lowest in all treatments compared to higher summer growth. The nutrient data indicated that phosphate and nitrate were lowest in the 65% recirculation treatment, but that ammonium was elevated compared to the other

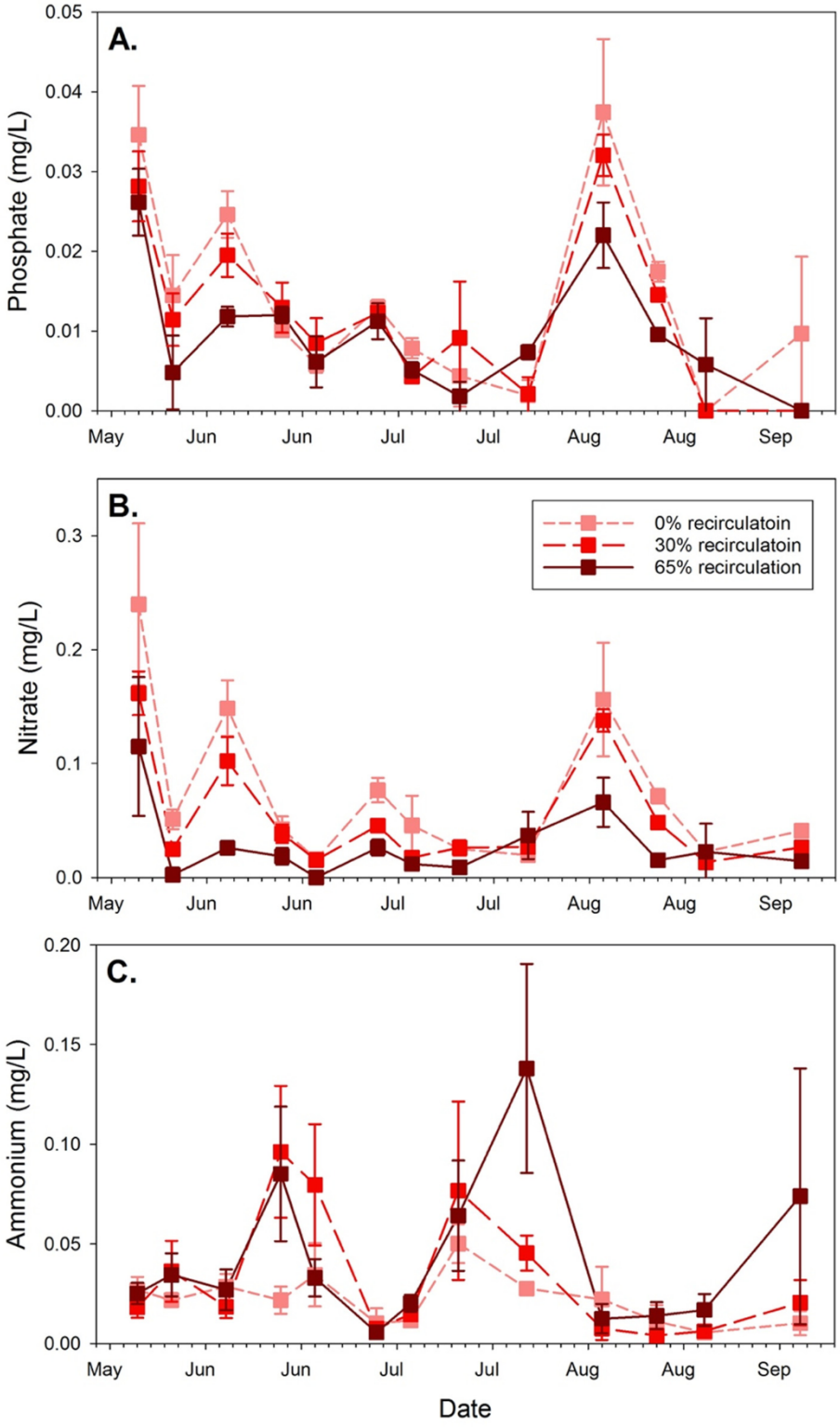

Fig. 7. Nutrient time series in the different recirculation treatments. (A) Phosphate, (B) Nitrate, and (C) Ammonium. Shown are the mean ± standard error of the nutrient concentrations measured in water samples every 2 weeks.

treatments. Thus, the trend for slightly reduced seaweed growth may be explained by the reduction in abiotic nutrients that occurred in the highest recirculation treatment and the shorter photoperiod at the beginning of the experiment. Seaweed metabolism can explain the observed nutrient depletion. Elevated ammonium in the 65% recirculation tanks is likely a biproduct of the excretion of metabolic waste products by the abalone, providing another usable source of nitrogen for seaweed growth, potentially offsetting the depletion in nitrate. Our IMTA experiment had a relatively low biomass of abalone given the small size of juvenile animals relative to that of grow out operations with adults. Higher biomass of larger individuals in later life stages in commercial operations would increase the ammonium concentration

further, potentially further offsetting the nutrient drawdown in nitrate that occurred in the highest recirculation treatment. At very high ammonium concentrations, levels could become toxic to the abalone. Thus, care should be taken to carefully monitor water quality conditions as abalone biomass increases in IMTA tanks. Periodic flushing of the tanks or increasing the biomass of seaweed could alleviate ammonium build up. The combined results indicate that optimal recirculation rates in commercial applications will be ones that balance nutrient uptake with the pH buffering capacity of the seaweeds and that provide temperature stabilization. Based on our experiments, this is likely to fall in 60–70% recirculation treatment range, given the tank sizes and set-up used for the experiments.

4.1. Benefits to the aquaculture industry

Faster growth of shellfish in IMTA tanks has the potential to decrease the time to reach market size, which will increase profits and reduce costs, while simultaneously buffering the shellfish industry from the effects of climate change. As discussed, we found that abalone/dulse co-culture yields a 22% increase in abalone growth. Farmed abalone produced in California typically reach a market size of 100 g in 4 years (Mau and Jha, 2018). A 22% increase in abalone growth could yield a market-sized abalone in approximately 3 years, decreasing the costs of labor and feed by one year per individual on average. Alternatively, this 22% increase could produce larger-sized abalone (~120 g) during the traditional 4 years to market, which may increase consumer demand and market value for the more desirable, larger abalone sizes.

Integrated land-based seaweed/abalone culture has numerous additional economic benefits over non-IMTA systems. First, all seaweed to be fed to the abalone can be produced on site, eliminating the need to harvest wild seaweed populations (Rosen et al., 2000). Procuring wild feed is currently one of the highest costs for commercial abalone farms. Farms can thus avoid costs of labor, equipment and boats for harvesting, transportation, processing, storage, insurance, and regulatory costs of harvesting a state-regulated natural resource (Nobre et al., 2010). Second, the increased recycling of ammonium (suggesting increased nitrate uptake) seen in the 65% recirculation treatment may yield both seaweed and abalone with more valued nutritional profiles (e.g., higher protein content) over non-IMTA systems (Rosen et al., 2000; Demetropoulos and Langdon, 2004a, 2004b). For example, in the 65% recirculation treatment, we observed elevated seawater nutrient concentrations of ammonium due to abalone excretion but lower concentrations of nitrate, due to seaweed uptake, indicating that seaweed coloration and protein content may have been further improved (Demetropoulos and Langdon, 2004a). The benefit of this cycle of nutrient uptake is that it contributes to increased abalone growth rates (Naidoo et al., 2006), product quality of shellfish and seaweeds, and consumer demand and market price. Finally, IMTA designs for shellfish culture can have ecosystem benefits, including decreasing the negative impacts of releasing shellfish aquaculture effluent into the coastal system, because seaweeds can be used to scrub those nutrients (Ray et al., 2015).

Nationally, although commercially-viable seaweeds are ubiquitous along U.S. coastlines, abalone are naturally restricted in distribution to the U.S. west coast. Thus, seaweed/abalone IMTA systems in California can inform the development of similar systems in Oregon, Washington, and Alaska, as well as help brand the IMTA seaweed and abalone products along this broad region. Given that this system is land-based, it could also broaden abalone aquaculture beyond the west coast. For example, although abalone are not native to Hawaii, California red abalone have been effectively established in economically-viable, land-based aquaculture systems on the Big Island of Hawaii (Mau and Jha, 2018), with safeguards to ensure that the non-native shellfish are not accidentally released into the native environment. Similar safeguards could be used to develop land-based seaweed/abalone IMTA systems along the U.S. Gulf and East coasts, broadening the geographic market of these high value seafood products.

More importantly, integrating seaweed and shellfish culture is likely to have benefits for many types of shellfish aquaculture, such as oysters and mussels, because ocean acidification is a global problem and because early life stages of shellfish (larvae and juveniles) are most susceptible to the deleterious effects of ocean acidification (Crim et al., 2011; Barton et al., 2012; Hettinger et al., 2012; Kim et al., 2013; Frieder et al., 2012; Kapsenberg et al., 2018). Thus, our techniques for land-based seaweed/abalone IMTA systems in California have the potential to increase survival rates and product quality for a variety of shellfish products, while providing insurance against environmental stressors throughout the life cycle.

4.2. Future directions

Additional improvements can be made to the IMTA design to further enhance abalone growth and increase system efficiency. For example, photosynthesis raises pH during the day but respiration lowers pH at night. Integrating a form of automated flow control with low flow (i.e., high residence time) during the day and high flow (i.e., low residence time) at night could raise the pH during the day and keep the system flushed to reduce the effects of respiration at night, thereby maximizing the growth potential for seaweeds and shellfish. Alternately, adding artificial light at night paired with a high residence time throughout a 24-h cycle could stimulate continual algal photosynthesis, elevating pH even higher, and mitigating diel fluctuations. This approach could further increase abalone and algal growth rates. However, adding lighting at night may create additional costs that need to be weighed against the potential benefits, such as the economic and environmental costs of lighting infrastructure, electricity generation, and battery and solar panel production, to name a few. Either approach to increasing tank pH can be coupled with nutrient additions or higher shellfish biomass and retention of metabolic waste products to further stimulate seaweed growth.

Our design included two paired tanks, integrated side-by-side, one stocked with seaweed and the other with abalone. Space efficiencies could be achieved by stacking the tanks on top of each other (using long troughs), with seaweed on top to capture sunlight and abalone on the bottom, or by co-culturing the seaweed and abalone in the same tanks (i.e. single-tank design). In addition to capital savings on equipment and land, a single-tank design would also significantly cut labor, as abalone in a single-tank system would be able to feed on the seaweed drifting around them, rather than having to be hand fed from a separate seaweed tank, and provide energy savings by running fewer pumps. What is unknown, however, is whether single-tank systems will have residence times long enough for the seaweed to absorb sufficient CO_2 and ammonium to effectively buffer ocean acidification and eutrophication conditions, thereby enhancing abalone growth. A secondary consideration for single-tank designs is that the seaweed biomass must be sufficient to compensate for abalone consumption, while providing the intended pH buffering capacity.

Finally, a logical extension of the growth findings is to determine if multiple aspects of animal condition beyond growth are similarly improved when abalone are farmed in co-culture with seaweeds. For example, disruptions to shell formation that occur in larvae and juvenile shellfish grown in low pH conditions may be ameliorated with land-based IMTA, which could further help reduce farming costs and shell breakage during handling (Auzoux-Bordenave et al., 2020; Tan and Zheng, 2020). This IMTA design may also reduce stress and increase the physiological performance of individual abalone, which is important for maintaining a high quality, palatable aquaculture product (Hettinger et al., 2012). Taken together, integrating the described improvements coupled with future studies on IMTA effects on abalone condition will increase the resiliency of abalone and shellfish aquaculture to impending climate change threats more broadly. Thus, this research provides the foundation for developing land-based shellfish aquaculture practices that will yield sustainable production well into the future.

CRediT authorship contribution statement

Scott L. Hamilton: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Matthew S. Elliott:** Investigation, Methodology. **Maya S. deVries:** Conceptualization, Writing – original draft, Writing – review & editing. **Jason Adelaars:** Investigation, Methodology. **Maxwell D. Rintoul:** Investigation. **Michael H. Graham:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738571.

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