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Growth, Productivity and Nutrient Uptake Rates of *Ulva lactuca* and *Devaleraea mollis* Co-Cultured with *Atractoscion nobilis* in a Land-Based Seawater Flow-Through Cascade IMTA System

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Abstract: To advance environmentally friendly technologies in the aquaculture of *Atractoscion nobilis*, and simultaneously to diversify seafood production, a 79-day trial was conducted to assess the performance of *Ulva lactuca* and *Devaleraea mollis* cultured in the effluent from *A. nobilis* in a land-based integrated multi-trophic aquaculture (IMTA) system in southern California, USA. Water quality and performance of macroalgae were measured weekly. The impacted factors on the growth of macroalgae and nutrient uptake rate of macroalgae were assessed. The specific growth rate of juvenile *A. nobilis* was 0.47–0.52%/d. Total ammonia nitrogen in effluents of *A. nobilis* tanks ranged from 0.03 to 0.19 mg/L. *Ulva lactuca* and *D. mollis* achieved an average productivity of 24.53 and 14.40 g dry weight (DW)/m^2/d. The average nitrogen content was 3.48 and 4.89% DW, and accordingly, the average nitrogen uptake rate was 0.88 and 0.71 g/m^2/d, respectively. Temperature and nutrient concentration were key factors impacting macroalgae growth, and light intensity also impacted the growth of *D. mollis*. The high protein content of *U. lactuca* and *D. mollis* would make them good for use as human or animal food, or for use in other industries. Research on the interaction effects between seawater exchange rates and aeration rates on the performance and nutrient uptake rates of macroalgae will be conducted in future studies.

Keywords: biofilter; nutrient dynamics; eutrophication; bioremediation; mariculture

Key Contribution: *Ulva lactuca* and *Devaleraea mollis* achieved high productivities; nutritional composition and nutrient uptake rates when co-cultured with *Atractoscion nobilis* in a land-based seawater flow-through cascade IMTA system. Increased nutrient concentrations are expected to improve the performance of macroalgae co-cultured with *A. nobilis*.

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Citation: Huo, Y.; Elliott, M.S.; Drawbridge, M. Growth, Productivity and Nutrient Uptake Rates of *Ulva lactuca* and *Devaleraea mollis* Co-Cultured with *Atractoscion nobilis* in a Land-Based Seawater Flow-Through Cascade IMTA System. *Fishes* **2024**, *9*, 417. https://doi.org/10.3390/fishes9100417

Academic Editor: Ronaldo Olivera Cavalli

Received: 12 September 2024
Revised: 11 October 2024
Accepted: 17 October 2024
Published: 19 October 2024

1. Introduction

In the next 30 years, global seafood demand is expected to grow 30%, and aquaculture is expected to meet nearly all the increased global demand [1]. To successfully expand seafood production, aquaculturists must continually try to meet high sustainability standards expected by the public [2]. For aquaculture producers, access to seawater and coastal space is very limited and difficult to acquire in most cases [3]. Any space and water that is available needs to be maximized in its use. Also, the seafood marketplace is highly competitive and one's ability to diversify product lines to include multiple, high-value products, as well as gain operational efficiencies, will be greatly beneficial [4]. Diversified product lines will also help buffer against production failures for any given single species [5].

Waste (e.g., solids and nutrients) produced from mono-cultured fed species can also be removed by a combination of assimilatory and dissimilatory processes, mediated by phototrophic and heterotrophic organisms. This modern form of polyculture is called integrated multi-trophic aquaculture (IMTA), in which phototrophic and heterotrophic organisms are intentionally used to remove waste from the system, while concurrently diversifying

production [6]. Among phototrophic organisms used in IMTA systems, macroalgae is demonstrated to be a good biological filter to remove nutrients from the water column and re-oxygenate the culture water, thereby providing a positive environment for cultured animals [6,7]. Many studies have already been conducted in open-water IMTA systems that integrated macroalgae with fish, bivalves, and sea cucumbers [8–11]. In recent years, much attention has been focused on land-based IMTA systems, and various models have been proposed that mostly seek to integrate macroalgae with finfish, shrimp, abalone, bivalves, and echinoderms [11–18]. In these studies, macroalgae showed great efficiencies in biomass production and nutrient waste uptake. Interest in this topic is growing rapidly, and more research publications should be forthcoming.

White seabass *Atractoscion nobilis* is the target of a commercial fishery within its range that extends from central California, USA to Baja California, Mexico [19]. *Atractoscion nobilis* has numerous aquaculture characteristics desirable for commercialization, and after years of research, the hatchery rearing protocols for *A. nobilis* from spawning through fingerling production are now among the most advanced for the intensive hatchery production of a marine fish species in the United States [19]. To advance environmentally friendly technologies in the culture of *A. nobilis*, and simultaneously diversify seafood production, it is worthwhile to evaluate the integration of *A. nobilis* with low trophic organisms. Drawbridge et al. [20] reported that the growth, productivity, nutritional quality, and nutrient waste removal rate of *Ulva lactuca* were improved when they were integrated into an IMTA system co-culturing with *A. nobilis* in southern California, USA.

Ulva species have been identified as an ideal biofilter due to their strong capacity for nitrogen and phosphorus uptake and robust resilience to environmental changes [21,22]. Additionally, *Ulva* has wide applications in human food, animal feed, biofuel, and medicine [18,23,24]. In addition to *Ulva* species, red macroalgae species have also been used in IMTA systems. *Devaleraea mollis* is a low-intertidal to subtidal red macroalgae species that is native to waters of the northeastern Pacific Ocean from Alaska to southern California and around Asia. Due to its high nutritional quality and growth rate [25,26], *D. mollis* has been used as a biofilter and feed in abalone polyculture as well as for human consumption [13,27,28].

Until now, except for the study conducted by Drawbridge et al. [20] using *Ulva* as a nutrient biofilter in three-week trials, no other studies on *A. nobilis* IMTA have been conducted for a relatively long period, and also no studies have co-cultured *D. mollis* with finfish in IMTA systems. In this study, *A. nobilis* was integrated with *U. lactuca* and *D. mollis* in a land-based flow-through cascade IMTA system. A 79-day trial was conducted to evaluate the growth, productivity, nutritional composition, and nutrient removal rates of *U. lactuca* and *D. mollis* cultivated in the effluent from *A. nobilis* culture tanks, and to confirm what environmental parameters were the controlling factors to impact the performance of *U. lactuca* and *D. mollis*. Based on the results of the present study, future worthwhile studies were suggested. The results of the present study can be used to help establish an efficient IMTA system integrating *A. nobilis* with multi-trophic level organisms that increases and diversifies farm production while reducing nutrient discharge into coastal areas.

2. Materials and Methods

2.1. The Cascade IMTA System Design

A flow-through seawater cascade IMTA system was designed as shown in Figure 1. Four tiers were constructed in a stepped manner to support black polyethylene tanks with a working volume of 700 L (height: 70 cm; surface diameter: 60 cm; floor diameter: 52 cm) and a surface area of 1.09 m^2 each. The tiered tanks allowed seawater to cascade by gravity from the first to the last prior to discharge such that water was only pumped once to the system. Each tiered unit of four tanks was set up in triplicate rows for a total of 12 tanks. Three tanks on the first tier were used to hold *A. nobilis* as the primary "fed" species. Incoming ambient seawater was pumped from Aqua Hedionda Lagoon through sand filters into a holding tank that allowed for degassing and also ensured uninterrupted

water flow to the fish tanks, and then seawater was pumped into each fish tank using a 1/3 HP pump (Sequence 1000 External, Colorado Springs, CO, USA) with ball valves at each tank inlet to control flow rate. The water inlet to each fish tank consisted of a horizontal spray bar (3.8 cm) to provide additional degassing and directional water currents. Each fish tank was also supplied with aeration using air stones and a standby supply of pure oxygen if needed. The water was discharged through a center standpipe that set the water level of the tank. A PVC sleeve (15 cm diameter) with slits at the bottom was placed around the 5 cm standpipe to facilitate self-cleaning, whereby a suction was created using airstones inside the sleeve as an air-lift. This drain design, combined with circular water currents in the tank, effectively pulled settled solids up and out of the tank into Tier-2. Tanks on Tier-2 were designed to culture invertebrates such as sea cucumbers, abalone, sea urchins, polychaetes, and filter feeders (e.g., oysters, mussels), but that was not part of this study. The seawater from Tier-2 tanks was directed out from the side of the tank at the surface into Tier-3 tanks which were used to support the tumbled-culture of seaweed as nutrient biofilters in this system. Each seaweed tank had a 5 cm side drain at a height of 0.71 m. The drain pipe was connected to a 0.56 m long 5 cm mesh tube (4 mm) on the interior of the tank to keep seaweed from going out the drain. An air ring was installed at the bottom of the standpipe to provide central aeration at a rate of 25 L/min to drive seaweeds up and around the entire tank equally in a vertical circular motion. The seawater from each tank on Tier-3 entered each tank on Tier-4 through a side drain similar to that in between Tier-2 and Tier-3. The tanks on the fourth tier were also designed to support the "second stage" seaweed cultivation as biofilters, which had the same structure design as the tanks on the third tier. The seawater effluent from Tier-4 was also screened and exited the side of the tanks before being discharged into Aqua Hedionda Lagoon.

Figure 1. A seawater flow-through cascade IMTA system containing four tiers for integrating fed fish species (**A**), invertebrates, and two-stage seaweeds (**B**). The working volume of all tanks was the same as 700 L with a surface area of 1.09 m2 each.

2.2. Fish and Seaweed Sourcing

White seabass *A. nobilis* were spawned and reared at the Hubbs-SeaWorld Research Institute (HSWRI) marine fish hatchery (Carlsbad, California, CA, USA). *Devaleraea mollis* was provided by The Cultured Abalone Farm (Goleta, California, CA, USA), and *U. lactuca* was provided by the San Diego State University's Coastal Marine Institute Laboratory (San Diego, California, CA, USA). *Devaleraea mollis* and *U. lactuca* were initially stocked into 175 L cone-bottom tanks and a larger 3300 L holding tank supplied with effluent from *A. nobilis* raceway at an exchange rate of 63 vol./day under ambient daylight conditions. *Devaleraea mollis* and *U. lactuca* were allowed to grow in these tanks until the start of this experiment. In this study, no invertebrates were integrated into this IMTA system. Rather, the three Tier-2 invertebrate tanks served as "sumps" to receive seawater containing nutrient and solid wastes from *A. nobilis* tanks (Tier-1). Seawater entered into the seaweed tanks by gravity from the top of Tier-2 and solid wastes that settled to the bottom of Tier-2 were siphoned out at regular intervals so they did not move into downstream tiers.

2.3. Experimental Design

2.3.1. IMTA Fish Component

Juvenile *A. nobilis* with an average body weight of 134.7 ± 16.0 g were stocked at a density of 30 kg/m^3 in each replicate Tier-1 tank. Individual total and standard lengths were measured to the nearest 1.0 mm and wet body weights to the nearest 1.0 g at days 0, 47, and 79. Fish were anesthetized using tricaine mesylate (MS-222) at 75 mg/L during the weighing and stocking density setting-up process [29]. Fish were fed a commercial growout diet (Skretting 4.0 mm Marine mix with a proximate composition of 46% protein and 12% fat) to satiation by hand twice daily (1–3% body weight per day) between 8:00 and 10:00 and between 14:00 and 16:00. Any mortalities were removed daily and general health status was monitored during feeding. To compensate for fish growth, the fish density was reset to 30 kg/m^3 on 8th May by removing an appropriate number of fish from each tank. This effectively divided the study period into two phases: Mar 23 to 8 May (47 days), and 8 May to 8 June (32 days). The seawater exchange rate was set to 63 vol./day (2100 L/kg) according to our previous study [20]. Each tank of *A. nobilis* had a 30% shade cover on top to keep fish from jumping out as well as a shade canopy above all the fish tanks. Average temperature ranged from 14.3 °C to 19.7 °C, dissolved oxygen (DO) was in the range of 8.08–10.55 mg/L, and photoperiod was 12 Light:12 Dark.

2.3.2. IMTA Seaweed Component

After rinsing with filtered seawater, *U. lactuca* was stocked at an initial density of 1 kg wet weight/m^2 according to our previous studies [6,20], and *D. mollis* was stocked at the initial density of 4 kg wet weight/m^2 based on other references [30,31]. *Devaleraea mollis* tanks were covered with standard 60% nursery shade cloth to control epiphytes, which was verified using a light meter (Extech 407026, Taibei, Taiwan). During the trial period, *D. mollis* and *U. lactuca* were harvested weekly and reset to their respective initial stocking densities mentioned above. To determine biomass, the seaweed was shaken gently in a basket to remove residual seawater and then it was allowed to air dry for 5 min before weighing to the nearest 1.0 g. For tissue analyses, 250 g sub-samples of each species were taken at the beginning of the trial, at each weekly sampling day, and at the end of the trial. Sub-samples were rinsed thoroughly with filtered seawater and then deionized water several times, and wiped with tissue paper before being dried in an oven at 60 °C for 48 h until a constant weight was achieved. Dried sub-samples were stored inside an oven at 60 °C until chemical composition was measured.

2.4. Data Collection

2.4.1. Environmental and Water Quality

Light and temperature levels at the surface of the water in *D. mollis* and *U. lactuca* tanks were measured and logged every 15 min using HOBO Pendant MX2202 Temperature/Light Data Logger (Onset, Bourne, MA, USA). Temperature was also logged separately in ambient air and in the *A. nobilis* tanks. DO and pH were measured twice daily (8:00 and 14:00) using a Hach HQ40d hand held multi-meter (Hach, Loveland, CO, USA) and a Pinpoint pH meter (American Marine, Ridgefield, CT, USA). Seawater samples were collected from the influent of the *A. nobilis* tanks, and influents and effluents of each seaweed tank once each week at 13:00 on sampling dates to measure the total ammonia nitrogen (TAN), nitrite (NO_2-N) and nitrate (NO_3-N), and phosphate (PO_4-P) concentrations. TAN, NO_2-N, NO_3-N, and PO_4-P concentration analyses were made in duplicates using a Hach DR 6000 spectrophotometer after filtering the sample through a 0.45 μm CA membrane filter.

2.4.2. Biological and Biochemical Measures

The specific growth rate (*SGR*) of *A. nobilis* was calculated based on the weight difference between the initial stocking density resetting day, and the end of the trial. Food conversion rate (*FCR*) was calculated by summing up the amount of food fed during the trial and dividing by the increase in population biomass. The specific growth rate (*SGR*)

and productivity of *D. mollis* and *U. lactuca* were assessed weekly by measuring the difference of wet-weight biomass. Seaweed tissue nitrogen and carbon were measured using a Costech 4010 gas chromatography elemental analyzer. The protein content of *D. mollis* and *U. lactuca* was determined by multiplying the nitrogen content by 6.25 [32].

2.4.3. Parameter Calculation

Nutrient removal efficiency and rate by *D. mollis* and *U. lactuca* was based on the difference of nutrient concentrations between influents and effluents of seaweed tanks, and tissue nitrogen, carbon content, and productivity. All parameters were calculated based on following equations:

$$\text{Specific growth rate } (SGR, \%/\text{day}) = 100 \times (\ln W_f - \ln W_i)/t \quad (1)$$

$$\text{Food conversion rate } (FCR) \text{ for } A.\ nobilis = Feed\ Given/Weight\ Gain \quad (2)$$

$$\text{Productivity (g dry weight (DW)/m}^2\text{/d) of seaweed} = DW \times (W_f - W_i)/a/t \quad (3)$$

$$\text{Nutrient removal efficiency } (\%) = [(C_{in} - C_{out})/C_{in}] \times 100 \quad (4)$$

$$\text{Nutrient uptake rate (g/m}^2\text{/day)} = C_{tissue} \text{ (or } N_{tissue}) \times \text{Productivity} \quad (5)$$

where W_f was the final wet weight, W_i was initial wet weight, and t was the experimental time (days); DW was the percentage dry weight of seaweed, a was the surface area of tank (m^2), C_{in} was the nutrient concentration in influents, and C_{out} was the nutrient concentrations in effluents; C_{tissue} and N_{tissue} was the seaweed tissue carbon and nitrogen concentrations.

2.5. Data Analysis

Data were expressed as mean ± standard deviation. Data analysis was performed using JMP Pro software (version 15.0, SAS Institute). Tests of homogeneity of variance were conducted and percentage data, such as nutrient removal efficiencies, were arsine transformed for normalization before analysis. ANOVA analysis and Pearson correlations were used in this study for data analyses. Tukey's least significant difference (LSD) was used to make post hoc comparisons between different combinations. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Environmental Conditions

The average seawater temperature ranged from 14.3 °C to 19.7 °C with a minimum of 12.6 °C and maximum of 25.8 °C during the experimental period (Figure 2 left). The average temperature increased gradually from 15.1 °C on 17 April to 19.6 °C at the beginning of June when the trial was finished. The PAR level varied significantly between days during the experimental period. Because of cloudy and raining days, the median PAR level of 22.4–127.8 μmol/m^{2}s was significantly lower during 3–5 April, 20 April–1 May and 26–28 May compared with other experimental days when the median PAR level ranged from 228.7 μmol/m^{2}s to 2260.4 μmol/m$^{/2}$s (Figure 2 right).

The average pH ranged from 7.75 to 8.16 in the influents of the seaweed tanks with the average value of 7.93 ± 0.03, and the average pH ranged from 7.81 to 8.55 in the effluents of seaweed tanks with the average value of 8.01 ± 0.05 (Figure 3). The seaweed tank influent was the same as the fish tank effluent.

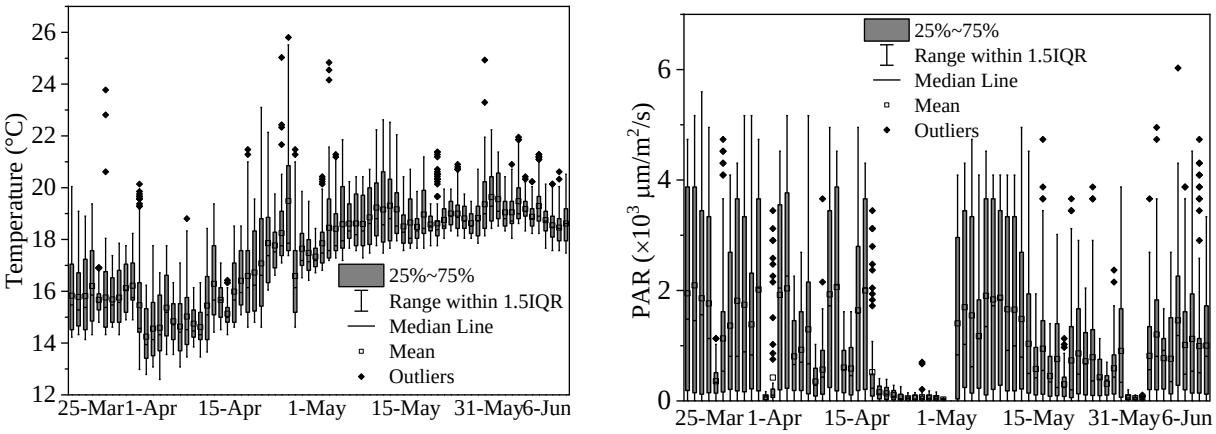

Figure 2. The change of daily seawater temperature (**left**) and light intensity (**right**) at the surface of the seaweed tanks throughout the experimental period from 25 March to 6 June (n = 3). The values 25–75% contain the middle 50% of the data. IQR means the interquartile range, and 1.5IQR means 1.5 points below the lower bound quartile or above the upper bound quartile is an outlier.

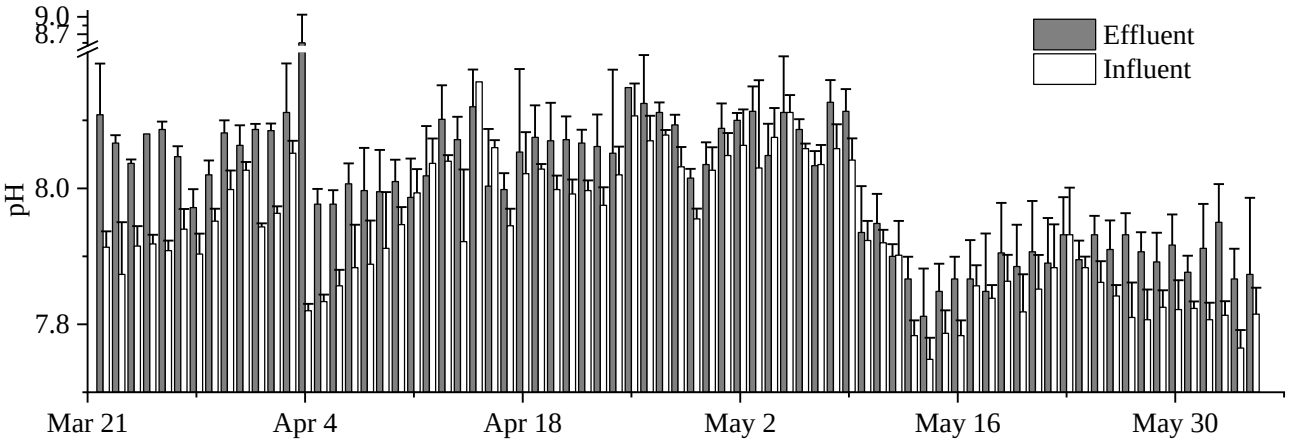

Figure 3. The change of monitored average pH values (measured twice daily at 8:00 and 14:00, respectively) in the influents and effluents of seaweed tanks throughout the experimental period where the seaweed tank influent is the same as the white seabass tank effluent (n = 3).

3.2. Atractoscion Nobilis Growth and Food Conversion Rate (FCR)

The weight gain of white seabass increased from 120.4 ± 4.4 g to 149.0 ± 3.0 g with the increasing percentage of 23.75% and from 149.0 ± 3.0 g to 188.1 ± 3.0 g with the increasing percentage of 26.24% in Phase 1 and 2, respectively, and consequently, the calculated *SGR* of white seabass was 0.47 ± 0.05%/d and 0.52 ± 0.05%/d and the *FCR* was 2.05 ± 0.27 and 1.64 ± 0.20 in Phase 1, and in Phase 2, respectively, but no significant difference was detected in *SGR* ($p = 0.37$) and *FCR* ($p = 0.10$) between the two phases (Table 1).

Table 1. The phase period, initial number with initial average weight (g), feeding rate (% body weight), final average weight (g), growth rate (SGR, %/day) and food conversion ratio (*FCR*) of white seabass (*Atractoscion nobilis*) in Phase 1 and Phase 2 during the experimental period (n = 3).

Phase	Period	Initial Number (No.)	Initial Average Weight (G)	Feeding Rate (% Body Weight)	Final Average Weight (G)	*SGR (%/Day)*	*FCR*
1	23 March–8 May	175 ± 6	120.4 ± 4.4	1.07 ± 0.002	149.0 ± 3.0	0.47 ± 0.05	2.05 ± 0.27
2	8 May–8 June	142 ± 3	149.0 ± 3.0	1.42 ± 0.002	188.1 ± 3.0	0.52 ± 0.05	1.64 ± 0.20

3.3. Growth and Productivity of Devaleraea Mollis and Ulva lactuca

The *SGR* ranged from 0.28 to 5.27%/d and 7.38 to 19.22%/d for *D. mollis* and *U. lactuca* with averages of 2.93 ± 1.69%/d and 12.95 ± 4.68%/d, respectively (Figure 4A). The productivity was in the range of 1.23–25.95 g DW/m^2d and 11.31–47.52 g DW/m^2d for *D. mollis* and *U. lactuca* with average values of 14.40 ± 9.09 g DW/m^2d and 24.53 ± 15.34 g DW/m^2d, respectively (Figure 4B). The *SGR* of *U. lactuca* was significantly higher than that of *D. mollis* ($p < 0.01$), and the productivity showed no difference between *U. lactuca* and *D. mollis* ($p = 0.059$).

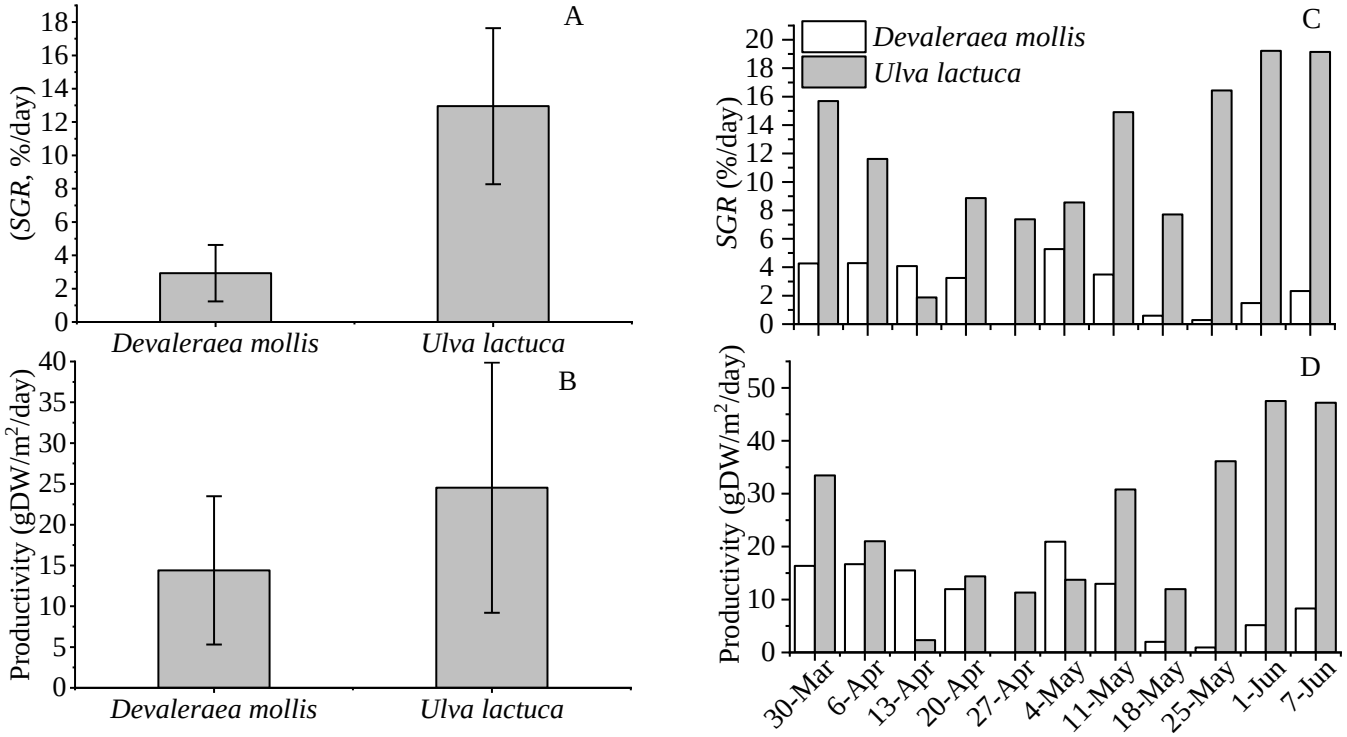

Figure 4. The average growth rate (*SGR*, %/day) (**A**) and productivity (g DW/m^2d) (**B**), and variation of growth rate (*SGR*, %/day) (**C**) and productivity (g DW/m^2d) (**D**) of *Devaleraea mollis* and *Ulva lactuca* cultivated with the effluent from *A. nobilis* tanks.

The results of correlation analysis between the *SGR* of *D. mollis* and *U. lactuca* and environmental factors is shown in Figure 5. The results showed that no relationship existed between TAN concentrations and the growth of *D. mollis* and *U. lactuca*, but it showed negative relationships between the growth of *D. mollis* and *U. lactuca* and PO_4-P concentrations. The *SGR* of *D. mollis* and *U. lactuca* showed a negative relationship with the pH. The *SGR* of *D. mollis* was negatively correlated to temperature, while the *SGR* of *U. lactuca* was positively correlated with temperature. Also, the *SGR* of *U. lactuca* showed a moderate positive relationship with NO_3-N concentrations.

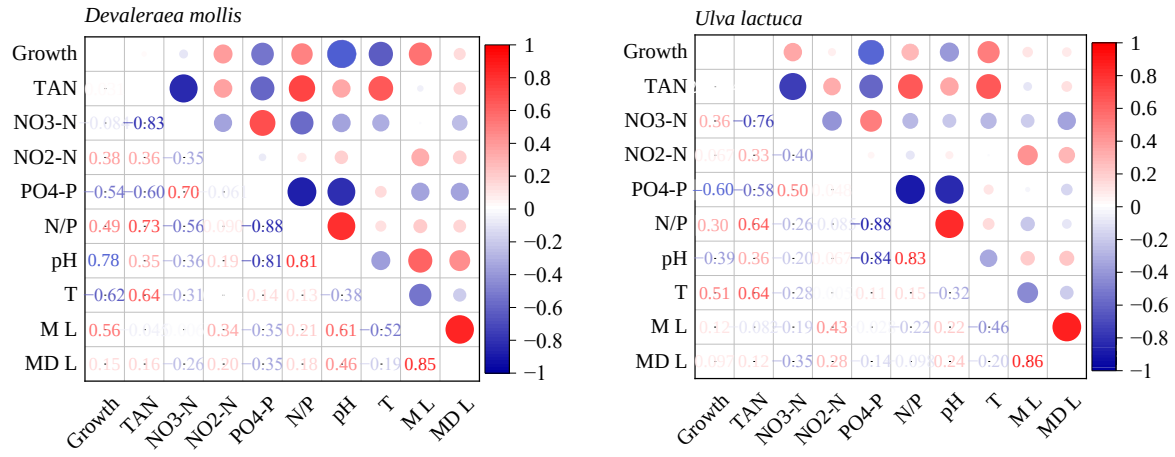

Figure 5. Correlation analysis between the growth rate of *Devaleraea mollis* and *Ulva lactuca*, and environmental factors. Red circles indicate a positive correlation, and blue circles indicate a negative correlation. Numbers in each cell represent the correlation coefficient. Note: M L indicates mean light intensity; MD L indicates median light intensity.

3.4. Nutrient Concentration and Removal Efficiency

The TAN, NO_3-N, NO_2-N, and PO_4-P concentrations in the influents of seaweed tanks varied greatly among sampling dates (Figure 6). Based on the difference between influent and effluent measurements, the nutrient removal efficiency was 37.27 ± 29.25% and 44.50 ± 30.70% for TAN; 56.67 ± 34.34% and 65.69 ± 31.81% for NO_3-N; 29.67 ± 12.61% and 22.92 ± 36.67% for NO_2-N; and 50.95 ± 32.47% and 44.26 ± 17.74% for PO_4-P by *D. mollis* and *U. lactuca*, respectively. The removal efficiency of each nutrient was not significantly different between *D. mollis* and *U. lactuca* (p = 0.64–0.88).

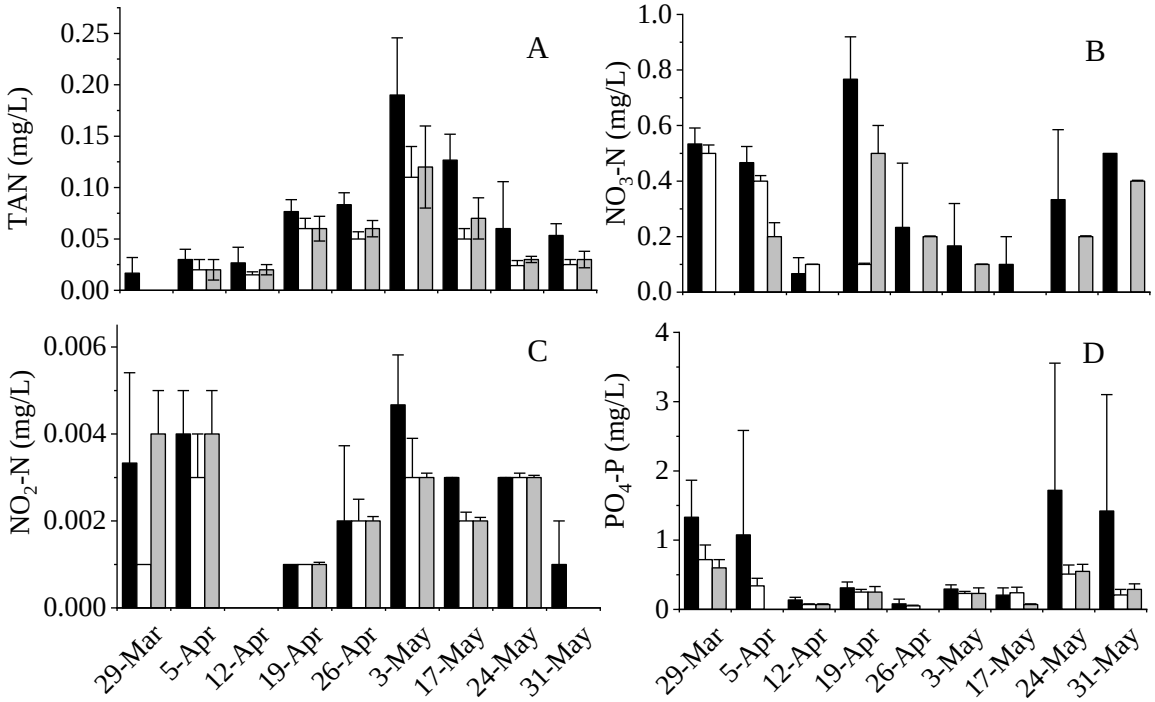

Figure 6. The change of TAN (**A**), NO_3-N (**B**), NO_2-N (**C**), and PO_4-P (**D**) concentrations in the influent and effluent of seaweed tanks throughout the experimental period. Black bar indicates the influents and seaweed tanks, white bar indicates the effluents of *Ulva lactuca* tanks, and grey bar indicates the effluents of *Devaleraea mollis* tanks (n = 3).

3.5. Tissue Chemical Composition and Nutrient Removal Rate

The average nitrogen content of *D. mollis* and *U. lactuca* was 4.89 ± 0.058% DW and 3.48 ± 0.10% DW, and the average carbon content was 30.98 ± 0.29% DW and 28.83 ± 0.78% DW, respectively (Figure 7). Based on the nitrogen and carbon content and the productivity of *D. mollis* and *U. lactuca*, the average nitrogen removal rate by *U. lactuca* was 0.88 ± 0.57 g/m^{2}d, and by *D. mollis* was 0.71 ± 0.46 g/m^{2}d, respectively, and the average carbon removal rate by *U. lactuca* and *D. mollis* was 7.21 ± 4.54 g/m^{2}d and 4.46 ± 2.78 g/m^{2}d, respectively (Table 2).

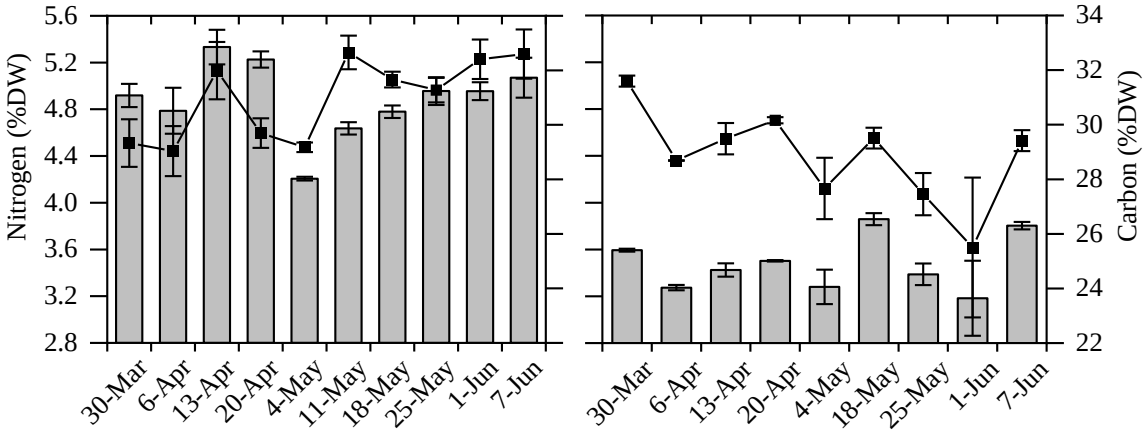

Figure 7. The nitrogen and carbon content (% DW) of *D. mollis* (left) and *U. lactuca* (right) cultivated in the effluent of white seabass tanks. The bars represent the nitrogen content (%DW), and the line with square symbols represents the carbon content (% DW) (n = 3).

Table 2. The average productivity (g DW/m^2/d), nitrogen (N) and carbon (C) contents (% DW), and nitrogen and carbon removal rates (g/m^2/d) by *Ulva lactuca* and *Devaleraea mollis* throughout the experimental period (n = 3).

Species	*Ulva lactuca*	*Devaleraea mollis*
Productivity (g DW/m^2/d)	24.53 ± 15.34	14.40 ± 9.09
Nitrogen (N, % DW)	3.48 ± 0.10	4.89 ± 0.058
Carbon (C, % DW)	28.83 ± 0.78	30.98 ± 0.29
N removal rate (g/m^2/d)	0.88 ± 0.57	0.71 ± 0.46
C removal rate (g/m^2/d)	7.21 ± 4.54	4.46 ± 2.78

4. Discussion

4.1. Performance of Atractoscion Nobilis

In the present study, the calculated *SGR* of *A. nobilis* increased from an average of 0.47%/d in Phase 1 to 0.52%/d in Phase 2, while the average temperature increased from 14.25–18.61 °C in Phase 1 to 18.46–19.66 °C in Phase 2; concurrently, the calculated *FCR* decreased from 2.05 in Phase 1 to 1.64 in Phase 2, which was in accordance with the results reported in other references [19,20,33].

TAN and NO_2-N are key indicators of water quality for marine finfish aquaculture [34]. Unfortunately, no studies have been published on the toxicity of un-ionized ammonia (NH_3-N) and NO_2-N to *A. nobilis*. The TAN concentrations of 0.03–0.19 mg/L in the effluents of *A. nobilis* tanks in the present study were similar to values reported by Drawbridge et al. [20] even though the stocking density of *A. nobilis* was increased to 30 kg/m^3; however, the NO_2-N concentration of 0–0.005 mg/L was significantly lower in the present study compared with previous results. The reasons for those differences are probably due to differences in the IMTA structures, system parameters, and experimental seasons. The highest NH_3-N concentration was estimated at 0.008 mg/L in the effluents of *A. nobilis* tanks based on

the TAN concentration, temperature, pH, and salinity in the present study, which was significantly lower compared with the accepted level of 0.07 mg/L in culturing juvenile European sea bass (*Dicentrarchus labrax*) [35] and 0.06 mg/L in the culture of juvenile longfin yellowtail (*Seriola rivoliana*) [36]. The NO_2-N concentration in the effluents of *A. nobilis* was also significantly lower than 0.21 mg/L of NO_2-N in one RAS in which *Seriola lalandi* was cultured for 488 days [34], and also considerably lower than the toxic levels of NO_2-N in other marine fish species [37–40]. Based on these results, it was indicated not only that dissolved nutrient concentrations in the present study were safe for *A. nobilis*, but also that the stocking density of *A. nobilis* could be increased to higher levels to produce more nutrients for downward co-culturing seaweeds to produce more biomass and improve their nutritional quality.

4.2. Growth and Productivity of Ulva lactuca and Devaleraea Mollis

The productivity of *U. lactuca* ranged from 11.31 to 47.52 g DW/m^2d with an average of 24.53 ± 15.34 g DW/m^2d in the present study, which was within the range of 6.73–55 g DW/m^2d reported by other researchers regardless of experimental conditions [6,20,22,41–45]. However, differences in experimental conditions and the types of culture tanks used among these studies can make comparisons difficult and the results hard to reproduce [6,20]. In tumbled-culture tanks, *U. lactuca* would have higher productivity when cultivated in shallow tanks compared with relatively deeper tanks under the same conditions because they can access sunlight for more time to process photosynthesis [6]. For example, the average productivity of *U. lactuca* reached up to 33.83 g DW/m^2d cultivated in 28 cm depth tanks supplied with 0.11–0.18 mg/L TAN [20]. Dissolved inorganic nutrient supplements can also influence *U. lactuca* productivity. When cultivated in 60 cm-depth tumbled-culture tanks, *U. lactuca* achieved the maximal production of 55 g DW/m^2d when the inflow ammonia concentration was at 78 µmol/L (≈1.41 mg/L), and it was suggested that the *U. lactuca* productivity was enhanced with the increasing of inflow ammonia concentrations [43]. In the present study, *U. lactuca* growth did not show any correlation with TAN concentrations mainly because of the low TAN concentrations. Under low TAN concentration conditions, *U. lactuca* will uptake NO_3-N to meet their requirements to produce biomass, which was indicated by the positive relationship we found between *SGR* and NO_3-N concentration (Figure 5). Dissolved inorganic nitrogen was the limiting factor for *U. lactuca* growth in this IMTA system because of the positive correlation with the ratio of N/P and the negative correlation with the PO_4-P concentrations. *Ulva lactuca* productivity could likely be increased with more TAN supplements, but this process could only be achieved through the combination effect of other environmental factors, mainly including light level, temperature, and pH [6]. Conversely, *U. lactuca* productivity might not be significantly improved at higher TAN concentrations if other environmental factors are unfavorable [6,22]. In the present study, *U. lactuca* growth and productivity increased significantly when seawater temperature gradually increased to what has been reported as an optimum of 19 °C at the end of this trial [46]. Low growth rates and productivity of *U. lactuca* from mid-April to the beginning of May were mainly due to low light levels [47]. Some researchers have indicated that low growth rates resulting from low nutrient uptake rates could be associated with a deficiency of dissolved inorganic carbon (DIC) [42,48]. In the present study, pH remained below 9.0 throughout the experimental period, which indicated that there was no DIC limitation for *U. lactuca* growth [49]. However, the negative correlation between *Ulva* growth and pH suggests that supplying more DIC might increase the growth and productivity of *U. lactuca*, which could be achieved by increasing the initial stocking densities of co-cultured *A. nobilis*, or aerating pure CO_2 into this IMTA system, especially during the daytime. Also, in this IMTA system, extra *A. nobilis* or invertebrates such as abalone, sea cucumbers, sea urchins, and filter feeders (e.g., oysters, mussels) can be cultured in second-tier tanks to produce additional TAN and DIC for *U. lactuca* to further enhance productivity. In addition to temperature, light level, nutrient type, and concentration, seawater exchange rate would be an important factor impacting the growth

and productivity of *U. lactuca* in tumbled culture tanks in IMTA systems. The seawater exchange rate in *U. lactuca* tanks was set to 63 vol./day in this study, which was demonstrated as the optimum (compared with 4 and 12 vol./day) by Drawbridge et al. [20]. Therefore, the effects of seawater exchange rates higher than 63 vol./day and values between 12 to 63 vol./day on the growth, productivity, and nutritional quality of *U. lactuca* should be tested in future studies.

The growth, productivity, and nutritional composition of *D. mollis* have already been studied by some researchers for the purpose of optimizing cultivation conditions, including nutrient concentrations, stocking density, light, salinity, temperature, seawater exchange rate, pH, aeration rate, and cultivation method [25,28,30,31,50–57]. Gadberry et al. [27] also cultivated *D. mollis* for one year in land-based tanks with effluents from a fish-rearing system, and the fish effluents were pulse-fertilized twice a week with 5.00 mg/L calcium nitrate as nitrogen, to evaluate seasonal growth, yield, nutritional composition, and contaminant levels. It is not practical to directly compare our results with these studies due to differences in experimental conditions and types of culture tanks. *Devaleraea mollis* is a temperate species and in general, grows well when seawater temperatures are lower than 16 °C. The optimal temperatures for maximum *SGR* were found to be a function of light, with increased light supporting higher growth rates at a higher temperature due to an interaction between light and temperature [31]. In the study by Demetropoulos and Langdon [31], *D. mollis* grew well at 18 °C at high specific light density (SLD, 0.021 mol photons/g/day). During the present study, the average temperature reached over 18 °C after April 25th, and the mean SLD maintained high levels (0.015–0.036 mol photons/g/day with an average of 0.020 mol photons/g/day) from the beginning of May except on three overcast days from May 26 to 28 (Figure 2). This can partially explain why *D. mollis* maintained a relatively high productivity at 5.98–21.50 g DW/m^{2}d (except for 1.23 g DW/m^{2}d evaluated on June 1 due to overcast days during that period) even though the seawater temperature went over 18 °C. Nutrient types (TAN, or NO_3-N and PO_4-P), as well as their concentrations, are other factors that will impact the growth and productivity of *D. mollis*, especially during high-temperature periods (e.g., >18 °C). Demetropoulos and Langdon [30] recommended providing high nitrogen (NO_3-N) fertilization rates of 2353–2942 µmol/d in order to maintain high growth rates of *D. mollis* at 16 °C and light levels of 300–1400 µmol/m^2/s. It is hard to apply this approach in a flow-through aquaculture system because the seawater exchange rate is another important factor that will significantly impact the growth and productivity of *D. mollis* in tumbled cultivation tanks. It was previously shown that 70–100% of the nitrogen requirement of macroalgae was provided by co-cultured fish [27], based on an influent ammonia–nitrogen concentration of 20–30 µmol/L (0.36–0.54 mg/L) [58]. It could be concluded that the TAN concentration (0.02–0.19 mg/L) was not sufficient for *D. mollis* to maintain a high growth rate in the present study, which was also indicated by the positive correlation between the growth rate and N/P ratio. The appropriate molar N/P ratio for nutrient supplementation was suggested to be approximately 70 and 35 for maximum *D. mollis* growth under low and high light conditions, respectively [30]. The molar N/P ratio of 0.60 to 6.11 in the influents of *D. mollis* culture tanks also indicated that dissolved inorganic nitrogen was the limiting factor for *D. mollis* to achieve high productivity in the present study. The negative correlation between the growth of *D. mollis* and pH suggests that supplying more DIC might increase the growth and productivity of *D. mollis*, even though DIC limitation was not indicated by pH values (<9.0). As mentioned above, the growth rate of *D. mollis* could also be significantly impacted by seawater exchange rates. The growth, productivity, and nutritional quality of *D. mollis* were shown to be significantly higher when cultivated at an exchange rate of 35 vol./day compared with those of 1 and 6 vol./day [54,55]. Demetropoulos and Langdon [31] stated that the high yield and growth of *D. mollis* were achieved at the seawater exchange rate of 60 vol./day in combination with moderate stocking density and high natural light. In the present study, the productivity and nutritional quality of *D. mollis* were only tested at 63 vol./day of seawater exchange.

Therefore, studies to determine differences in the growth, productivity, and nutritional quality of *D. mollis* across a wide range of seawater exchange rates should be conducted.

Seaweed culture can be successful in relatively deep tanks when properly "tumbled" in the water column using aeration to bring suspended seaweeds from deeper layers to the surface to access light for photosynthesis [20]. Strong aeration evens out light exposure and facilitates solute diffusion [59]. The turbulence generated by the aeration also thins the diffusive boundary layer (DBL) around the frond surfaces, accelerating the inflow of nutrients to the fronds and removal of excess oxygen from them [42], which ultimately enhances the nutrient uptake rate, growth, and productivity of seaweeds in tumble culture tanks [25,60]. In this present study, the growth of *U. lactuca* and *D. mollis* was only evaluated at one aeration rate of 25 L/min in 700 L tanks (0.035 L air/L of seawater/min), but it is expected that the performance of these two species would be impacted by the interaction between nutrient concentration, seawater exchange rate, and aeration rate in tumble culture tanks. These interactions are worthy to be evaluated in future studies.

4.3. Water Quality Remediation and Nutrient Uptake Rates

Co-cultured seaweeds can significantly increase pH values in the effluent from primary-fed species by utilizing DIC during the photosynthesis process. In one study conducted by Huo et al. [6], seawater pH in *U. lactuca* tanks gradually increased from 7.84 to a peak of 8.59 during the daytime, and gradually decreased to a low of 7.76 before dawn the next day when integrated into an IMTA system co-culturing with *S. dorsalis.* In an IMTA system integrating red abalone (*Haliotis rufescens*) and *D. mollis*, the mean seawater pH increased by 0.2 pH units due to the biological activity of *D. mollis* [13]. In the present study, *U. lactuca* and *D. mollis* increased pH by 0.01–0.73 units in the seawater effluents compared to the influents. The pHs of the effluents from *A. nobilis* tanks were lower from mid-May to the end of the trial because the feeding rate of *A. nobilis* increased from 1.07 to 1.42% body weight per day when water temperatures increased (Table 1). During this time, the pH values only increased 0.01–0.14 pH units higher in the effluents from the seaweed tanks (Figure 3). This was mainly caused by the reduced growth and productivity of *D. mollis* at higher water temperatures. As discussed above, increasing light levels and/or nutrient concentrations, optimizing seawater exchange rates and/or aeration rates, might increase the growth and productivity of *D. mollis*, but because of its intrinsic properties, the growth of *D. mollis* would continue to decrease with increasing temperature. During seasons when water temperature is >18 °C, some other economically valuable, and temperature-tolerant red seaweeds, such as *Gracilaria parvispora* [61] or *Gracilaria pacifica* [62], could be integrated into the IMTA system to replace *D. mollis*.

Nitrogen removal rates are dependent on the productivity and tissue nitrogen concentration of seaweeds cultivated in the effluents from primary-fed species in IMTA systems. The average productivity was 24.53 ± 15.34 g DW/m^2d and 14.40 ± 9.09 g DW/m^2d for *U. lactuca* and *D. mollis* during the present study, which could be enhanced by optimizing the cultivation parameters discussed above. The tissue nitrogen concentration of seaweeds functions as dissolved inorganic nitrogen load levels within a certain range under suitable conditions of other parameters in tumbled culture tanks. In the present study, the average nitrogen content of *U. lactuca* (3.48% DW) was similar to another study in which fish effluent TAN concentrations were similar [20]. However, the nitrogen content in the present study was significantly lower compared with that of Huo et al. [6] where *Ulva* nitrogen content reached 4.85% DW under TAN concentrations that reached as high as 1.19 mg/L. Similarly, nitrogen content was lower compared with 4.41–7.27% DW when TAN in effluent from a fishpond ranged 1.18–2.23 mg/L [22]. Neori et al. [43] conducted a series of trials to demonstrate that the nitrogen content of *U. lactuca* improved significantly when TAN concentration increased from 10 μM to 48 μM in effluents from fishponds. Similar to findings for *U. lactuca*, the nitrogen content of *D. mollis* was shown to be a function of nitrate (NO_3-N) concentrations and averaged between 3.07 and 5.01% DW [52], which indicates that the protein content of *D. mollis* will improve with increasing dissolved

inorganic nitrogen. The average nitrogen content of *D. mollis* (4.89% DW) in this study was higher than most values (3.07–5.01% DW) reported by Demetropoulos and Langdon [52]. It was also higher than all values (3.76–4.11% DW) across four seasons by [27] when *D. mollis* was cultivated in the effluent of fish tanks. It was also reported that the nitrogen content of *D. mollis* was a function of nutrient application rate [30]. Specifically, when *D. mollis* was cultivated at nutrient supplementation intervals of daily and every 3 and 5 days, the nitrogen content of 4.99–5.83% DW was higher than other nutrient supplementation intervals and also higher than the values obtained in the present study. This demonstrated that continuously supplying high concentrations of dissolved inorganic nitrogen to *D. mollis* can significantly increase tissue nitrogen contents, which will ultimately increase the protein content of *D. mollis* and promote higher nutrient removal rates. In the present study, TAN and NO_3-N concentrations in effluents from *A. nobilis* tanks varied greatly between experimental days with relatively low levels on some days, which slowed down the growth and productivity of *D. mollis* and decreased the tissue nitrogen content. Based on this discussion, we concluded that the tissue nitrogen content of *U. lactuca* and *D. mollis* could be further enhanced by supplying higher concentrations of nutrients through increased initial stocking density of *A. nobilis* and culturing invertebrates in second-tier tanks to increase nutrient supplementation.

5. Conclusions

Atractoscion nobilis is a marine fish species that is ready to be cultured commercially in the United States, but little research has been done to study the integration of *A. nobilis* with low trophic level organisms. The present study reported the growth, productivity, nutritional quality, and nutrient removal rate of economically valuable seaweeds *U. lactuca* and *D. mollis* when integrated with *A. nobilis* in a land-based flow-through cascade IMTA system. *U. lactuca* and *D. mollis* achieved high growth rates and productivities, nutritional qualities, and nutrient removal rates. Due to its temperature constraints, *D. mollis* can be integrated into IMTA systems during late fall and early summer (November to June) in southern California. The performance of *D. mollis* can be enhanced by increasing light levels (reducing shade) during overcast days and during high-temperature periods. The performance of *U. lactuca* and *D. mollis* can both be enhanced through consistently supplying high concentrations of nutrients in influents. The effects of aeration rates and their interactive effects with seawater exchange rates on the performance of *U. lactuca* and *D. mollis* should be investigated to further maximize the nutrient removal rates and diversify the seafood production in IMTA systems in future studies. Moreover, nitrogen and phosphorus concentrations in the environment of *A. nobilis* tanks would be significantly reduced by the assimilation of *U. lactuca* and *D. mollis*, which will be qualified in future studies.

Author Contributions: Y.H., M.S.E. and M.D. designed the experiment. M.S.E. and Y.H. conducted the experiment and data analysis. Y.H. drafted the manuscript. Y.H., M.S.E. and M.D. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Builders Initiative (BIF Grant 2022-6439).

Institutional Review Board Statement: All experiments were conducted according to the Institutional Animal Care and Use Committee (IACUC) of HSWRI on the care and use of experimental animals. Animal research in this study gained approval from the IACUC (identification code: 2022-05).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: This study received additional support from California's Ocean Resources Enhancement and Hatchery Program. The authors would like to thank Matthew Edward's laboratory at San Diego State University for measuring the nutritional composition of the seaweeds. Many technicians supported the studies reported here; we are thankful for their contributions.

Conflicts of Interest: The authors declare no conflicts of interest.

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