INTEGRATION OF EXTRACTIVE (*HOLOTHURIA SCABRA, PERNA VIRIDIS,* AND *SARGASSUM SILIQUOSUM*) WITH A FED SPECIES (*CHANOS CHANOS*) IN A LAND-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE RECIRCULATING SYSTEM

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Abstract. This study explored the environmental sustainability potential of integrated multi-trophic aquaculture (IMTA) utilizing extractive species in a re-circulating aquaculture system, with physico-chemical parameters and growth models used as indicators of effective waste assimilation. Control and experimental glass tanks, each filled with 210-L of filtered seawater, were set-up to maintain the test organisms for 42 days: tank 1 (fed species, *Chanos chanos,* and *Holothuria scabra*), tank 2 (*Perna viridis*), and tank 3 (*Sargassum siliquosum*). Physico-chemical parameters such as temperature, dissolved oxygen, salinity, and total dissolved solids showed no significant differences between tanks, while pH differed significantly ($p = 0.028$). Nutrient analysis revealed significant reductions in ammonia and phosphate ($p < 0.05$), but nitrate levels, although reduced in the integrated multi-trophic aquaculture tanks, were not statistically different from the control. *C. chanos* exhibited low survival (17.39%) and a growth rate (0.017% d^{-1}). Conversely, *H. scabra* and *P. viridis* showed high survival rates (100% and 69.64%) with growth rates of 0.46% d^{-1} and a net decrease of -0.98% d^{-1}, respectively. *S. siliquosum*'s nitrate removal was low (15.65%), but phosphate removal was high (79.61%), with tissue N and P contents significantly higher ($p < 0.05$) than initial values. Findings indicated that the cultured species' growth and assimilation performances suggest IMTA potential for a sustainable marine aquaculture.
Keywords: *water quality, growth rate, biofiltration potential, macroalgal tissue, inorganic nutrients*

Introduction

Water pollution is a significant issue in intensive aquaculture production in open marine waters (Sanz-Lazaro and Sanchez-Jerez, 2017). Recognizing the role this sector plays in providing food and livelihood to humanity, current research is pressing for more sustainable aquaculture practices and concepts (Fernandez-Gonzalez et al., 2018) that seek to balance economic benefits and environmental gains (Cranford et al., 2013; Park et al., 2012). One form of aquaculture technology considered to be environmentally sustainable is integrated multi-trophic aquaculture (IMTA) (Park et al., 2012). This management tool utilizes several extractive species of different trophic levels (Kerrigan and Suckling, 2018) to assimilate nutrients released by fed species such as fish and shellfish in traditional fish farms (Sanz-Lazaro and Sanchez-Jerez, 2017; Yu et al., 2016), thereby contributing to a harmonious ecosystem function (Mustafa et al., 2019).

Most of the IMTA system established in Asia and is adapted by many countries in the world used deposit feeders (e.g., holothurians), suspension feeders (e.g., bivalves) and/or macroalgae (e.g., seaweeds) to maximize biofiltering capacities of dissolved and organic wastes released from farming operations. Experimental studies found potentials of highly marketable species as effective organic extractive components. These include sea cucumbers like *Apostichopus japonicus* (Yuan et al., 2015), *Actinopyga bannwarthi*

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(Israel et al., 2019), *Holothuria scabra* (Chary et al., 2020) and mussels such as *Mytilus edulis* (Min, 2011). The latter, however, is debatable as more studies supported the claim of insignificant assimilation rate of wastes on the mussel's diet (Irisarri et al., 2015; Sanz-Lazaro and Sanchez-Jerez, 2017). Moreover, a large-scale study of *Sargassum* spp. revealed its efficiency to mitigate the total negative impact of aquaculture to the coastal environment (whole bay) while giving a quality seaweed product with better yield (Yu et al., 2016).

In the Philippines, mariculture using commercial feeds (Largo et al., 2016) has been promoted and supported by the government as means of livelihood diversification for coastal fishers (Salayo et al., 2012). Consequently, the release of organic and inorganic wastes from cages in the surrounding water column was also intensified. Despite its launching for nearly two decades now, the lack of institutionalized intervention (Largo et al., 2016) to areas utilized for such operations aggravates water pollution problem (Salayo et al., 2012). As a matter of fact, waters in the traditional culture areas are now heavily polluted (Layugan et al., 2018). While integrated aquaculture has long been done in freshwater systems, the application of IMTA to address environmental sustainability issues of open sea culture systems is relatively a new concept (Largo et al., 2016). The research of Largo et al. (2016) reported the potential use of economically-important red seaweeds, *Gracilaria heteroclada* and *Eucheuma denticulatum* as biofilters for nitrate and ammonia concentrated in open water cages where abalone species (*Haliotis asinina*) was cultivated.

Determination of the IMTA potential of marine species in a recirculating aquaculture system has been explored by several studies, but mostly, one extractive for one or more fed organisms. Recent findings revealed a decrease of inorganic nutrients such as total ammonia, nitrate, nitrite, and phosphate in nutrient-enriched system as species like *H. scabra* (Senff et al., 2020; Thuy et al., 2024), *P. viridis* (Srisunont and Babel, 2015), and *S. siliquosum* were integrated (Edwards et al., 2024). At varying densities, sandfish (Neofitou et al., 2019; Senff et al., 2022; Chatzivaasileiou et al., 2024), green mussels (Rejeki et al., 2021), and *Sargassum* also exhibited survival and growth after assimilating wastes from grouper (Adharini et al., 2021) and shrimp (Yasir and Adharini, 2021) as fed species.

The present study is intended to lay the groundwork for setting up an open water multi-trophic aquaculture system (IMTA) by first investigating in a more controlled environment the suitability of extractive, high-value species namely sand fish (*Holothuria scabra*), green mussel (*Perna viridis*) and brown seaweed (*S. siliquosum*) which were integrated as components in fed aquaculture namely, milkfish (*Chanos chanos*). Particularly, this work sought to determine the biofiltration efficiencies of the extractive species of the wastewater coming from the fed species in an indoor glass tank set-up. This was carried out by: (1) investigating the growth of each cultured species; (2) determining the biofiltration potential of extractive species *S. siliquosum*; and (3) estimating the total amount of ammonia, nitrate and phosphate removed as excess nutrients from the fed species that enters the ambient seawater and as part of the seaweed biomass removed during harvest.

Methods

Extractive species used in the experiment

The three extractive species that were utilized in this research (*Fig. 1*) were obtained from the nearest possible source within the Visayas region. For the culture trials, species of juvenile *H. scabra* (a benthic deposit-feeding sea cucumber) were obtained from a

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fisher in Cordova, Cebu who identified the sea cucumber using a (laminated) photograph of the *H. scabra*. Young adults of *P. viridis* (a suspension feeding bivalve) were obtained from a mussel farm in Villareal, Samar Province. As for the brown seaweed *S. siliquosum*, vegetative fragments were obtained from Maribago, Lapulapu City (Mactan Island, Cebu) after securing a permit from the Bureau of Fisheries and Aquatic Resources – Region 7 (BFAR 7). All species were gradually acclimatized with sand-filtered seawater from the supply source to the transplant study area for seven days following the procedure of Layugan et al. (2018).

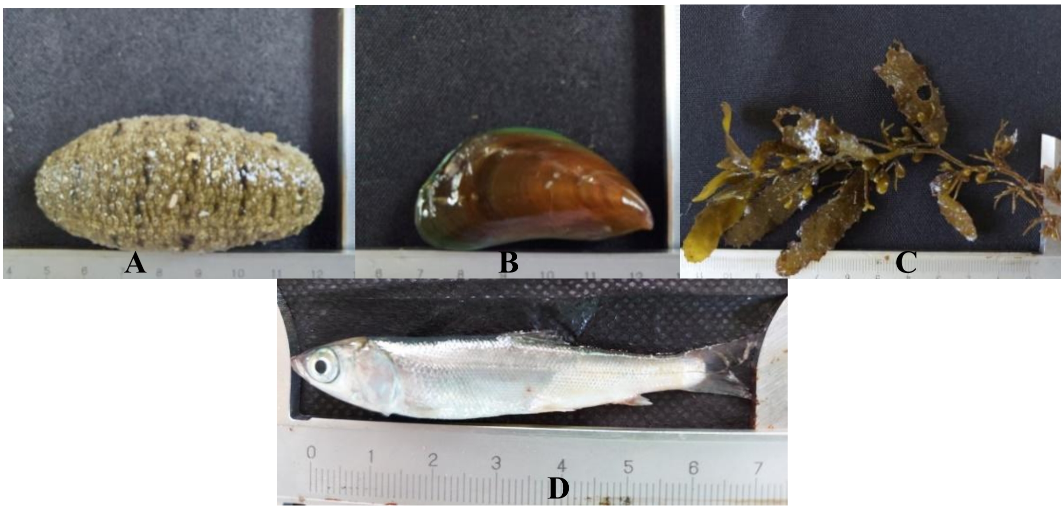

Figure 1. *Extractive and fed species integrated in an indoor laboratory setup: (A) Tank 1 - sandfish, H. scabra, (B) Tank 2 - green mussel, P. viridis and (C) Tank 3 - brown macroalgae, S. siliquosum, and (D) C. chanos. (Source: authors)*

Tank experiment on IMTA system with fed and extractive species

A 42-day laboratory feasibility study was conducted in the Marine Research Station of the University of San Carlos adopting the methods of Correia et al. (2020) with some modifications. Four glass tank units (210 L; dimension: 89.2 cm × 56.1 cm × 60 cm) was set up to investigate the potential of IMTA concept for the local fish farms. The system involved juvenile (~7 cm) milkfish (*Chanos chanos*) as fed species, and sea cucumber (*H. scabra,* ~7 cm; 53.9 g), green mussel (juvenile *P. viridis,* ~ 5 cm shell length) and brown seaweed (*S. siliquosum*, vegetative thalli fragments, ~ 10 cm in length) as extractive species. After an acclimatization period of one week, both milkfish and sea cucumber were placed together in a single tank and the other species were placed in separate tanks – all tanks were connected with each other in a closed, re-circulating system (*Fig. 2*). All tanks were supplied with common seawater that passed first through a filtration tank with sand and gravel before it goes into the individual tank. Each tank was aerated using air pumps connected with tubes. A water pump placed at the end of the last tank re-circulated the seawater back to the first tank containing the milkfish and sea cucumber after passing the filtration tank (*Fig. 2*).

In the tank where milkfish and sandfish were co-cultured, 2 cm layer of clean sand (Dobson, 2020) were obtained from where the sandfish were sourced. During the observation period, physico-chemical parameters were maintained in an outdoor condition where water temperature ranged from 27.17°C to 31.63°C, pH between 6.07 and 8.27,

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salinity at around 35 psu, dissolved oxygen between 4.41 and 7.30 mg/L, and concentrations of total dissolved solids at a range of 1.02–3.29 mg/L. These parameters were determined using pH meter, refractometer, DO meter and Bante 900 benchtop multiparameter meter, respectively. As for the seaweed *Sargassum*, photoperiod was maintained at 12/12 h light/dark cycle (Correia et al., 2020) was irradiated by 100.69 ± 21.38 µmol m^{-2} s^{-1} (mean ± SD) using light meter. The control tank contained milkfish only and was maintained with similar environmental conditions as the treatment tanks.

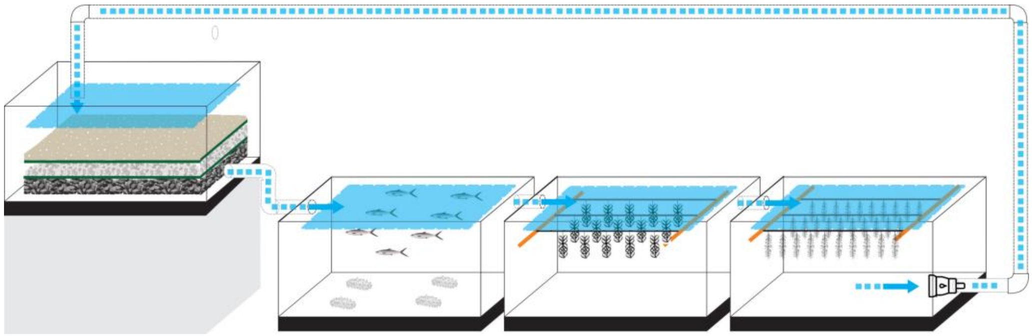

Figure 2. *Schematic diagram of IMTA treatment tanks. The broken line with an arrow indicates the recirculation flow from the filtration tank to IMTA tanks, supporting the maintenance of fed and extractive species. The blue layers represent the water levels maintained within each tank*

The fish tank was stocked with 7 juveniles of *C. chanos* of ~ 7 cm in length at a stocking density of 1657 g/m^3, based on the total water volume of 120 L. Stocking densities for other IMTA species were calculated considering their initial biomass and the tank surface area: *H. scabra* at 40.1 g/m^2 (initial biomass of 100 g distributed over the tank surface, n = 4; Senff et al., 2020), *P. viridis* juveniles at 19 500 g/m^3 of (initial total biomass of 2 116 g; Correia et al., 2020), and *S. siliquosum* at 1 298 g/m^3 (Correia et al., 2020) (*Fig. 2*) with modifications to optimize biomass and resource use within the 120 L tank. The fish was fed with a commercial diet (*Santeh*) containing crude protein (31%) and crude fat (8%) (FAO, 2021) three times a day (8:00 AM, 12:00 NN, and 16:00 PM) at a feeding rate of 4% of biomass per day (FAO, 2021). Total feed intake was measured and recorded daily. From each tank, water samples were collected weekly at 9:00 o'clock in the morning, after feeding the milkfish (Correia et al., 2020). All water samples were chilled and sent to FAST Laboratory (Subangdaku, Mandaue City) within 6 h of collection for ammonia, nitrate, and phosphate analyses. The results were compared to the 2016 DENR Water Quality Standards for marine waters.

Growth rate of extractive species

At the start and at the end of the experiment, the fish, sea cucumber, mussels and *Sargassum* were group-weighed using an analytical balance to get the total biomass of each species. The diameter and length of each sea cucumber and length and width of each green mussel shell were measured with a Vernier caliper (Correia et al., 2020). Except for the seaweed *Sargassum*, all test organisms' daily growth rate (DGR) and survival rate (SR) were calculated using the following equations (Largo et al., 2016):

$$\mathrm{DGR} = \frac{[(W_f - W_i)]}{t} \times 100 \quad \text{(Eq.1)}$$

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where W_f is the wet weight after t days culture and W_i is the initial weight

$$SR = N_I - N_F/N_I \times 100 \quad \text{(Eq.2)}$$

where N_I = initial number of species and N_F = final number of species

As for the brown alga, *S. siliquosum*, the increase in biomass yield (Y) and daily growth rate (DGR) were determined using the following equation (Bermejo et al., 2020):

$$Y\ (\text{mg dw m}^{-1}\ \text{day}^{-1}) = [(B_t - B_0)\ (\text{dw/fw})/\text{tL}] \quad \text{(Eq.3)}$$

where B_t is the final algal wet weight for each rope (mg), B_0 is the initial biomass, t is the cultivation period in days, dw is the dry weight, fw is the fresh weight and L is the length of the ropes. After the cultivation period, samples of *Sargassum* were dried for 48 h in a Memmert drying oven at 60°C.

The biofiltering efficiency (%) of the seaweed was calculated based on nutrient concentrations in the effluents from both the control and IMTA systems using *Equation 4* (Hernandez et al., 2005, as cited in Kang et al., 2011);

$$\text{Biofiltering efficiency (\%)} = (A - B) / A \times 100 \quad \text{(Eq.4)}$$

where A and B are the nutrient concentrations (total nitrate and phosphate) in the effluent of the control and IMTA system, respectively;

To determine the total N and P content in the macroalgal tissue, a sample containing 30 g (dry weight) from the initial and final stocks were analyzed for total nitrogen and total phosphorus using macro-Kjeldahl and spectrophotometric method, respectively. The bio-mitigation capacity of the same seaweed was calculated using *Equation 5* (Bermejo et al., 2020):

$$\text{Bio-mitigation capacity (mg N m}^{-1}\ \text{month}^{-1}) = [N_t\ (\text{dw/fw})_t\ B_t - N_0\ (\text{dw/fw})_0\ B_0] \quad \text{(Eq.5)}$$

where N_t and N_0 (mg N g^{-1} dw) are the tissue N (or P) contents at the end and at the beginning of the cultivation period.

Statistical analysis

All statistical analyses were performed using Microsoft Excel (version 2021). Differences of the environmental conditions in control and IMTA tanks, as well as the initial and final inorganic nutrients accumulated by *S. siliquosum* tissues were compared using Student's t-test. To test the variability of the concentrations of inorganic nutrients in the IMTA tanks one-way ANOVA was performed. Significance level is at $p < 0.05$.

Results

Growth of the cultured species in the IMTA tanks

Growth performance of the species maintained inside tanks were evaluated for their survival rate, daily growth rate, and yield after being exposed to organic and inorganic wastes coming from the milkfish fed with commercial feeds (*Table 1*). In the control

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set-up, the milkfish showed limited growth and 0% survival rate prior to the termination of the observation period likely due to potential microbial infection originating from the source, which will be described in detail in the subsequent subsection. In the IMTA set-up, on the other hand, the initial total or combined biomass (fresh) of *C. chanos* as fed species and the extractive species, namely, *Holuthuria scabra*, *Perna viridis* and *Sargassum siliquosum* were 39.8, 215.6, 2 116.0 and 1 298.0 g, respectively. Their combined biomass was 3 669.4 g. After a 42-day period, the final biomass of each species was recorded as follows: *C. chanos* = 19.2 g, *H. scabra* = 257.1 g, *P. viridis* = 1 250 g, and *S. siliquosum* = 458.5 g. The overall biomass of the setup at the end of the period was 2 382 g, indicating a 35.08% reduction in total biomass.

Table 1. *Growth data of fed and extractive species in the recirculating IMTA system*

Parameters	*Fed species		Extractive species		
	Control	IMTA			
	C. chanos (N = 7)	*C. chanos* (N = 7)	*H. scabra* (N = 4)	*P. viridis* (N = 112)	*S. siliquosum*
DGR in weight (% fw d^{-1})	-	- 0.017	0.46	- 0.98	- 1.54
Initial weight (g)	40.5 ± 0.36	39.8 ± 0.37	215.6 ± 14.16	2 116 ± 1.99	1 298 ± 83.67
Final weight (g)	-	19.2 ± 0.29	257.1 ± 17.60	1 250 ± 2.31	458.5 ± 36.42
DGR in length (cm^{-1} d^{-1})	-	0.11	1.54	0.00003	-
Initial mean length (cm)	7.00 ± 0.0	7.00 ± 0.00	7.75 ± 0.99	5.656 ± 0.80	-
Final mean length (cm)	-	11.67 ± 0.01	12.75 ± 1.65	5.658 ± 1.21	-
DGR in width (cm^{-1} d^{-1})	-	-	0.0162	0.001	-
Initial mean width (cm)	-	-	3.93 ± 0.21	2.62 ± 0.37	-
Final mean width (cm)	-	-	3.25 ± 0.62	2.58 ± 0.41	-
Survival rate (%)	0	17.39	100 ± 0.00	69.64	-
Yield (mg dw m^{-1} d^{-1})	-	-	-	-	- 276.1
Initial algal wet weight (mg)	-	-	-	-	432, 670
Final algal fresh weight (mg)	-	-	-	-	150, 500
Final algal dry weight (mg)	-	-	-	-	17, 800
Total weight reduced (%)	-	-	-	-	35.08
Initial weight (milkfish + IMTA species in g)	-	-	-	-	3 669.4
Final weight (milkfish + IMTA species in g)	-	-	-	-	2 382.0

DGR means daily growth rate of the parameter measured
*The initial sample size was 7 individuals; however, the remaining samples at the end of the observation period was 0 (4^{th} week) in the control and 4 in the IMTA set-up

In this study, the juvenile milkfish demonstrated poor growth rates in terms of body length and weight. The survival rate of *C. chanos* was 17.39%, with only 4 individuals remaining out of the original number of 23. Its average initial length of 7 cm and weight of 5.68 g increased to 11.67 cm and 6.4 g, respectively, after 42 days. These values accounted for a total increase in length of 1.59% and weight of 0.30% d^{-1}, respectively.

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Noticeably, prior to the death of the milkfish grown in tanks, some form of microbial infection (*Fig. 3A*) was manifested as external hemorrhagic red spots in the fish's body surface, accompanied with skin lesions associated with fin rot. Mucus secretions near the gills and concentrated in the pectoral fin towards the vent was also noticed. The microbial organism isolated from the surface mucus of the dead milkfish (obtained from the Tank 1) appeared as microscopic filaments (*Fig. 3B*).

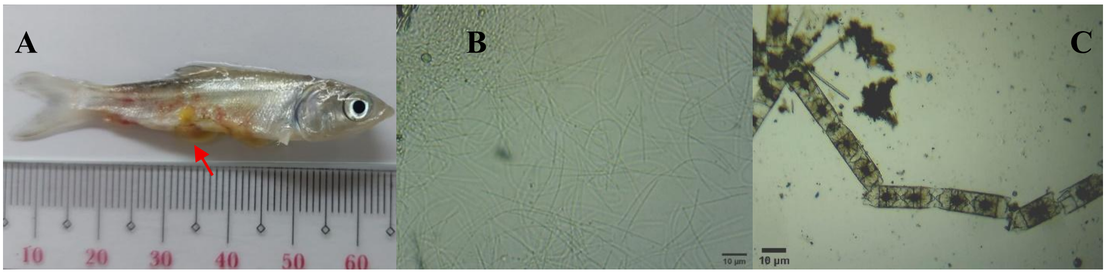

Figure 3. *(A) Chanos chanos sample from Tank 1 with multiple red spots on its body with microbial colony (yellowish) as pointed by the red arrow, (B) photomicrograph of freshly-mounted mucoid sample containing the suspected pathogen, and (C) Biddulphia sp. obtained from the S. siliquosum thalli. (Source: authors)*

On the other hand, the survival rates of the extractive species varied among the species used (*Table 1*). *H. scabra* registered 100% survival rate. Its mean body length and weight increased from an initial mean length of 7.75 cm to 12.75 cm (9.09%) and from the initial mean weight of 215.6 g to 257.1 g (16%). *Perna viridis* recorded a 69.64% survival rate with a daily growth rate (DGR) of -0.98% d^{-1} indicating loss in weight. While its mean initial shell length remained at 5.66 cm, shell width increased from 2.58 cm to 2.62 cm. Although there was an observed increase in shell width of 1.62%, the mean initial and final shell weights (in grams) decreased from 18.89 to 16.03 (15.1%). As for the *S. siliquosum*, the vegetative thalli fragments were replaced with new ones every three weeks (three sets of replacements): 399, 500, and 399 g. The adjustment of initial biomass for the second set was tried to observe whether there will be changes to the concentrations of the inorganic nutrients tested from the water samples. Thalli of *S. siliquosum* showed signs of deterioration and disintegration within the first three weeks of study period. Overgrowth of epiphytic diatom, *Bidulpphia* sp. (*Fig. 3C*) collected from the seaweeds' thalli was also observed. The total initial algal fresh weight used in the set-up was 1 298 g and the total final harvested fresh weights from the three sets were 458.5 g or 35.32% difference as compared to the initial fresh weight (*Table 1*) showing a negative net growth of -1.54%. The final computed yield is -276.1 mg dw m^{-1} d^{-1} indicating a net biomass loss over the study period.

Biofiltration potential of Sargassum siliquosum

In this study, *S. siliquosum* showed biofiltering efficiency for inorganic nutrient (nitrate and phosphate) concentrations in the IMTA tanks when compared to the control tanks (*Figs. 5D*, *6D*, *7B-C*). Nitrate removal was relatively low, with an overall reduction of 15.65%, although the highest nitrate biofiltration occurred during the 2nd week of the experimental period. Conversely, the phosphate biofiltering efficiently was higher (79.61%), indicating a more effective removal by *S. siliquosum*.

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Chemical analyses further showed that there was a considerable increase of total tissue nitrogen and phosphorus of *S. siliquosum* thalli cultured in IMTA tanks at the start and end of the culture period (*Fig. 4*). Results showed that the total nitrogen and phosphorus in the macroalgal tissue (initial = 0.91 mg N / g ± 0.082 and final = 1.72 mg N /g ± 0.050; initial = 0.02 mg P / g ± 0.003 and final = 0.16 mg P / g ± 0.003) are statistically different (Total N is $p = 0.014$; Total P is $p = 0.001$). Computed from these values, the biofiltration rates were estimated at 183.3 mg N m^{-1} $month^{-1}$ and 31.7 mg P m^{-1} $month^{-1}$.

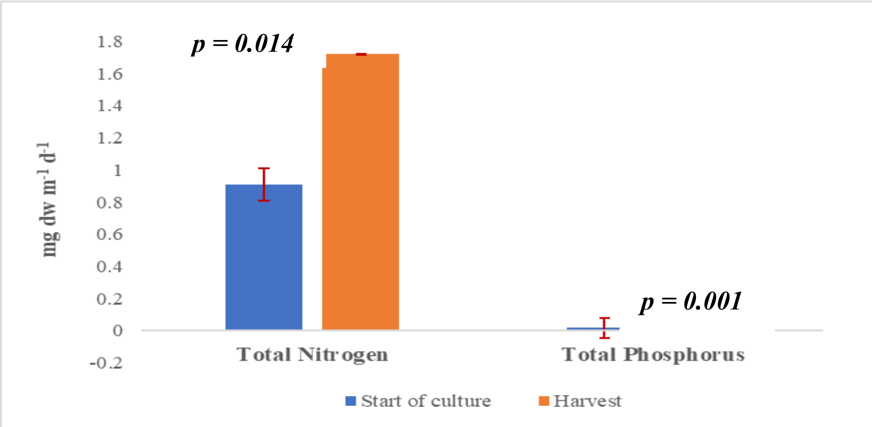

Figure 4. *Estimate of the total nitrogen and phosphorus content in S. siliquosum tissue. Data presented are means ± SD (n = 3) of dry weight samples at initial and final stocks*

Environmental conditions

Figure 5 shows the results of the weekly average value of the water quality parameters measured in both control and IMTA tanks over a 42-day period, indicating the parameters to be within the optimal range of milkfish aquaculture production including changes in temperature, salinity, dissolved oxygen, pH, and total dissolved solids (Astuti and Warsa, 2020). The values of temperature, DO, salinity, and TDS were not significantly different ($p > .05$) between control and treatment (IMTA) tanks and all were within the Water Quality Standards of DENR (DAO 2016-08) for Marine Waters Class SC category. As for the pH levels, while the values between control and IMTA tanks differed significantly ($p = 0.028$), both are within the pH range of DENR DAO 2016-08 water quality standards for marine waters under the Class SC category.

Inorganic nutrients in each tank, monitored weekly from day 0 to day 42, showed ammonia and phosphate concentrations to be significantly different between control and experimental set up ($p < .05$) (*Fig. 5D*), while that of nitrate concentration, although higher in IMTA (mean 0.79 ± 0.004 mg/L) than that of the control (mean 0.72 ± 0.001 mg/L), their values were not statistically different ($p = 0.428$). The primary sources of ammonia were the uneaten feeds (*Santeh* feeds, with 39% crude protein and 7% crude fat according to its nutrient profile label) and fish feces. With reference to the DENR Water Quality Standards for Class SC category, the levels of ammonia in both control (1.56 mg/L) and IMTA (0.56 mg/L) were relatively high; nitrate concentrations, on the other hand, were comparatively low, while phosphate levels were high in the control, but low in the IMTA tanks (*Fig. 6A–C*).

Weekly monitoring of nutrients showed ammonia to be fluctuating but generally increasing for nitrate in both control and IMTA tanks and for phosphate in IMTA tank

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only (*Fig. 6A–C*). Concentrations of ammonia were highest in 4th (2.56 ± 0.006 mg/L) and 5th (1.59 ± 0.004 mg/L) weeks for control and IMTA tanks, respectively (*Fig. 6A*). Nitrate levels also increased but only up to the 2nd week in the control tank, after which it gradually went down towards the 4th week (1.04 ± 0.004 mg/L) (*Fig. 6B*) while it generally went up in the IMTA tanks until the 5th week (1.60 ± 0.003 mg/L) (*Fig. 6B*). Phosphate, on the hand, consistently increased up to 4th week (6.6 ± 0.014 mg/L) followed by a slight decline in the following week in the control tank, whereas it dramatically went up in the IMTA tank of up to 1.75 ± 0.0002 mg/L (*Fig. 6C*). Overall, nutrient levels for ammonia and phosphate were significantly high in the control tank than in the IMTA tanks ($p < 0.05$; *Fig. 5D*); however, within IMTA tanks 1, 2, and 3, nutrient concentration were not statistically different ($p < 0.05$; *Fig. 6D*). In *Figure 6D*, nutrient concentrations in IMTA tanks were presented separately together with the control to illustrate tank-specific differences resulting from the biological responses of different IMTA species placed in tanks.

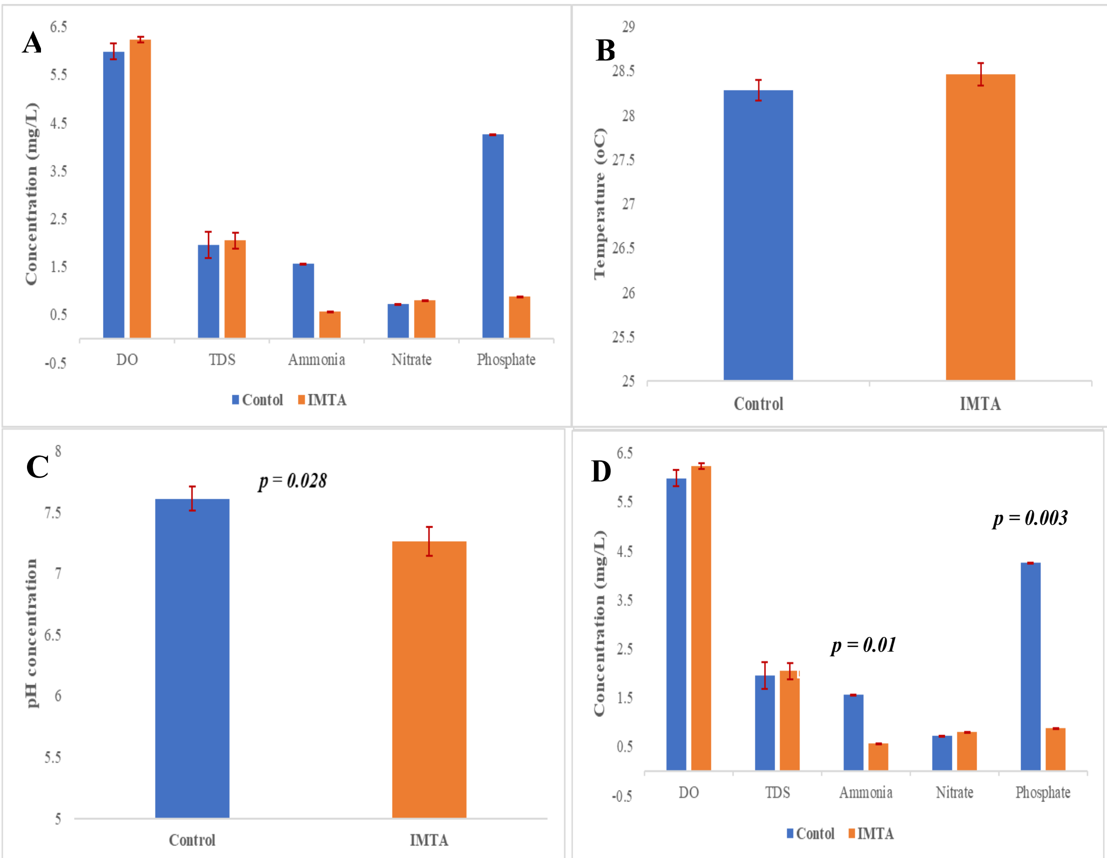

Figure 5. *Environmental conditions in water samples between control and IMTA tanks during the observation period, showing significant difference in the concentrations of pH (C), nutrients ammonia, and phosphate (D). Data presented are means ± SD (n = 6) at p < 0.05*

Discussion

Growth of fed and IMTA species

Decreased biomass (35.08%) of both fed and IMTA species after 42 days indicates poor growth and mortality of some species used in the system. Since *H. scabra* and *P.*

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viridis have high tolerance to environmental pollution (Wolkenhauer et al., 2010; Layugan et al., 2018), and *S. siliquosum* was recorded to have high reproductive and vegetative wet biomass (Hurtado and Ragaza, 1999), multiple environmental stressors that are either natural or anthropogenic in origin could have affect the health of these marine species such as increase of water temperature, salinity variation, and nutrient enrichment (Carrier-Belleau et al., 2021).

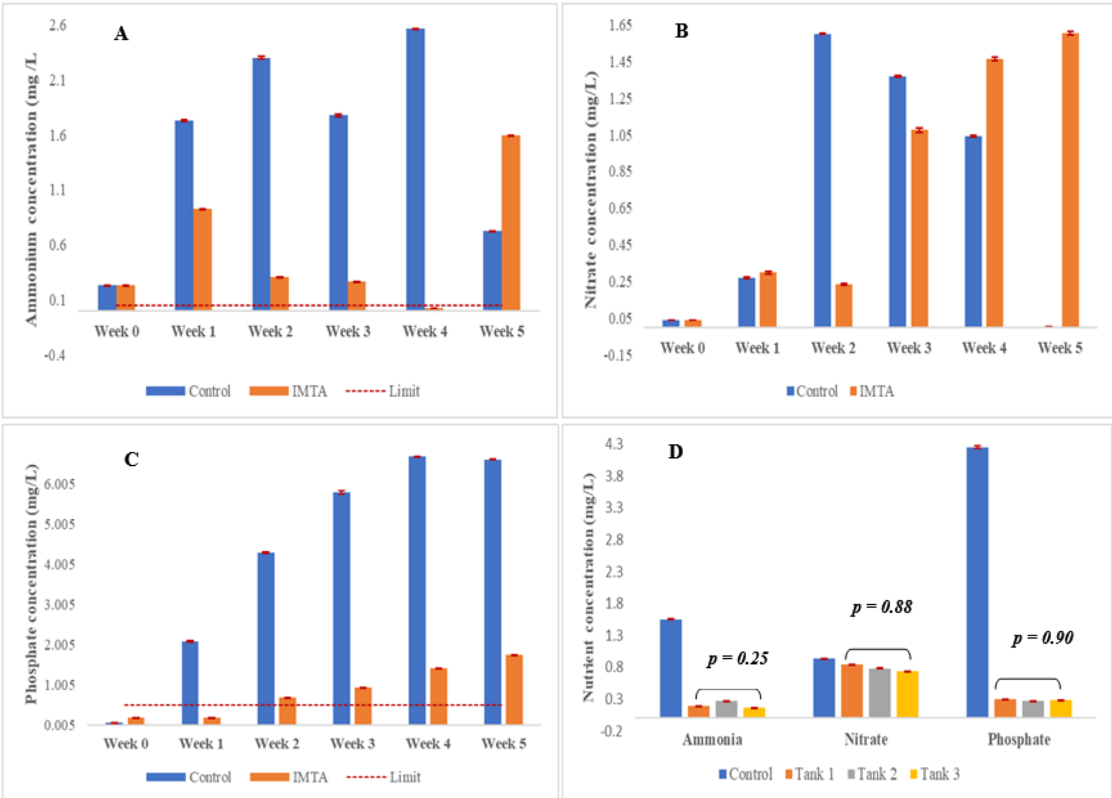

Figure 6. *Inorganic nutrients accumulated in the control and IMTA tanks based on weekly measurements of total ammonia, nitrate, and phosphate, showing a general increase but fluctuating values for ammonia in both control and IMTA tanks (A), an upward trend up to the second week for nitrate in the control tank but up to the 5th week for the IMTA tank (B), while consistently increasing for phosphate in the control up to the 4th week and to the 5th week in the IMTA tank (C); all three nutrients dominated in mean concentrations in the control tank but with significantly lower in values up to the end of the culture period in IMTA tanks 1, 2 and 3 (D). The broken red lines represent the acceptable limits of nutrients based on the DENR Water Quality Standard for aquaculture farms. Data presented are means ± SD (n = 3). Statistical groupings indicated by brackets are based only on data from IMTA tanks ($p < 0.05$)*

While high mortality of juvenile *C. chanos* in this study could be attributed to combined effect of high temperature, poor water quality demonstrated by elevated environmental ammonia, and the presumed ascendance of opportunistic bacterial pathogens (*Fig. 3B*) isolated from the surface skin mucus of the fed species (Hanke et al., 2019; Estante-Superio et al., 2021) present in the IMTA system. Results showed that growth rate per day in terms of weight (0.30%) of the milkfish was much lower than the average rate (2.4-2.7% d^{-1}) stipulated by FAO for milkfish fingerling (FAO, 2022).

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Daily temperature during the experiment is substantially varied reaching a maximum of 31.7°C at mid-day. In an indoor-based system, this was within the reported range (26°C to 33°C) of Hanke et al. (2019) where chronic thermal stress induced increase in ontogenetic and regenerated cortisol levels resulting to poor growth of juvenile milkfish.

On the other hand, the overall growth of *H. scabra* in weight and length indicates its potential to effectively assimilate organic benthic deposits, thus, reduce particulate organic matter settling in aquaculture farms. Robinson et al. (2019) highlighted high biomass densities of *H. scabra* reared under organic enriched land-based RAS. In this study, the remarkable 100% survival of *H. scabra* is in agreement with previous studies demonstrating holothurian's growth by assimilating organic wastes from sediments (Neofitou et al., 2019; Senff et al., 2022; Chatzivaasileiou et al., 2024) at low stocking density (Namukose et al., 2016). As observed throughout the experiment, sandfish moved on the glass surfaces, on surface and inside the sediments while feeding. This behavior caused bioturbation of the sediments and is believed to simultaneously reduce organic nutrients on sediments while oxygenating it (Purcell and Kirby, 2006).

Mussels cultured in open seawater grow at an average of > 1% d^{-1} (Srisunot and Babel, 2015; Tantanasarit et al., 2013) and in tanks at 0.5 d^{-1} and 0.8 d^{-1} (Rejeki et al., 2021) at lower and higher densities, respectively. For this experiment, mussel tanks were grown at very high densities (19, 500 g m^{-2}) achieved a negative daily growth rate of -0.74 g d^{-1}. Although there was an observed increase in shell width (1.62%), the mean final shell weight (in grams) decreased at 15.1%. The slight increase of shell width is very low compared to the shell width increase of similar species recorded before and after IMTA study of Melendres and Largo (2021). No increase of shell length and decrease of shell weights indicates the species' stress condition, including food source and space attachment (Srisunot and Babel, 2015; Rejeki et al., 2021).

Previous studies explored the feasibility of open sea suspension culture of *Sargassum* seedlings and vegetative cuttings using long-line. For tank experiments, research endeavors of *Sargassum* were focused to develop technologies for sexual (Largo et al., 2020; Ko et al., 2020) and asexual reproduction (Kavale et al., 2023; Guo et al., 2024). In this study, we found that vegetative thalli grown in IMTA tanks revealed a daily growth rate of -1.54% d^{-1}, which is similar to what was reported by Adharini et al. (2021) grown in aquarium; however, lower compared to open sea culture using raft method (1.12 ± 0.40% d^{-1}), but higher SGR in fixed-off bottom method (-2.03 ± 0.23% d^{-1}) (Yangson et al., 2022).

Thalli degradation of *Sargassum* was observed after three weeks which could be attributed to light limitation and nutrient competition. Mean light intensity that was made available to mature plants in the treatment tanks was lower to the light requirement for benthic *Sargassum* (150 mmol photons m^{-2} s^{-1}) (Redmond et al., 2014). Along with the noticeable overgrowth of diatom *Biddulphia* sp. (*Fig. 3C*), low light and microscopic epiphytes influence photosynthetic capabilities affecting growth and biomass of the host plant species (Chen et al., 2019; El-din et al., 2015).

For future cultivation of similar species in tanks, microbial factors that could have affected the physiological performances of species utilized in the system can be eliminated by applying appropriate disinfection treatments (e.g., ultraviolet irradiation) of seawater to control microbial sources (Douillet and Pickering, 1998).

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Also, to add data to the growth of *S. siliquosum* inside the IMTA recirculating system, it is recommended that weighing of the seaweeds will be done after seven days and vegetative thalli will be adjusted when needed to maintain the initial density (Mai et al., 2008; Correia et al., 2020).

Practical modifications to enhance species growth

The consistent weight loss observed across species in the current IMTA system suggests that further refinement of the design is necessary to promote species growth and enhance productivity. To address these limitations, practical modifications should be considered. For instance, replacing glass tanks with alternative materials or design modifications in milkfish tanks can help lower water temperatures, which are critical for optimal fish growth (Kodama et al., 2021; A'yun and Takarina, 2017). Additionally, sourcing water from the marine environment with lower nutrient levels can mitigate eutrophication risks and better mimic natural conditions, potentially reducing stress on cultured species (Duarte and Krause-Jensen, 2018). Incorporating mussel spat instead of juveniles can increase biofiltration capacity (Lauzon-Guay, 2005), while increasing light exposure for mature *Sargassum* can promote higher biomass productivity (Cheung-Wong et al., 2022), both of which are key for optimizing nutrient uptake. Furthermore, increasing water movement within the tanks can enhance nutrient circulation, especially benefiting filter feeder such as mussels, by improving mass transfer and waste removal (Maar et al., 2023).

Future research should focus on systematically identifying the key limiting factors within the system, such as water quality, nutrient availability, and species-specific environmental needs. Optimizing species selection, adjusting stocking densities, and refining feeding strategies are crucial steps toward improving growth performance (Ghosh et al., 2025). Addressing these challenges will be essential for advancing IMTA systems from conceptual models to practical solutions capable of supporting sustainable aquaculture development. Despite current challenges, the core principles of nutrient sharing and waste reduction inherent in IMTA was observed in the study, thus, continue to offer significant potential for environmentally sustainable aquaculture when integrated and managed effectively.

Accumulation of inorganic nutrients in macroalgal tissues

There is limited land-based study that evaluated nutrient removal efficiency of *Sargassum siliquosum* integrated in an IMTA system using macroalgal tissue as indicator. In this study, total nitrogen and phosphorus contents in *S. siliquosum* tissue increased by 89.01% and 700%, respectively, as response to the feed input in the fed species tank. The relative increases of values in both nutrients showed changed nitrogen: phosphorus ratio, from the initial 46:1 to 11:1, suggesting a lower nitrogen and higher phosphate uptake rates. Although these findings demonstrated that, indeed, mature vegetative thalli can significantly assimilate total nitrogen and phosphorus from enriched aquaculture water, the changed N:P ratio at the end of the experimental period may indicate decreased status of seaweed health. Similarly, other studies also reported positive absorption of inorganic nutrients, nitrate, phosphate, and even urea and ammonium in tissues of *Sargassum* species in integrated recirculated aquaculture systems (Edwards et al., 2024; Theobald et al., 2024; Adharini et al., 2021; Yasir and Adharini, 2021). As far as we are aware, past studies did not compute for bio-

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mitigation capacity of *Sargassum* in IMTA system. Analysis of our results estimated bio-mitigation capacities of 183.3 mg N m^{-1} $month^{-1}$ and 31.7 mg P m^{-1} $month^{-1}$. These findings suggested that *S. siliquosum* is a good IMTA candidate in tropical aquaculture farms.

Inorganic nutrient levels show potentials of extractive species as IMTA candidates

Enrichment of the control and IMTA tanks with residual commercial feed led to the significant differences of the pH, inorganic nutrients ammonia, and phosphate in the recirculating aquaculture system. Results of the study demonstrated that integration of species such as *Holothuria scabra, Perna viridis*, and *Sargassum siliquosum* in the system potentially accumulated and reduced the nutrients from the water. In this study, feed and fish feces were the primary sources of ammonia. Absorption of nutrients from feeds by cultured fishes alone, including *Chanos chanos*, was reportedly at limited capacity (20%–30% only) (Effendi et al., 2020). This resulted to statistically significant higher concentrations of the nutrients in control tanks as compared to that of the IMTA tanks. This further suggests that the elevated pH values were consequence of the increasing total ammonia in waters (Salama et al., 2013), not the increase of nitrate and phosphate in water. However, noticeably, the total phosphate in the control tank after 42 days (4.25 ± 0.01 mg P/L) was alarming. This values strongly indicate water pollution as total phosphates in water bodies recommended for aquaculture should not exceed 0.05 mg/L (Boyd, 2015; FAO, 2024; Edwards et al., 2024).

Between IMTA tanks, the increased levels of ammonia (0.187 ± 0.005) where *C. chanos* is placed exceeded the prescribed maximum limit of ammonia in aquaculture waters in the Philippines and most part of the world (DENR, 2016; Edwards et al., 2024). This concentration can be attributed to the observed lost balance, tissue erosion, slow growth and eventually, death of the fed species. High environmental ammonia can disrupt the homeostatic mechanisms that fishes regulate. Aside from the overall effects of overabundant ammonia to *C. chanos* growth, reproduction and immune responses, other physical manifestations such as hemorrhage and darkened body color, damage to gill morphology, and loss of equilibrium (Yan et al., 2021; Zeitoun et al., 2016) were observed in this study.

In the present study, the total ammonia and nitrate concentrations were lowest in IMTA tanks 1 and 3, respectively, where extractive species *H. scabra* and *S. siliquosum* are placed, and total phosphate was lowest in tank 2 where *P. viridis* was integrated. The decreased level of ammonia in the surrounding water in IMTA tank 1 contradicted the findings of Robinson et al. (2019) where concentrations of ammonia continue to increase in the first two months of sandfish culture, but comparable with other studies (Senff et al., 2020; Thuy et al., 2024). Nitrate uptake in recirculating nitrogenous wastewater was also reported successful in other study that utilized *S. siliquosum* (Edwards et al., 2024). Separate studies utilizing holothurians and green mussels mentioned significant increase of nitrates in experimental tanks (Robinson et al., 2019; Setyarini and Adharini, 2022). The latter could be a result of phosphate excretion as metabolic product of mussels (Setyarini and Adharini, 2022). Phosphate concentration, which was lowest in IMTA tank 2, agrees to the reported phosphorus absorption efficiency of *P. viridis* in aquatic systems (Srisunont and Babel, 2015). The biofiltering efficiency of *S. siliquosum* for inorganic nutrients, nitrate and phosphate, is indicative for its potential effectivity in reducing both nutrients within the system. While this study observed a relatively low nitrate biofiltration (15.65%) by *Sargassum* grown at higher

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densities in land-based RAS, other studies has reported higher efficiencies ranging from 50-80% when population of similar genus were cultured in open marine environments (Abdulwahid et al., 2023; Yasir and Adharini, 2021). This suggests that environmental variables within the set-up may inhibit optimal nitrate uptake. Factors such as low light intensities may limit photosynthetic activity for mature *Sargassum* thalli (Yasir and Adharini, 2021); elevated pH levels above 6.0 in RAS water potentially decreasing removal of nitrate ions adsorption capacity of the thalli (Meirinawati and Wahyudi, 2023); and even water motion, which can interfere water uptake (Edwards et al., 2024), all likely contribute to *S. siliquosum* nitrate biofiltration efficiency. On the other hand, the biofiltering efficiency for phosphate in this study was higher compared to that of nitrate efficiency. This result supports the findings reported by Yasir and Adharini (2021), where genus *Sargassum* reducing rate for phosphate was 86%. Comparably, this study indicates higher phosphate biofiltration than other species of macroalgae (*Ulva pertusa, Saccharina japonica, Gracilariopsis chorda*) grown in similar recirculating system at different water levels that exhibit lower biofiltration efficiencies ranging from 22.0% to 65.2% (Kang et al., 2011). Though phosphorus pollution does not directly the fed fish species, it does play a major role to seaweed growth and effective removal of phosphate from eutrophicated water (Lopez-Miranda et al., 2025; Yasir and Adharini, 2021; Ohtake et al., 2020; Kang et al., 2011). This suggests that *S. siliquosum* is well-suited as a biofilter in IMTA system.

Finally, the differences of the inorganic nutrients in the study set-up promised efficacy of the chosen organisms to decontaminate coastal ecosystems in tropical regions. Further investigations to understand better the contribution of each species in the IMTA system can include determination of total suspended solids in filter-feeder's tank and sediment analyses of organic matter in deposit-feeder's tank.

Conclusion

As the health of the marine ecosystem continually faces multiple stressors, the exploration of IMTA system using locally available species known to bioremediate polluted marine waters is important. Findings revealed that integration of *C. chanos, H. scabra, P. viridis* and *S. siliquosum* is viable for a marine aquaculture set-up. Growth of *C. chanos* is influenced primarily by both the fingerling's physical condition at the time of transfer and water quality (i.e., temperature, nutrients) in the maintaining tanks rather than by the species present within the system. Co-culture of *C. chanos* and *H. scabra* is possible showing high economic potential and environmental benefits. The remaining samples of *P. viridis* proved its wide tolerance to changing environmental conditions. *S. siliquosum* also indicated biofiltration efficiency as shown in the reduced inorganic nitrate and phosphate levels in IMTA tanks. Assimilated nutrients in the macroalgal tissues at biomass after harvest added evidence to the bio-mitigation capacity of *S. siliquosum* as IMTA candidate.

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