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Effect of mixed leaf litter of four mangrove species on shrimp post larvae (*Penaeus monodon*, Fabricius, 1798) performance in tank and mesocosm conditions in Bangladesh

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ABSTRACT

Mangrove leaf litter is a potential source of nutrients for shrimp post larvae. To introduce mangrove trees in shrimp farms it is necessary to identify the combination of trees which is most beneficial for shrimp. The present study evaluated the effects of mixed leaf litter of four mangrove species (*Avicennia officinalis* (Ao), *Sonneratia apetala* (Sa), *S. caseolaris* (Sc) and *Heritiera fomes* (Hf) on shrimp post larvae performance and water and soil quality. Leaf litter with and without supplemental feed was applied to shrimp culture tanks according to a 4 × 2 factorial design and followed by testing a subset of treatments in mesocosm pond conditions. Shrimp post larvae of 15-days old (PL_{15}) with an average weight of 0.01 g were used for both experiments, each with a 4-week duration without water exchange. Under controlled conditions in the tanks, leaf litter and feed resulted in 22 to 32% higher weight gain of PL shrimp than combined weight gain realized when receiving only leaf litter or only feed, indicating synergism. Based on this, the pond experiment was designed with combined application of leaf litter and feed. The pond experiment resulted in higher shrimp weight gain than realized in the tanks. In tanks, the highest average individual weight gain of PL was observed for the leaf litter mixture SaAoHf (0.23 g) followed by SaScHf (0.21 g), ScAoHf (0.21 g) andSaScAoHf (0.20 g). Paralleling the results of the tank experiment, SaAoHf leaf litter also gave the highest average individual weight gain (1.2 g) of PL shrimpin the ponds but other leaf litter treatments followed by SaScAoHf (0.95 g), ScAoHf (0.84 g) and SaScHf (0.69 g) leaf litter. The different mixtures of mangrove leaf litter also resulted in significant differences in biological oxygen demand (BOD_5), phytoplankton and zooplankton concentrations in pond water and organic carbon in soil. Both phytoplankton ($P < 0.01$, Pearson correlation $r = 0.910$)and zooplankton ($P < 0.05$, $r = 0.535$) abundance was positively correlated to shrimp weight gain. The low feed conversion ratio (FCR) in the treatments combining leaf litter and supplemental feed as compared to treatments using only feed indicated extra food benefits for shrimp PL from decomposing leaf litter. Overall, mixed mangrove leaf litter had a positive effect on shrimp performance and this effect was highest for SaAoHf leaf litter.

1. Introduction

Aquaculture is the world's fastest-growing food sector with an annual growth rate of 5.3% during the past two decades (FAO, 2020). Crustacean aquaculture is dominated by shrimp species. They are typically farmed in coastal areas of tropical and subtropical countries (Viet Nguyen et al., 2020) and are an important source of foreign-exchange earnings for a number of developing countries in Asia and Latin America (He et al., 2021). Despite economic benefits, shrimp farming has been under intense criticism because of various negative

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impacts on ecosystems, biodiversity and society (Naylor et al., 2021; Ahmed et al., 2018; Troell et al., 2014; Bush et al., 2010). Many authors have argued against coastal aquaculture including shrimp farming in recent years by pointing out it's typically devastating effects on mangrove forests in a number of countries, including Bangladesh, Brazil, China, India, Indonesia, Malaysia, Mexico, Myanmar, Sri Lanka, the Philippines, Thailand, and Vietnam (UNEP, 2014; FAO, 2007; Hossain et al., 2001). In response, much effort has been devoted to developing mangrove friendly silvo-aquaculture systems in the above-mentioned countries (Bosma et al., 2016; Primavera, 2000).

Mangrove ecosystems are dynamic and highly productive and rich in floral and faunal diversity (Mahmood et al., 2021; Nagarajan et al., 2008). Mangroves provide feeding, resting and breeding grounds, as well as shelter, for many aquatic organisms and supply organic matter to coastal fish populations (Mahmood et al., 2021; Athithan and Ramadhas, 2000). In the mangrove ecosystem, the prime nutrients are provided by adjacent river and surface run-off from land. Additional nutrients are also brought in with tidal water from adjacent estuaries. The influx of nutrients stimulates both mangrove and algal growth. Mangrove trees take up nutrients, and release part of these nutrient through the roots and senescent leaves falling from the trees, contributing to internal nutrient cycling within the mangrove forest (Srisunont et al., 2017; Reef et al., 2010;). Mangrove leaf litter is one of the main components contributing to the nutrient flux through the water bodies in the mangrove area (Kamruzzaman et al., 2019). Mangrove roots and fallen leaf litter also provide substrate for biofilm development and release nutrients in the water column supporting fish production (Gatune et al., 2014; Gatune et al., 2012; Hutchison and Spalding, 2014; Verweij et al., 2008; Nordhaus et al., 2006).

Next to supporting production, multi-dimensional ecosystem services provided by mangrove include climate regulation, biodiversity conservation, coastal protection, timber production, tourism, fuel, medicine, etc. (UNEP, 2014; Nagarajan et al., 2008; FAO, 2007). Even though mangroves are known to provide a multitude of provisional, regulating, supporting and cultural services, it remains necessary to demonstrate convincing incentives to shrimp farmers before they will be motivated to actually incorporate mangroves into their shrimp culture system and transition to economically and ecologically more-resilient mangrove-based silvo-aquaculture or silvo-fisheries. The main idea behind the latter culture systems is to enhance aquatic production while also enhancing the broader potential benefits provided by mangroves.

Mangroves and aquaculture have been found to be compatible (Rejeki et al., 2019; Bosma et al., 2016; Hai and Yakupitiyage, 2005). However, not all mangrove tree species have positive impacts on aquaculture production under all conditions. Firstly, leaf litter in some mangrove species contains anti-nutrients such as tannins which can negatively impact shrimp performances (Rejeki et al., 2019; Hai and Yakupitiyage, 2005). On the other hand, many tree species have positive impacts on shrimp performance and aquaculture-based livelihoods (Rahman et al., 2020). Alam et al. (2021a, 2021b) identified the nutrients and anti-nutrients in four mangrove species and tested their effect on shrimp post larvae performance. Nutritional and anti-nutritional content of leaf litter differed markedly between mangrove species. However, these experiments were done in tanks, where the influx from different mangrove species was controlled. To mimic the environmental conditions in a mangrove ecosystem, where numerous mangrove species co-exist, all shedding leaves, we here conducted experiments to examine the effect of mixtures of leaf litter from different mangrove species on shrimp performance in outdoor ponds. The two main research questions asked were: (i) what happens to shrimp production if mixtures of mangrove leaf litter from different tree species are supplied? and (ii) can the results obtained from indoor tank experiments be replicated in outdoor pond experiments for production of shrimp juvenile with mixed mangrove leaf litter?

To answer these questions, two experiments were conducted. An indoor tank experiment under controlled conditions using a factorial design and considering mangrove species and supplemental feed as the main factors. Using insights derived from these tank experiment, we subsequently designed experiments to test the effects of selected combinations of mangrove litter and feed under pond conditions.

2. Methodology

2.1. Experimental design

For the tank experiment, the rearing tanks were under a transparent plastic tarpaulin roof to control against large fluctuations in salinity due to heavy rain, while still maintaining the natural diurnal variation in light incidence. The experiment was set up according to a 4×2 factorial design with different combinations of mangrove leaf litter serving as the first factor and the presence or absence of formulated feed serving as the second factor (Table 1). In addition, there was one control treatment with only formulated feed. All treatments were executed in triplicate. The control treatment was used to explore for potential synergy between formulated feed and leaf litter addition.

Brackish water collected from a nearby canal was stocked in a pond and left to settle for one week. The top water layer from this pond was transferred to the experimental tanks, passing it through a 25 μm mesh screen to remove possibly present predators or their eggs or larvae. The shrimp rearing tanks were 1100-L fibre-reinforced polyethylene tanks containing 1000 L of brackish water with a salinity of 10 ppt and a water depth of 0.9 m. Each tank was aerated with a 2-cm diameter air stone connected to an air blower (RESUN, LP-100).Mangrove leaf litter was mixed at equal percentages for each species and directly added in the culture tanks at a concentration of 1 g/L (wet weight). This loading rate was standardized following Hai and Yakupitiyage (2005). On the same day, 100 specific pathogens free (SPF), 15-days old shrimp post larvae (PL_{15}) were stocked at a rate of 1 PL/10 L of water in each tank. The post larvae of an average weight of 0.01 g were obtained from Desh Bangla Shrimp Hatchery, Batiaghata, Khulna. The experiment assessing growth and survival was conducted over a four-week period in dry season (January–April 2020). The water was not exchanged during the experiment. The survival and growth indices were calculated after harvest at the end of the experiment.The tank experiments showed the great importance of adding feed. Therefore the pond experiments were adapted to all include feed and hence feed was no longer a factor for comparison (Table 1). In the pond experiment, the concentration of leaf litter was adjusted to apply leaf litter according to natural falling rates as observed in the Sundarbans mangrove forest. The falling rate and amount of leaf litter was calculated based on Kamruzzaman et al. (2019), taking into account the water surface area of our experimental ponds. The experiment was carried out in fifteen 21-m^2 ponds. Including

Table 1
Design and treatments applied in the tank and pond experiment.

Experiment in tanks					
Tanks with mangrove leaves					
Feeding type	Mangrove species				
	SaAoHf	SaScHf	ScAoHf	SaScAoHf	
Feed (F)	SaAoHf-F	SaScHf-F	ScAoHf-F	SaScAoHf-F	
No Feed (nF)	SaAoHf-nF	SaScHf-nF	ScAoHf-nF	SaScAoHf-nF	
Tanks without mangrove leaves					
Formulated feed only (F)					
Experiment in ponds					
Treatments	T1	T2	T3	T4	T5
Feeding composition	SaAoHf-F	SaScHf-F	ScAoHf-F	SaScAoHf-F	F

SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter;

the dike, the surface area of each pond was 30 m^2. All the ponds were fenced with bamboo sticks and 1-cm mesh, 1.5 m heigh, nets to avoid nuisance animals like cattle, goats, dogs and frogs. Brackish water from a nearby river was transferred to the ponds through a 25 μm mesh size screen to keep out predators and their eggs and larvae. The final depth of water in the pond was 1.2 m. Mangrove leaf litter (wet weight) was directly added in the pond every day to mimic natural mangrove litter fall. Equal proportions of mangrove leaf litter from each species was mixed and 71.5 g leaf litter (wet weight) was added to each pond directly during 28 consecutive days. On day 1, 1000 specific pathogens free (SPF), 0.01 g shrimp post larvae (PL_{15}) obtained from the Desh Bangla Shrimp Hatchery, were stocked in each pond. The experiment was also run for a four-week period in dry season (January–April 2020) without any exchange of water.

2.2. Selection of mangrove species, collection of leaf litter and sample preparation

Selection of mangrove species was done following Alam et al. (2021a, 2021b) and Rahman et al. (2020). Senescent leaves that fell down naturally, after changing color from greenish to yellowish, were collected from the selected mangrove species from the Sundarbans mangrove forest. The traps were 2 by 2 m, and installed beneath selected mangrove species during winter (November 2019-January 2020). At regular intervals, the fallen leaves were recovered from the traps, separated by species and prepared for use in the experiments. Our leaf litter was collected from traps at the identical place where Kamruzzaman et al. (2019) had their litter traps.

2.3. Soil sample analysis

Soil samples were collected from each newly excavated pond before filling and after harvesting. Soil pH was determined electrochemically with the glass electrode pH meter maintaining the ratio of soil to water at 1:2.5, following Jackson (1962). Soil redox potential (Eh) was determined following Rowell (1981). Total organic carbon (TOC) was determined following Walkley and Black's wet oxidation method as outlined by Jackson (1962). Total phosphorus (P) was analysed by the vanadomolybdophosphoric yellow color method as described by Jackson (1973). Total nitrogen (N) was determined by the Kjeldahl digestion+ Alkali distillation method as described by Michałowski et al. (2013).

2.4. Feeding the shrimp PL and calculation of FCR

Shrimp growth was monitored weekly in the control treatment that received feed at 5% body weight per day. Each week, five shrimp PLs were taken randomly from the control treatment tanks or mesocosms and weighed to determine the feeding rate for all fed treatments. The feed used was"Titas Tiger" from Bismillah Feed Mills Limited, Mollahat, Bagerhat, witha content of 12% moisture, 36% protein, 10% lipid, 7% fibre, 18% ash, 1.9% calcium and 1.7% phosphorus. This was fed to the shrimp once daily. After harvest, FCR was calculated as the total feed given divided by total shrimp biomass gain.

2.5. Water quality monitoring

Temperature, salinity, pH, and dissolved oxygen (DO) in each tank and pond were measured daily, shortly after sunrise using, respectively, a Hanna digital thermometer, an Atago (Japan) hand refractometer, a pH (Eutech, Singapore) meter, and a Lutron (Taiwan) DO meter. Total Ammonia Nitrogen (TAN) and Nitrite-N (NO_2-N) were measured weekly by the colorimetric Nessler method, with color card and sliding comparator: HI 3826|TAN, HI 3873|Nitrite test; HANNA instruments.

Biochemical (biological) oxygen demand (BOD) was measured weekly (as BOD_5 – i.e. a 5-day incubation) at the Khulna University water quality laboratory following APHA (1998). Water samples were collected from the tanks and ponds at a depth of 10–30 cm below the surface. Two BOD bottles (300 ml) from each tank and pond were filled carefully with sample water without allowing air bubbles to form. In one bottle, DO was fixed following the Winkler method to measure initial DO while another bottle was left to incubate for 5 days.

Chemical oxygen demand (COD) was measured bi-weekly. Samples were collected from the middle of the tank or pond at a depth of 10–30 cm below the surface and transported to the laboratory for analysis. The analysis was done following the open reflux (OR) method outlined in APHA (1998).

2.6. Sampling and analysis of plankton

Phytoplankton and zooplankton samples were collected on day 1 and 28. Samples (15 L per sample) were collected between 9.00 and 11.00 h from three points in each tank or pond and passed through a 45 μm mesh plankton net and combined. The concentrated samples were preserved in plastic bottles with 1 ml of Lugol's solution. The abundance estimations of plankton (individual L^{-1}) were done using a one milliliter Sedgewick-Rafter (S-R) counting chamber. One ml of sample was put in the S-R cell and left undisturbed for 15 min to allow the plankton to settle. The plankton in 10 randomly selected cells were then counted using a compound microscope (Lx 400; magnification-4×-100×, USA) and identified (where possible to genus level) using 5.1 M C-Mount CMOS Camera- Aptina MT9P001 CMOS (Color). Plankton was identified using determination tables by Prescott (1962), Edmondson (1982), Bellinger (1992) and Tomas (1997). Plankton abundance was calculated using the following formula:

$$N = (P \times C \times 100)/V$$

Where, N = the number of plankton cells or units per liter of original water, P = the number of plankton counted in 10 fields, C = the volume of final concentrate of the sample (ml), V = the volume of the water sample in liters.

2.7. Assessment of shrimp larval performances

Shrimp growth and survival indices were calculated at the end of the four-week period using the formulas described by Busacker et al. (1990). After harvesting, the shrimp PLs were placed in tissue paper to remove excess water for accurate wet-weight determination. Weight gain was calculated by deduction of initial weight from the final weight. Weight gain per day was calculated from final weight gain divided by experiment duration (days). The formulas for calculation of survival rate (SR) and specific growth rate (SGR) were as follows:

$$SR\ (\%) = \frac{Nf}{Ni} \times 100.$$

$$SGR\ (\%BW/day) = \left(ln\left(BW_f\right) - ln(BW_i)\right)/D \times 100$$

In these, SR is the survival rate; N_f is the number of shrimp collected at final sampling time; N_i is the number of PLs stocked; SGR is specific growth rate (% BW day^{-1}); BW_f is the final body weight (g); BWi is the initial body weight (g); and D is the duration of the experiment (days).

2.8. Calculation of synergy between feed and leaf litter

The calculation of individual and synergistic contributions of leaf litter and feed was based on total weight gain in shrimp juveniles, following Alam et al. (2021b).

2.9. Statistical analysis

The data were analysed using the IBM SPSS statistical software package version 26. One-way ANOVA was conducted to compare the synergistic effects of feed and mangrove leaf litter between the four combinations of leaf litter used as well as to compare the performances among the different treatments under pond conditions. A factorial analysis was carried out for the experiment under tank conditions, feed presence or absence as one factor and mangrove leaf litter species and the sampling date as the second, a repeated measure factor, by means of a general linear model (GLM). A post-hoc Tukey HSD test was used to examine for pair-wise differences ($P < 0.05$). Correlations among the different variables were assessed using the Pearson's correlation coefficient while linear regression between selected variables allowed us to examine their effect.

3. Results

3.1. Tank experiments

The tank experiments showed the effects of combined application of leaf litter and supplemental feed on shrimp performances. In terms of survival, addition of mangrove litter showed a significant effect ($P < 0.01$) but feed did not ($P > 0.05$). There was also no interaction effect of mangrove species x feed ($P > 0.05$) on survival. Shrimp survival over the four weeks experimental period ranged from 80 to 89% while the highest survival rate was observed for SaAoHf and the lowest for SaScAoHf (Table 2). The average survival rate without application of supplemental feed was 84% (Table 2). In the treatment with feed only, the survival rate was 88%. In contrast to the results for survival, for individual weight gain there was a significant effect of both mangrove species ($P < 0.001$) and feed ($P < 0.01$) but there was no interaction effect ($P > 0.05$) (Table 2). The same was observed for SGR (Table 2). The highest average individual body weight gain was recorded in treatment SaAoHf (0.23 g) and the lowest in treatment SaScAoHf (0.20 g) (Table 2). The average individual weight gain in fed treatments was 0.33 g and in non-fed treatments was 0.09 g. The average individual weight gain in the treatment with feed only was 0.15 g. When looking at FCR, SaAoHf-F showed the best performances of all treatments (Fig. 1). The highest value of FCR (0.48) was recorded for the treatment with formulated feed only, whereas the lowest (0.19) was recorded for treatment SaAoHf-F.

The total weight gain based on feed only was 12.8 g and the total weight gain based on leaf litter ranged from 6.7 to 9.4 g ($P < 0.01$; Table 3). The contribution of leaf litter to total weight gain ranged between 27 and 29%. The contribution of feed ranged between 39 and 51%. Combined, leaf litter and feed resulted in 22 to 32% higher weight gain than based on the combined contribution of leaf litter alone and feed alone. Though there were no significant differences ($P > 0.05$) between mangrove species in their contribution (%) to shrimp juvenile weight gain (Table 3), the different combinations of mixed leaf litter did give significant difference. SaAoHf contributed most to total weight gain, ScAoHf the least, with SaScAoHf and SaScHf at intermediate levels (Table 3).

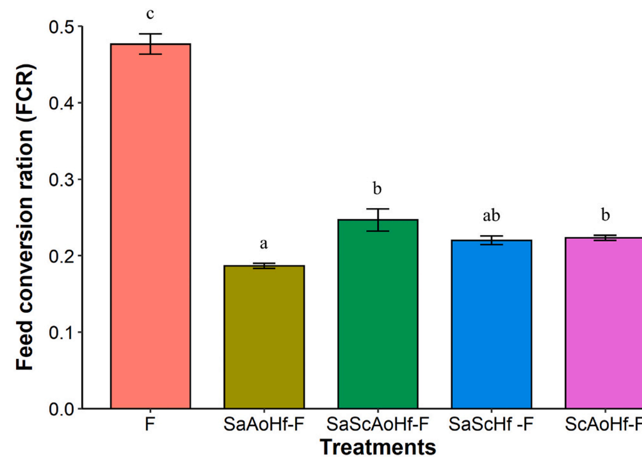

Fig. 1. Feed conversion ratio (FCR) in fed treatments in tank experiment. Values are means (± SD) of three replicate. The bars in graph sharing the same superscript letter or absence of superscripts are not significantly different ($P > 0.05$). SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; F = Only feed.

In the case of water quality parameters, significant principal effects were observed for pH, BOD_5, NO_2-N and phytoplankton but not for DO, COD, TAN and zooplankton concentration (Table 4). A significant interaction ($P < 0.05$) between mixed mangrove species and feed was found for pH ($P < 0.01$) and BOD_5 ($P < 0.001$), while all water quality parameters changed over time ($P < 0.05$). The average pH in different treatments ranged from 7.58–7.84 and differed significantly ($P < 0.01$) between different combinations of mixed leaf litter. The pH decreased over time in all treatments ($P < 0.01$) (Table 4) and was affected ($P < 0.01$) by both mangrove species and feeding.

For BOD_5, there were effects of mangrove species combination ($P < 0.001$) and feed ($P < 0.05$) as well as their interaction ($P < 0.001$). Among mangrove species litter combinations, the highest BOD_5 (2.55 mg/L) was observed for SaAoHf and the lowest (2.34 mg/L) for SaScAoHf (Table 4). Overall, the BOD_5 increased with time ($P < 0.001$), and different combination of mangrove species affected the BOD_5 differently with the increase of experimental period (MS x T, $P < 0.001$), while this was not the case with feeding (F x T, $P > 0.05$).

Feeding did influence the TAN concentration ($P < 0.01$), whereas mangrove species combination did not ($P > 0.05$). The TAN concentration increased over time ($P < 0.001$), and the increase was greater with feed than without feed (FxT, $P < 0.05$) (Table 4).

NO_2-N concentrations ($P < 0.05$) increased faster with feeding than without feeding (FxT, $P < 0.05$). In our experiments the concentrations never rose above 1 mg/L, thus never reaching toxic levels. Among the mangrove leaf litter combinations, SaScAoHf was higher in NO_2-N

Table 2
ANOVA table (repeated measure) for shrimp performances observed in shrimp nursery tanks during a 4-week incubation period, with different combinations of feed and leaf litter mangrove species.

Parameter	Leaf litter mangrove species (MS)				Feed (F)		*P*-values		
	SaAoHf	SaScHf	ScAoHf	SaScAoHf	Yes	No	MS	F	MS x F
Survival rate (%)	89.2 ± 2.14[b]	84.7 ± 2.94[ab]	82.3 ± 2.94[a]	80.2 ± 4.75[a]	84.6 ± 5.40	83.6 ± 3.85	**	ns	ns
Individual weight gain (g)	0.23 ± 0.14[b]	0.21 ± 0.13[a]	0.21 ± 0.13[a]	0.20 ± 0.13[a]	0.33 ± 0.02[b]	0.09 ± 0.01[a]	**	*	ns
Specific growth rate (% bw /d)	13.2 ± 2.31[b]	12.8 ± 2.49[a]	12.7 ± 2.53[a]	12.5 ± 2.51[a]	15.2 ± 0.21[b]	10.7 ± 0.40[a]	**	*	ns

Presented values are the mean ± SD. Means in each rows sharing the same superscript letter or absence of superscripts are not significantly different for main effect mangrove species (MS) and feed (F) according to Tukey HSD test ($P > 0.05$). P value is expressed as a symbol ($P < 0.001$: **; $P < 0.01$: *; ns: not significant, $P > 0.05$). SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter

Table 3
Contribution of mixed leaf litter and feed in weight gain in culture tanks during nursery from PL_{15} to juvenile shrimp for 04-week.

Considered factors	Mangrove species				P-value
	SaAoHf	SaScHf	ScAoHf	SaScAoHf	
Total weight gain with leaf and feed (g)	32.7 ± 0.10^{b}	28.2 ± 2.82^{a}	27.5 ± 0.44^{b}	25.1 ± 2.86^{a}	**
Total weight gain with leaf litter only (g)	9.4 ± 0.39^{b}	7.7 ± 0.38^{a}	7.2 ± 0.51^{a}	6.7 ± 0.47^{a}	**
Total weight gain with feed only	12.8 ± 0.71				n.a.
Contribution of leaf litter (%) to weight gain	28.9 ± 1.15	27.3 ± 2.43	26.0 ± 1.78	26.8 ± 2.20	ns
Contribution of feed (%) to weight gain	39.0 ± 2.08^{a}	45.3 ± 1.37^{ab}	46.4 ± 3.30^{ab}	51.2 ± 6.43^{b}	*
Synergistic effect (%)	32.1 ± 2.25	27.4 ± 3.80	27.6 ± 3.14	22.0 ± 7.68	ns

Presented values are the mean ± SD. Means in each rows sharing the same superscript letter or absence of superscripts are not significantly different according to Tukey HSD test ($P > 0.05$). P value is expressed as a symbol ($P < 0.01$: **; $P < 0.05$: *; ns: not significant, $P > 0.05$). SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter.

concentrations, followed by SaAoHf, SaScHf and ScAoHf ($P > 0.05$), in that order (Table 4).

Both the factors mangrove leaf litter and feed affected the phytoplankton concentration ($P < 0.001$) but there was no significant interaction between the two ($P > 0.05$). The highest phytoplankton concentrations were observed with SaAoHf leaf litter (27 inds/ml) and the lowest with SaScAoHf leaf litter (20 inds/ml). Phytoplankton concentrations increased over time, with the combination of leaf litter and feeding causing the fastest increase in phytoplankton concentration by the end of the experiment (MS x T and F x T; $P < 0.001$). The three most abundant phytoplankton species identified were *Cladophora nitellopsis, Closterium tumidium and Pediastrum tetras.* For zooplankton, concentration was not significantly different between main effects ($P > 0.05$) but the concentration increased over time (T; $P < 0.001$). The variations in zooplankton were less and the most abundant species identified was *Acartia tonsa.*

3.2. Pond experiments

The shrimp survival in ponds with different treatments ranged between 63% and 76% across the 4-week experimental period (Table 5) and differed significantly ($P < 0.05$) among the treatments. In contrast, the shrimp survival (%) in the ponds applied with mixture of leaf litter from different mangrove species and supplemental feed was higher than in the ponds applied with supplemental feed only. There were also significant differences ($P < 0.05$) in survival of shrimp juvenile between ponds being fed different combination of mixed leaf litter. The highest survival rate (76%) was observed in the ponds with mixtures of all four (*S. apetala, S. casiolaris, A. officinalis* and *H. fomes*) types of leaf litter and the lowest survival rate (72%) was found in the ponds with the mixture of *S. apetala, A. officinalis* and *H. fomes* leaf litter (Table 5). Survival in the ponds with *S. apetala, S. casiolaris* and *H. fomes* leaf litter was better than the ponds with *S. casiolaris, A. officinalis* and *H. fomes* leaf litter.

The growth rates of shrimp PL differed significantly ($P < 0.05$) depending between treatments used in the ponds. The application of mangrove leaf litter combined with feed gave higher growth rates than the treatments with only feed (Table 5). The highest average individual growth (1.2 g) was observed in the ponds applied with *S. apetala, A. officinalis* and *H. fomes* leaf litter and the lowest (0.7 g) was observed in the ponds applied with *S. apetala, S. casiolaris and H. fomes* leaf litter. For the other two treatments, weight gain was intermediate and similar to each other (Table 5). The specific growth rate (SGR) of shrimp juvenile also differed significantly ($P < 0.05$) between treatments. The highest SGR was recorded for the juvenile in the ponds receiving *S. apetala, A. officinalis* and *H. fomes* leaf litter and the lowest SGR was recorded in the ponds receiving only supplemental feed (Table 5).

The ponds with leaf litter showed better FCR performances than the ponds applied with feed only (Table 5). The highest value of FCR (0.32) was found for the treatment with formulated feed only whereas the lowest (0.13) was found for treatment with *S. apetala, A. officinalis* and *H. fomes* leaf litter.

No differences ($P > 0.05$) in water quality parameters were found between the different treatments except for BOD_5, phytoplankton and

Table 4
ANOVA table (repeated measure) for water quality parameters observed in shrimp nursery tanks during a 4-week incubation period, with different combinations of feed and leaf litter mangrove species.

Parameter	Leaf litter mangrove species (MS)				Feed (F)		P-values						
	SaAoHf	SaScHf	AoScHf	SaAoScHf	Yes	No	MS	F	MS x F	Time (T)	MS x T	F x T	MS x F x T
pH	7.84 ± 0.11^{c}	7.68 ± 0.07^{ab}	7.70 ± 0.09^{b}	7.58 ± 0.04^{a}	7.63 ± 0.18^{a}	7.71 ± 0.09^{b}	**	**	**	**	ns	ns	*
DO(mg/L)	5.42 ± 0.06	5.41 ± 0.03	5.40 ± 0.03	5.42 ± 0.06	5.41 ± 0.04	5.41 ± 0.05	ns	ns	ns	**	ns	**	*
BOD_5 (mg/L)	2.55 ± 0.05^{c}	2.40 ± 0.08^{b}	2.37 ± 0.12^{ab}	2.34 ± 0.07^{a}	2.30 ± 0.28^{a}	2.41 ± 0.10^{b}	***	*	***	***	***	ns	*
COD (mg/L)	35.6 ± 5.02	37.2 ± 9.76	35.6 ± 8.07	31.7 ± 6.24	34.2 ± 9.55	33.3± 4.71	ns	ns	ns	***	ns	ns	ns
TAN (mg/L)	0.19 ± 0.22	0.21 ± 0.13	0.19 ± 0.25	0.17 ± 0.19	0.38 ± 0.13^{b}	0.03± 0.06^{a}	ns	**	ns	***	ns	**	ns
NO_2-N(mg/L)	0.31 ± 0.13^{b}	0.29 ± 0.19^{ab}	0.25 ± 0.14^{a}	0.33 ± 0.13^{c}	0.33 ± 0.15^{b}	0.20± 0.11^{a}	*	**	ns	***	ns	**	ns
Phytoplankton (inds/ml)	26.7 ± 12.2^{c}	21.3 ± 14.5^{ab}	23.8 ± 16.9^{bc}	20.0 ± 9.4^{a}	28.7 ± 12.6^{b}	12.1 ± 5.4^{a}	***	***	ns	***	***	***	ns
Zooplankton (inds/L)	2.55 ± 0.05	2.40 ± 0.08	2.37 ± 0.12	2.34 ± 0.07	2.30 ± 0.28	2.41 ± 0.10	ns	ns	ns	***	ns	ns	ns

Presented values are the mean ± SD.Means in each rows sharing the same superscript letter or absence of superscripts are not significantly different for main effect mangrove species (MS) and feed (F) according to Tukey HSD test ($P > 0.05$). P value is expressed as a symbol ($P < 0.001$: ***; $P < 0.01$: **; $P < 0.05$: *; ns: not significant, $P > 0.05$).). SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter.

Table 5
Shrimp performance observed in mesocosm ponds with different leaf litter mangrove species and supplemental feed.

Parameter	Treatments					P-value
	SaAoHf-F	SaScHf-F	ScAoHf-F	SaScAoHf-F	F	
Survival rate (%)	72.3 ± 0.86[b]	75.4 ± 1.28[c]	74.3 ± 1.76[bc]	76.5 ± 0.91[c]	63.4 ± 0.65[a]	***
Individual weight gain (g)	1.18 ± 0.07[c]	0.69 ± 0.21[ab]	0.84 ± 0.21[ab]	0.95 ± 0.10[bc]	0.57 ± 0.08[a]	**
Specific growth rate (% bw /d)	16.9 ± 0.36[c]	15.0 ± 0.96[ab]	15.7 ± 0.98[abc]	16.2 ± 0.36[bc]	14.5 ± 0.55[a]	*
Feed conversion ratio (FCR)	0.13 ± 0.00[a]	0.23 ± 0.06[bc]	0.19 ± 0.05[ab]	0.16 ± 0.01[ab]	0.31 ± 0.05[c]	**

Presented values are the mean ± SD. Means in each rows sharing the same superscript letter are not significantly different according to Tukey HSD test ($P > 0.05$). P value is expressed as a symbol ($P < 0.001$: ***; $P < 0.01$: **; $P < 0.05$: *). SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; F = only Feed.

zooplankton concentration (Table 6). TAN and NO_2-N concentration were below the detection level over the experimental period of four weeks. The highest BOD_5 (2.58 mg/L)was measured in the ponds applied with *S. casiolaris, A. officinalis* and *H. fomes* leaf litter and lowest (1.29 mg/L) in the ponds applied with supplemental feed only. Though there were significant BOD_5 differences ($P < 0.05$) between experimental ponds, there were no significant differences that could be ascribed to different combinations of leaf litter (Table 6). For phytoplankton concentrations significant differences ($P < 0.01$) were found between treatments. There were also significant differences between the ponds applied with different combination of leaf litter. While highest phytoplankton concentrations (135 inds/ml) were found in the ponds with *S. apetala, A. officinalis* and *H. fomes* leaf litter and the lowest (77 inds/ml) were found in the ponds with *S.apetala, S. casiolaris* and *H. fomes* leaf litter. The ponds with the combination of all four types of leaf litter had higher phytoplankton concentrations than those with *S. casiolaris, A. officinalis* and *H. fomes*. Zooplankton concentrations also showed significant differences ($P < 0.05$) between different pond treatments. Ponds with mangrove leaf litter had higher zooplankton concentrations than ponds with only supplemental feed. Between ponds mixed mangrove leaf litter, the highest zooplankton (48 inds/ml) concentrations were observed in ponds with *S. casiolaris, A. officinalis* and *H. fomes* while the lowest (37 inds/ml) were observed in the ponds with all four types of leaf litter. The ponds with *S. apetala, A. officinalis* and *H. fomes* leaf litter had higher zooplankton concentrations than the ponds with *S. apetala, S. casiolaris* and *H. fomes* leaf litter (Table 6).

3.3. Soil quality

The soil quality was quantified before the start of the pond experiment (Table 7) and after four weeks when the experiment was terminated (Table 8). Before start the experiment, the soil quality of the experimental ponds did not show any significant differences ($P > 0.05$) between ponds (Table 7). However, after the experiment, the soil quality was different ($P < 0.05$) between treatments for some of the parameters (such as Eh and OC content). The highest Eh levels were observed in the soil of the ponds with only supplemental feed (278 mV). Between leaf litter treatment, the highest Eh (274 mV) was observed in ponds with *S. casiolaris, A. officinalis* and *H. fomes* leaf litter and lowest (265 mV) in the ponds with *S. apetala, A. officinalis* and *H. fomes* leaf litter. For OC, there were also significant differences ($P < 0.05$) between treatment types. Between ponds with mangrove leaf litter, the highest OC was observed in the soil of ponds with *S. apetala, A. officinalis* and *H. fomes* leaf litter and the lowest in the soil of ponds with *S. apetala, S. casiolaris* and *H. fomes* leaf litter (Table 8).

Pearson correlation analysis among different parameters showed

Table 7
Soil quality before experiment.

Soil quality parameters	Treatments					P-Value
	SaAoHf-F	SaScHf-F	ScAoHf-F	SaScAoHf-F	F	
pH	7.26 ± 0.04	7.27 ± 0.03	7.28 ± 0.03	7.25 ± 0.03	7.27 ± 0.05	ns
Eh (mV)	284.3 ± 3.45	283.8 ± 3.38	286.0 ± 2.89	282.8 ± 4.18	285.2 ± 4.05	ns
Organic Carbon (%)	0.95 ± 0.00	0.95 ± 0.00	0.95 ± 0.01	0.95 ± 0.01	0.94 ± 0.00	ns
Nitrogen (%)	0.13 ± 0.02	0.14 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.15 ± 0.02	ns
Phosphorus (%)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	ns

Presented values are the mean ± SD. Means in each rows absence of superscripts are not significantly different according to Tukey HSD test ($P > 0.05$). ns:not significant, $P > 0.05$. SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter: F = only Feed.

Table 6
Average water quality parameter values observed in different shrimp PL nursery ponds.

Water quality parameters	Treatments					P-value
	SaAoHf-F	SaScHf-F	ScAoHf-F	SaScAoHf-F	F	
Temp (°C)	28.1 ± 0.03	28.2 ± 0.10	28.0 ± 0.57	28.4 ± 0.03	28.4 ± 0.01	ns
pH	7.63 ± 0.04	7.53 ± 0.06	7.60 ± 0.02	7.57 ± 0.05	7.67 ± 0.10	ns
DO (mg/L)	4.43 ± 0.10	4.45 ± 0.03	4.45 ± 0.06	4.54 ± 0.05	4.43 ± 0.06	ns
BOD_5 (mg/L)	2.55 ± 0.14[b]	2.52 ± 0.03[b]	2.58 ± 0.19[b]	2.53 ± 0.16[b]	1.29 ± 0.06[a]	**
COD (mg/L)	34.17 ± 3.82	23.33 ± 2.89	28.33 ± 2.89	23.33 ± 9.46	26.67 ± 2.89	ns
TAN (mg/L)	–	–	–	–	–	–
NO_2-N (mg/L)	–	–	–	–	–	–
Phytoplankton (inds/ml)	135.0 ± 10.0[d]	76.7 ± 12.6[ab]	96.7 ± 7.64[bc]	110.0 ± 5.0[c]	56.7 ± 7.64[a]	**
Zooplankton (inds/ml)	40.8 ± 6.29[ab]	38.3 ± 6.29[ab]	48.3 ± 3.82[b]	37.2 ± 4.51[ab]	28.3 ± 3.82[a]	*

Presented values are the mean ± SD. Means in each rows sharing the same superscript letter or absence of superscripts are not significantly different according to Tukey HSD test ($P > 0.05$). P value is expressed as a symbol ($P < 0.01$: **; $P < 0.05$: *; ns: not significant, $P > 0.05$; "-": below the detection limit). SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; F = Feed only.

Table 8
Soil quality after 4-weeks experimental periods.

Soil quality parameters	Treatments					P-Value
	SaAoHf-F	SaScHf-F	ScAoHf-F	SaScAoHf-F	F	
pH	7.43 ± 0.06	7.43 ± 0.08	7.43 ± 0.06	7.40 ± 0.04	7.33 ± 0.02	ns
Eh (mV)	264.5 ± 1.97[a]	273.1 ± 2.63[b]	273.5 ± 1.92[b]	267.1 ± 2.06[ab]	278.0 ± 3.22[c]	*
Organic Carbon (%)	1.09 ± 0.02[d]	1.01± 0.01[b]	1.04 ± 0.03[bc]	1.07 ± 0.01[cd]	0.95 ± 0.01[a]	*
Nitrogen (%)	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.02	0.15 ± 0.01	0.13 ± 0.00	ns
Phosphorus (%)	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.06 ± o.00	ns

Presented values are the mean ± SD. Means in each rows sharing the same superscript letter or absence of superscripts are not significantly different according to Tukey HSD test ($P > 0.05$). *P* value is expressed as a symbol ($P < 0.05$: *; ns: not significant, $P > 0.05$). SaAoHf = *Sonneratia apetala, Avicennia officinalis, Heritiera fomes* leaf litter; SaScHf = *Sonneratia apetala, S. caseolaris, Heritiera fomes* leaf litter; ScAoHf = *Sonneratia caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter; SaScAoHf = *Sonneratia apetala, S. caseolaris, Avicennia officinalis, Heritiera fomes* leaf litter: F = Feed.

that the majority of variables were correlated (Table 9). The regression also showed significant correlations ($P < 0.05$) between different pairs of variables. We found a positive correlation between soil organic carbon and phytoplankton concentrations, phytoplankton concentrations and shrimp weight gain as well as a negative correlation between feed conversion ratio and phytoplankton concentration (Fig. 2).

4. Discussion

4.1. Effect of leaf litter on shrimp performances

In all tank experiments using exclusively leaf litter as a potential food source, a high survival (80–88%) indicated that leaf litter directly or indirectly contributed to the nutrition for shrimp through production of natural food. Alam et al. (2021a, 2021b) found that without any application of supplemental feed, shrimp PL managed to survive well on food produced through decomposition of mangrove leaf litter. The average survival (%) (Table 2) identified in our experiment using mixed mangrove leaf litter did not differ substantially from the survival values (75–94%) observed by Alam et al. (2021b) where leaf litter of individual mangrove species were used. In our experiments, shrimp survival was found to be higher in ponds with mixed mangrove leaf litter plus feed than in ponds with only feed (Table 5). Such higher survival rate with leaf litter could be due to numerous reasons including, that litter might create shelter with which shrimp PLs were able to avoid predation and cannibalism (Hai and Yakupitiyage, 2005).

The natural food produced from the decomposition of leaf litter supported growth in shrimp PL as we observed 26 to 29% weight gain based on the contribution of leaf litter alone (Table 3). This might be due to the release of nutrients through decomposition of leaf litter and the development of a microbial biofilm of direct use as food for the shrimp and/or the growth of plankton in the water column. Zooplankton, phytoplankton and bacteria are natural foods for shrimp PL and juveniles (Porchas-Cornejo et al., 2012) that can contribute up to 50–70% of the nutritional needs of shrimp (Martinez-Cordova and Enriquez-Ocana, 2007; Enríquez, 2003; Tacon, 2002). Others such as Gatune et al. (2014, 2012) and Nga et al. (2006) also documented that the decomposition of mangrove leaf litter releases nutrients, supports natural food production and microbial biofilm development which serve as food for shrimp PL. In this work we again documented the synergistic effect of mixed leaf litter and supplemental feed in weight gain of shrimp juvenile, just as Alam et al. (2021b) did for single-species mangrove leaf litter.

The differences in shrimp performances among the different leaf litter treatments applied both in tank and pond experiment, as we found in this study, might be due to the differences in decomposition rate of leaf litter of different species. For instance, Alam et al. (2021a) determined that the contribution of single-species mangrove leaf litters to shrimp PL performances differed depending on the decomposition rate of leaf litter. In concordance with the higher decomposition rates documented for Sa and Ao by Alam et al. (2021a), in this study, we observed comparatively higher individual growth in the leaf litter treatments that included Ao and Sa (Table 2, Table 5). Alam et al. (2021a) further found that the higher the decomposition rate of the litter, the higher the growth of phytoplankton and the higher the growth of shrimp PL. In the experiments described here we also observed a positive correlation between phytoplankton concentration and shrimp growth (Table 9). The average effects of mixed-species leaf litter on shrimp weight gain in our tank experiments were comparatively higher than the average effects of single-species litter as found by Alam et al. (2021b). Alam et al. (2021a, 2021b) identified *H. fomes* asproviding the lowest benefit to weight gain of all four mangrove species tested. Our results with mixed-species litter that included this species, suggest that with mixed-species leaf-litter input into shrimp ponds, *H. fomes* can also be effectively used to support shrimp pond production. This is interesting, not only as this is the most common species in the Sundarbans mangrove area, but also because it is a highly preferred species for planting by farmers due to its high value for various construction purposes (Hossain, 2015; Islam and Wahab, 2005; Rahman et al., 2020). Incorporation of this species for use in the shrimp pond setting might help facilitate for farmers to transition to use of mangroves in shrimp pond settings (Bosma et al., 2016).

In our results we observed higher growth performances under pond

Table 9
Pearson's correlations among different important variables ($n = 15$).

		Weight gain	Survival	pH	BOD_5	COD	Phytoplankton	Zooplankton
Weight gain	Pearson Correlation	1						
	Sig. (2-tailed)							
Survival	Pearson Correlation	0.428	1					
	Sig. (2-tailed)	0.111						
pH	Pearson Correlation	−0.004	**−0.517**[*]	1				
	Sig. (2-tailed)	0.989	0.048					
BOD_5	Pearson Correlation	**0.538**[*]	**0.901**[**]	−0.457	1			
	Sig. (2-tailed)	0.038	0.000	0.087				
COD	Pearson Correlation	0.502	−0.180	0.000	0.100	1		
	Sig. (2-tailed)	0.057	0.522	1.000	0.722			
Phytoplankton	Pearson Correlation	**0.910**[**]	**0.526**[*]	−0.114	**0.684**[*]	0.450	1	
	Sig. (2-tailed)	0.000	0.044	0.685	0.005	0.092		
Zooplankton	Pearson Correlation	**0.535**[*]	**0.601**[*]	−0.346	**0.653**[*]	0.307	0.512	1
	Sig. (2-tailed)	0.040	0.018	0.207	0.008	0.266	0.051	

** Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed);

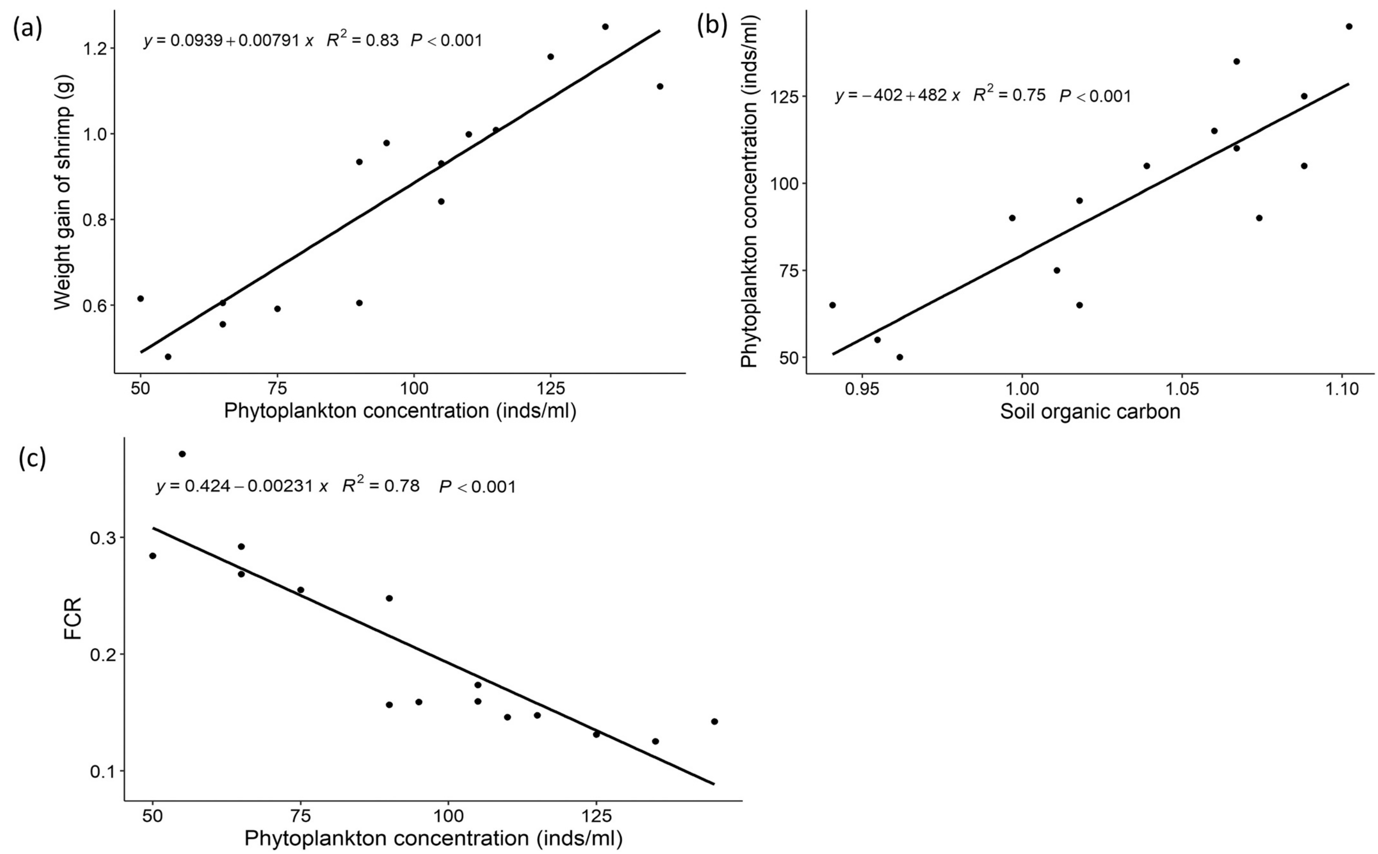

Fig. 2. (a-c): Linear regression of (a) Soil organic carbon and phytoplankton concentration, (b) Phytoplankton concentration and individual weight gain of shrimp, and (c) Phytoplankton concentration and Feed conversion ratio.

conditions than in culture tanks (Table 2 and Table 5). This might be due to a number of supporting factors. For instance, the pond setting facilitated a higher growth of both phytoplankton and zooplankton than the tank setting. The pond setting was also favorable to the development of soil organic matter by the addition of leaf litter. This was evidenced by the positive correlation regression between soil organic matter and phytoplankton growth as documented (Fig. 2b). The lower FCR in the treatment with the application of leaf litter as compared to treatments with only feed in both tank and pond experiments demonstrated the added-value of supplementation with natural food and/or shelter as a result of adding leaf litter. This finding correspond to those of Alam et al. (2021b) and Martinez-Cordova et al. (2011) who observed the addition of leaf litter lowers the FCR. The positive correlation between phytoplankton concentrations and shrimp juvenile weight gain (Fig. 2a) and the negative correlation between FCR and phytoplankton concentration (Fig. 2c) suggest that the growth of phytoplankton correlated to the weight gain of shrimp juvenile and the lowering of the FCR. Phytoplankton has been found not only to be nourishing but even vital to shrimp nutrition during the post larvae stages (Thong, 2017).

4.2. *Effect of leaf litter on water quality*

The average DO level in our tank experiment (Table 4) was significantly higher than the DO levels measured in the pond experiments (Table 6). While we usedan air stone to maintain DO level in experimental tanks we used no artificial aeration in the ponds. Even so, in both cases the DO level well-exceeded the minimum level needed for shrimp growth and survival (Allan and Maguire, 1991). The water temperature in both experiments was within the range conducive to shrimp performance (FAO, 1986).

While significant effects of mangrove species composition on pH was observed in shrimp rearing tanks (Table 4) both with and without supplemental feed, no effects of mangrove species composition on pH were found in the pond setting (Table 6). This is similar to the results by Marschner and Noble (2000), Deano (1985) and Alam et al. (2021b) who documented significant effects of mangrove species composition on pH but contrast with the results of Alam et al. (2021b) in which no similar effect was observed for feed. The effect of mangrove species on pH differed according to the species (Table 3). This might be due to the differences in decomposition rates of the different leaf litter as previously observed by Alam et al. (2021a). In both our tank and pond experiments, the pH observed was well within in the optimum range (7.5–9.0) for shrimp production (FAO, 1986).

The BOD_5 level in our both experiments was observed to be higher in the treatments that included mangrove leaf litter than those with only supplemental feed. This was likely due to the differential rate of leaf litter decomposition as suggested by Alam et al. (2021a). We also observed significant differences among the different mixture of leaf litter in tank water (Table 3) but not in pond water (Table 6). The higher BOD level in water might cause stress for shrimp PL due to the rapid oxygen depletion (Boyd, 2018; Banrie, 2012) but it did not happen in our tank experiment due to continuous aeration support. For both experiments, the BOD_5 was within the acceptance level (< 25 mg/L) recommended by Kasnir et al. (2014).

Nitrite (NO_2-N) and total ammonium nitrogen (TAN) were detected in our tank experiment but for the pond experiment both were below the detection level. In case of the pond experiment, either this went to the pond bottom or returned to the atmosphere by denitrification (Kabir et al., 2019). In the tank experiment, with the similar concentration of leaf litter but of a single species, Alam et al. (2021b) also observed an almost similar tendency of presence of TAN and NO_2-N in water column as we observed in our tank experiment. According to Hai and

Yakupitiyage (2005) and and Nga et al. (2006), the concentration of TAN and NO_2-N in water column might be increased with the increase of concentration of leaf litter. Both mangrove leaf litter and feed are sources of NO_2-N and TAN (Dutra and Ballester, 2017). Hari et al. (2004) also stated that the amount of fed nitrogen (N) that is not retained in animal weight gain, increases the TAN and NO_2-N concentrations in the water column. The higher presence of TAN and NO_2-N concentrations affect the shrimp performances but this might not happened in our experiments as both of those were within safe concentrations as suggested by Chen and Lei (1990) and Banrie (2012).

4.3. Effect of leaf litter on soil quality

Mangroves contribute essential nutrients to the soil through litter fall that is incorporated into sediments (Srisunont et al., 2017; Reef et al., 2010;) through decomposition. In our pond experiment, the addition of leaf litter also changed the pond soil quality as before addition of litter there were no difference ($P > 0.05$) in soil parameters whereas after of different litter combinations there were significant differences ($P < 0.05$) between treatments. Among the nutrients, the change in OC was significant as the leaf litter of mangrove species contain more organic carbon than nitrogen and phosphorus (Alam et al., 2021a). The contribution of organic carbon by the different mangrove species were significantly different. This might be due to the differences in decomposition rate of different leaf litters. Alam et al. (2021a) identified Sa leaf litter as having a higher decomposition rate than Ao, Sa and Hf, in that order. We also observed a higher contribution of OC in the treatments with leaf litter species which had higher decomposition rate as identified by Alam et al. (2021a).

5. Conclusions and recommendations

This study shows that shrimp post larvae performed better in terms of weight gain in ponds than in tanks. Even so, the pond experiments confirmed that the beneficial effects of mangrove litter as documented for tanks were quite applicable to the pond setting. We tested four combinations of mixed-species mangrove leaf litter as a potential supplement for shrimp culture and found the combination of *S. apetala*, *A. officinalis* and *H. fomes* to yield the best results. In addition, leaf litter not only served as a food source but also demonstrated significant synergy when used in combination with commercially formulated pelleted shrimp feed. Combining mangroves into the shrimp pond culture setting thus presents major potential benefits to shrimp production, particularly when used in combination with commercial feed. In addition, our results demonstrate that incorporating the commercially favored mangrove lumber species *H. fomes* into the species mix still results in good shrimp performance, even though *H. fomes* alone is not one of the best mangrove species for supporting shrimp culture (Alam et al., 2021a, 2021b). However, the species is highly favored by the farmers (Rahman et al., 2020) and inclusion of it in silvo-cultural approaches to making shrimp culture more profitable and more sustainable, can likely provide an additional incentive for farmers to transition to using mangroves to their greater economic benefit. Additional research questions needed to further investigate the economic potential of mangrove-based silvo-aquaculture are:- (i) what cover proportion of trees will provide the optimum leaf litter fall for shrimp culture?; (ii) what would be the water management strategy to a) ensure high utilization of released nutrition from leaf litter and b) limit biological oxygen demand for the jointly best culture conditions for shrimp; (iii) will the synergistic benefit continue through the life stages of shrimp or is most suited to nursery stage?; (iv) what is the length of time needed before newly planted mangrove can contribute to the pond environment via the provision of leaf litter?

CRediT authorship contribution statement

Md. Iftakharul Alam: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Moin Uddin Ahmed:** Funding acquisition, Project administration, Writing – review & editing, Resources. **Sanjida Yeasmin:** Data curation, Investigation, Methodology. **Adolphe O. Debrot:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Md. Nazmul Ahsan:** Funding acquisition, Project administration, Writing – review & editing. **M.C.J. Verdegem:** Conceptualization, Data curation, Formal analysis, Project administration, Supervision, Writing – review & editing, Validation.

Declaration of Competing Interest

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

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References

Ahmed, N., Thompson, S., Glaser, M., 2018. Integrated mangrove-shrimp cultivation: potential for blue carbon sequestration. Ambio 47, 441–452. https://doi.org/10.1007/s13280-017-0946-2.

Alam, M.I., Ahsan, M.N., Debrot, A.O., Verdegem, M.C.J., 2021a. Nutrients and anti-nutrients in leaf litter of four selected mangrove species from the Sundarbans, Bangladesh and their effect on shrimp (*Penaeus monodon*, Fabricius, 1798) post larvae. Aquaculture 542, 736865. https://doi.org/10.1016/j.aquaculture.2021.736865.

Alam, M.I., Debrot, A.O., Ahmed, M.U., Ahsan, M.N., Verdegem, M.C.J., 2021b. Synergistic effects of mangrove leaf litter and supplemental feed on water quality, growth and survival of shrimp (*Penaeus monodon*, Fabricius, 1798) post larvae. Aquaculture 545, 737237. https://doi.org/10.1016/j.aquaculture.2021.737237.

Allan, G.L., Maguire, G.B., 1991. Lethal levels of low dissolved oxygen and effects of short-term oxygen stress on subsequent growth of juvenile *Penaeus monodon*. Aquaculture 94, 27–37. https://doi.org/10.1016/0044-8486(91)90126-R.

APHA: American Public Health Association, 1998. Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF, Washington D. C.

Athithan, S., Ramadhas, V., 2000. Bioconversion efficiency and growth in the white shrimp, *Penaeus indicus* (Milne Edwards), fed with decomposed mangrove leaves. NAGA 23 (1), 17–18 (ISSN: 0116-290X).

Banrie, 2012. Monitoring Pond Water Quality to Improve Production. The Fish Site, 15 June, 2012.

Bellinger, E.G., 1992. A key to Common Algae: Freshwater, Estuarine and Some Coastal Species. Institution of water and environmental management London.

Bosma, R.H., Nguyen, H.T., Siahainenia, A.J., Tran, H.T.P., Tran, H.N., 2016. Shrimp-based livelihoods in mangrove silvo-aquaculture farming systems. Rev. In Aquac. 8, 43–60. https://doi.org/10.1111/raq.12072.

Boyd, C.E., 2018. Dissolved oxygen dynamics. Global Aquacult. Alliance 20 (August 2018).

Busacker, G.P., Adelman, T.R., Goolish, E.M., 1990. Methods for Fish Biology. American Fishery Society, Bethesda, MD, pp. 363–387.

Bush, S.R., van Zwieten, P.A.M., Visser, L., van Dijk, H., Bosma, R., de Boer, W.F., Verdegem, M., 2010. Scenarios for resilient shrimp aquaculture in tropical coastal areas. Ecol. Soc. 15 (2), 15. https://doi.org/10.5751/ES-03331-150215.

Chen, J.C., Lei, S.C., 1990. Toxicity of Ammonia and nitrite to *Penaeus monodon* juveniles. World Aquac. Soc. 21 (4), 300–306. https://doi.org/10.1111/j.1749-7345.1990.tb00543.x.

Deano, P.M., Robinson, 1985. The effect of decaying leaves on the pH and buffer capacity of waters. J. Env. Sci. Hea. Part A: Envl Sci and Eng 20, 903–911. https://doi.org/10.1080/10934528509375266.

Dutra, F.M., Ballester, E.L.C., 2017. Determining safe levels of ammonia and nitrite for shrimp culture. Global Aquacult. Alliance 16 (January 2017).

Edmondson, W., Kiefer, F., Fryer, G., 1982. 1978. Das Zooplankton der Binnengewässer. Teil 2. E. Schweizerbartsche Verlag, Stuttgart. 380 p. DM108. Lim and Oceano, 27, p. 196.
Enríquez, F., 2003. Variación del Bentos, con Enfasis en la Comunidad de Poliquetos en una Laguna de Descarga de una Granja Camaronícola. Universidad de Sonora, Hermosillo, Sonora, Tesis de Maestría, p. 98.
FAO, 1986. NACA Training Manual Series No. 2. Shrimp Culture: Pond Design, Operation And Management. Food and Agriculture Organization (FAO) of the United Nations.
FAO, 2007. The world's Mangrove 1980–2005. Food and Agriculture Organization of the United Nations, Rome.
FAO, 2020. The state of world fisheries and aquaculture 2020. In: Sustainability in Action. Fisheries and Aquaculture Technical paper (Rome).
Gatune, W.C., Vanreusel, A., Cnudde, C., Ruwa, R., Bossier, P., Troch, M.D., 2012. Decomposing mangrove litter supports a microbial biofilm with potential nutritive value to penaeid shrimp post larvae. J. Exp. Mar. Biol. Ecol. 426–427, 28–38. https://doi.org/10.1016/j.jembe.2012.05.015.
Gatune, W.C., Vanreusel, A., Ruwa, R., Bossier, P., Troch, M.D., 2014. Fatty acid profiling reveals a trophic link between mangrove leaf litter biofilms and the post-larvae of giant tiger shrimp *Penaeus monodon*. Aquac. Environ. Interac. 6, 1–10. https://doi.org/10.3354/aei00117.
Hai, T.N., Yakupitiyage, A., 2005. The effects of the decomposition of mangrove leaf litter on water quality, growth and survival of black tiger shrimp (*Penaeus monodon* Fabricius, 1798). Aquaculture 250, 700–712. https://doi.org/10.1016/j.aquaculture.2005.04.068.
Hari, B., Madhusoodana Kurup, B., Varghese, J.T., Schrama, J.W., Verdegem, M.C.J., 2004. Effects of carbohydrate addition on production in extensive shrimp culture systems. Aquaculture 241, 179–194. https://doi.org/10.1016/j.aquaculture.2004.07.002.
He, Z., Chen, X., Zhao, J., Hou, D., Fu, Z., Zhong, Y., Sun, C., 2021. Establishment of infection mode and *Penaeus monodon* hemocytes transcriptomics analyses under decapod iridescent virus 1 (DIV1) challenge. Aquaculture 542, 736816. https://doi.org/10.1016/j.aquaculture.2021.736816.
Hossain, M., 2015. Handbook of Selected Plant and Species of the Sundarbans and the Embankment Ecosystem. SDBC-Sundarbans, GIZ, Germany and Dhaka, Bangladesh.
Hossain, M.H., Lin, C.K., Hussain, M.Z., 2001. Goodbye Chakaria Sunderban: the old mangrove Forest. Wet. Sci. and Prac. 18 (3), 19–22. https://doi.org/10.1672/0732-9393(2001)018[0019:GCSTOM]2.0.CO;2.
Hutchison, J., Spalding, M., Zu Ermgassen, P., 2014. The Role of Mangroves in Fisheries Enhancement. The Nature Conservancy and Wetlands International, 54 pages.
Islam, M.S., Wahab, M.A., 2005. A review on the present status and management of mangrove wetland habitat resources in Bangladesh with emphasis on mangrove fisheries and aquaculture. Hydrobiologia 542, 165–190. https://doi.org/10.1007/s10750-004-0756-y.
Jackson, M.L., 1962. Soil Chemical Analysis. Prentice Hall Inc., Englewood cliffs, New Jersey, USA.
Jackson, M.L., 1973. Soil Chemical Analysis. Prentice-Hall of India Pvt. Ltd, New Delhi, India.
Kabir, K.A., Schrama, J.W., Verreth, J.A.J., Phillips, M.J., Verdegem, M.C.J., 2019. Effect of dietary protein to energy ratio on performance of nile tilapia and food web enhancement in semi-intensive pond aquaculture. Aquaculture 499, 235–242. https://doi.org/10.1016/j.aquaculture.2018.09.038.
Kamruzzaman, M., Basak, K., Paul, S.K., Ahmed, S., Osawa, A., 2019. Litterfall production, decomposition and nutrient accumulation in Sundarbans mangrove forests, Bangladesh. For. Sci. Tec. 15 (1) https://doi.org/10.1080/21580103.2018.1557566.
Kasnir, M., Harlina, H., Rosmlati, R., 2014. Water quality parameters analysis for the feasibility of shrimp culture in Takalar regency, Indonesia. J. Mod. App. Sci. 8 (6), 321–325. https://doi.org/10.5539/mas.v8n6p321.
Mahmood, H., Ahmed, M., Islam, T., Uddin, M.Z., Ahmed, Z.U., Saha, C., 2021. Paradigm shift in the management of the Sundarbans mangrove forest of Bangladesh: issues and challenges. Trees For. People 5 (100094). https://doi.org/10.1016/j.tfp.2021.100094.
Marschner, B., Noble, A.D., 2000. Chemical and biological process leading to the neutralisation of acidity in soil incubated with litter materials. Soil Biol. Biochem. 32, 805–813. https://doi.org/10.1016/S0038-0717(99)00209-6.
Martinez-Cordova, L.R., Enriquez-Ocana, L.F., 2007. Study of benthic fauna in the discharge slagoon of a shrimp farm with special emphasis on the polychaetes. J. Biol. Sci. 7, 12–17.
Martinez-Cordova, L.R., Campana-Torres, A., Martinez-Porchas, M., 2011. Effect of supplying four copepod densities (*Acartia* sp. and *Calanus pacificus*) on the productive shrimp consumption of natural and artificial *food* response of *Litopenaeus vannamei* pre grown intensively at microcosm level. Ciencias Marinas 37 (4A), 415–423 (in Spanish and English).
Michałowski, T., Asuero, A.G., Wybraniec, S., 2013. The titration in the Kjeldahl method of nitrogen determination: base or acid as titrant? J. Chem. Educ. 90 (2), 191–197. https://doi.org/10.1021/ed200863p.
Nagarajan, B., Pandiarajan, C., Krishnamoorthy, M., Sophia, P., 2008. Reproductive fitness and success in mangroves: implication on conservation. In: Sengupta, M., Dalwani, R. (Eds.), Proceedings of Taal 2007: the 12th World Lake Conference. International Lake Environment Committee Foundation, Jaipur (India), pp. 29–33.
Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Troell, M., 2021. A 20-year retrospective review of global aquaculture. Nature 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6.
Nga, B.T., Roijackers, R., Nghia, T.T., Ut, V.N., Sheffer, M., 2006. Effects of decomposing *Rhizophora apiculata* leaves on larvae of the shrimp *Penaeus monodon*. Aquac. Int. 14, 467–477. https://link.springer.com/article/10.1007%2Fs10499-006-9049-y.
Nordhaus, I., Wolff, M., Diele, K., 2006. Litter processing and population food intake of the mangrove crab *Ucides cordatus* in a high intertidal forest in northern Brazil, Estuarine, Coast. Shelf Sci. 67 (1–2), 239–250. https://doi.org/10.1016/j.ecss.2005.11.022.
Porchas-Cornejo, M.A., Martinez-Porchas, M., Martinez-Cordova, L.R., Ramos-Trujillo, L., Barraza-Guardado, R., 2012. Consumption of natural and artificial foods by shrimp (*Litopenaeus vannamei*) reared in ponds with and without enhancement of natural productivity. The Israeli J. Aquacult. - Bamidgeh, IJA. _64.2012.709 (7).
Prescott, G.W., 1962. Algae of the Western Great Lakes Area. Wm. C. Brown Co, Dubuque, lowa, 977 pp.
Primavera, J.H., 2000. Integrated mangrove-aquaculture system in Asia. Integrated coastal zone Management Autumn Edition 121–130.
Rahman, K.S., Islam, M.N., Ahmed, M.U., Bosma, R.H., Debort, A.O., Ahsan, M.N., 2020. Selection of mangrove species for shrimp based Silvo-aquaculture in the coastal areas of Bangladesh. J. Coast. Conserv. 24 (59) https://doi.org/10.1007/s11852-020-00770-8.
Reef, R., Feller, I.C., Lovelock, C.E., 2010. Nutrition of mangroves. Tree Physiol. 30 (9), 1148–1160. https://doi.org/10.1093/treephys/tpq048.
Rejeki, S., Middeljans, M., Widowati, L., Ariyati, R.W., Elfitasari, T., Bosma, R.H., 2019. The effects of decomposing mangrove leaf litter and its tannins on water quality and the growth and survival of tiger prawn (*Penaeus monodon*) post larvae. Biodiversitas 20, 2750–2757. https://doi.org/10.13057/biodiv/d200941.
Rowell, D.L., 1981. Oxidation and reduction. In: Greenland, D.J., Hayes, M.H.B. (Eds.), The Chemistry of Soil Processes. John Wiley & Sons, Chichester, pp. 401–461.
Srisunont, T., Srisunont, C., Jaiyen, T., Tenrung, M., Likitchaikul, M., 2017. Nutrient accumulation by litter fall in mangrove forest at Klong Khone, Thailand. Thammasat Int. J. Sci. Tech. 22, 9–18. https://doi.org/10.14456/tijsat.2017.2.
Tacon, A.G.J., 2002. Thematic Review of Feed and Feed Management Practices in Shrimp Aquaculture. A Report for FAO. World Bank, WWF, and NACA, Kanehoe, HI.
Thong, P.Y., 2017. Phytoplankton in aquaculture ponds: friend or foe? Global Aquacult. Alliance. April 24, 2017 (6 pages).
Tomas, C., 1997. Identifying Marine Phytoplankton, 1st edition.
Troell, M., Naylor, R.L., Metian, M., Beveridge, M., Tyedmers, P.H., Folke, C., de Zeeuw, A., 2014. Does aquaculture add resilience to the global food system? PNAS 111 (37), 13257–13263. https://doi.org/10.1073/pnas.1404067111.
UNEP, 2014. The Importance of Mangrove to People : A Call to Action. UNEP World Conservation Monitoring Centre, Cambridge.
Verweij, M.C., Nagelkerken, I., Hans, I., Ruseler, S.M., Mason, P.R.D., 2008. Seagrass nurseries contribute to coral reef fish populations. Limnol. Oceanogr. 53 (4), 1540–1547.
Viet Nguyen, T., Ryan, L.W., Nocillado, J., Le Groumellec, M., Elizur, A., Ventura, T., 2020. Transcriptomic changes across vitellogenesis in the black tiger prawn (*Penaeus monodon*), neuropeptides and G protein-coupled receptors repertoire curation. Gen. Comp. Endocrinol. 298, 113585 https://doi.org/10.1016/j.ygcen.2020.113585.