The contribution of mangrove leaf litter to juvenile shrimp (*Penaeus monodon*) production in mangrove-shrimp aquaculture systems

Md. Iftakharul Alam

Propositions

1. Selection of a beneficial mix of local mangrove species is mandatory before implementing silvo-aquaculture.
   (this thesis)

2. The combination of mangrove leaf litter and commercial shrimp feed is the best for nourishing juvenile shrimp.
   (this thesis)

3. Letting forest to grow old is a sign of wisdom.

4. Research in developing countries deals often with problems in developed countries, not with urgent problems at home.

5. The Sundarbans is a natural gift from Bangladesh to the world.

6. The pursuit of wealth destroys our planet.

Propositions belonging to the thesis, entitled

”The contribution of mangrove leaf litter to juvenile shrimp (*Penaeus monodon*) production in mangrove-shrimp aquaculture systems”.

Md. Iftakharul Alam
Wageningen, 13 June 2022

The contribution of mangrove leaf litter to juvenile shrimp (*Penaeus monodon*) production in mangrove-shrimp aquaculture systems

Md.Iftakharul Alam

Thesis committee

Promotor

Dr M.C.J. Verdegem

Associate Professor, Aquaculture and Fisheries Group

Wageningen University & Research

Co-promotor

Dr Adolphe (Dolfi) O. Debrot

Tropical Marine Ecologist, Wageningen Marine Research

Wageningen University & Research

Other members

Prof. Dr S.J. Oosting, Wageningen University & Research

Prof. Dr G. van Stappen, Ghent University, BE

Prof. Dr M.A. Wahab, WorldFish-CGIAR, BD

Dr A.A. van Dam, IHE Delft Institute for Water Education

This research was conducted under the auspices of the Graduate School Wageningen Institute of Animal Sciences (WIAS).

The contribution of mangrove leaf litter to juvenile shrimp (*Penaeus monodon*) production in mangrove-shrimp aquaculture systems

Md.Iftakharul Alam

Thesis

Submitted in fulfilment of the requirements for the degree of doctor

at Wageningen University

by the authority of the Rector Magnificus,

Prof. Dr A.P.J. Mol,

in the presence of the

Thesis Committee appointed by the Academic Board

to be defended in public

on Monday 13 June 2022

at 11 a.m. in the Omnia Auditorium.

Md. Iftakharul Alam

The contribution of mangrove leaf litter to juvenile shrimp (*Penaeus monodon*) production in mangrove-shrimp aquaculture systems, 168 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2022)

With references, with summary in English.

ISBN: 978-94-6447-134-2

DOI: https://doi.org/10.18174/566007

To my Parents (Md. Zulfiker Ali and Mrs. Angura Ali)

Table of contents

Chapter 1

General introduction

1.1. Mangroves in Bangladesh

Mangrove forests are complex and also highly productive ecosystems, harboring distinctive biophysical environments in intertidal coastal regions of the tropics and subtropics (Nagarajan et al., 2008). The total mangrove area in the world covers about 15 million hectares distributed across 100 countries (FAO, 2003). Bangladesh accommodates approximately 4%, or 6000 km^2, of the world mangrove forests and is placed at the sixth position in terms of mangrove area coverage (Hossain, 2015). Mangroves present in Bangladesh largely represent naturally developed mangroves (The Sundarbans, Chakaria Sundarbans and scattered mangroves) except for 170 km^2 of mangroves planted specifically for coastal protection (Hoque and Datta, 2005).

Aside from being a top-ranking mangrove country, Bangladesh, along with neighboring India, is home to the world's largest uninterrupted single tract mangrove forest, "The Sundarbans". In all, 62% of the Sundarbans forest pertains to Bangladesh, located between 21°38′10.18′′ and 22°29′51.65′′ north and 89°02′22.87′′ and 89°53′13.93′′ east (Aziz and Paul, 2015). The Sundarbans is a diversified and unique ecosystem comprising dynamic fauna and flora communities (Nagaranjan et al., 2008). Apart from mangrove species, the Sundarbans is home to an extensive diversity of plant and animal species (Table 1.1).

Table 1.1

Number of major species contributing to the flora and fauna in the Sundarbans, Bangladesh (Islam et., 2016; Aziz and Paul, 2015).

Flora/Fauna	Total number of species
Plant	61,189
Mangrove	182
Wildlife	1136
Terrestrial	289
Aquatic	678
Others	619

Among 111 plant families contributing to mangrove forest formation, eight are dominant dicotylodon woody trees, i.e., *Heritiera fomes, Excoecaria agalocha*, *Xylocarpus mekongensis*, *X. granatum*, *Bruguiera gymnorhiza*, *Sonneratia apetala*, *Avicennia officinalis* and *Ceriops decundra* while one species is the only mangrove tree from the monocotylodon Palmae (Arecaceae) family (Rahman et al., 2015; Chaffey et al., 1985).

1.2. Contribution of mangroves to fisheries

Mangroves are highly productive ecosystems, with mangrove trees and algae growing on tree roots and on the forest floor and phytoplankton in the water column contributing to the net primary production (NPP) (Verweij et al., 2008; Nordhaus et al., 2006). The total NPP along the oligohaline zone of the Sundarbans reserve forest (SRF) was estimated at 21.0 $t.ha^{-1}.yr^{-1}$ (Kamruzzaman et., 2017). The mangrove ecosystem also receives and traps nutrients form the land (river, run-off) and from the sea (estuary/tides/waves) which also contribute to its high biological productivity (Victor et al., 2004) and fisheries production (Reef et al., 2010; Kristensen et al., 2008; McKinnon et al., 2002). Mangrove leaf litter is one of the main contributors to biological productivity and fisheries production, especially via the detritus pathway (Hutchison et al., 2014; Ellis et al., 2006).

1.3. Destruction of mangroves

Although mangrove forests are among one of the most productive and biologically important ecosystems, this unique ecosystem has suffered great worldwide losses in coverage and habitat quality based on unsustainable overexploitation and alternative land use development (Kibria, 2013). Unsustainable shrimp aquaculture is by a considerable margin, the greatest single cause of worldwide mangrove loss (Fig 1.1).

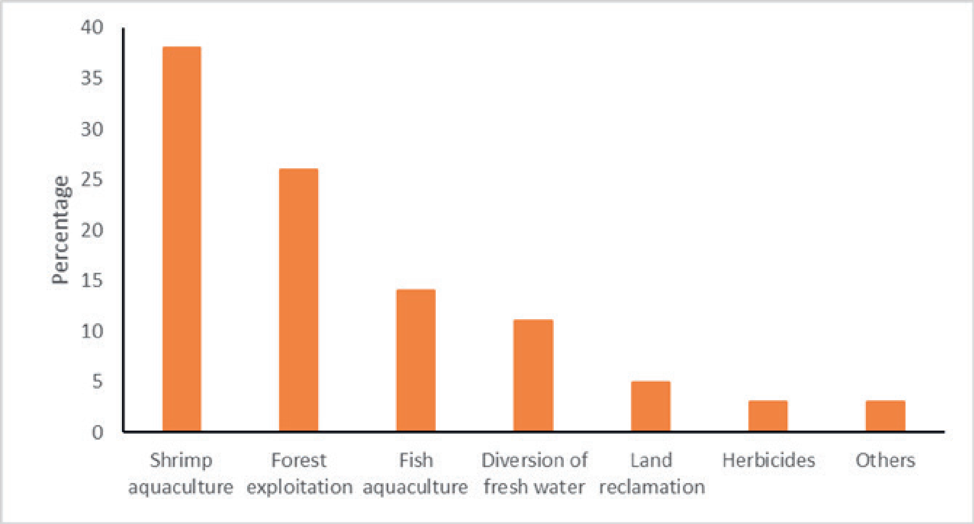

Fig 1.1. Area of mangrove habitat destroyed worldwide by different human activities (modified from IUCN, 2005).

Recent estimates are that about 50% of the historic global coverage of mangroves has already been lost (Zabbey and Tanee, 2016), with more than 35% of this loss having occurred during the 1980s and 1990s alone (Romanach et al., 2018). The same is true for Bangladesh which also underwent an unprecedented expansion of export-oriented shrimp culture activities, including conversion of mangrove areas into pond areas between 1977 and 1996 (Hossain et al., 2001). For instance, 75 km^2 of mangrove forest in the Chakaria Sundarbans, Cox's Bazar, Bangladesh, has been totally deforested (Hossain et al., 2001), of which one third of the aera was converted to shrimp ponds and more elevated land was converted into croplands by the end of 2016. The remaining land largely remains bare, with small areas used for salt production (Fig. 1.2).

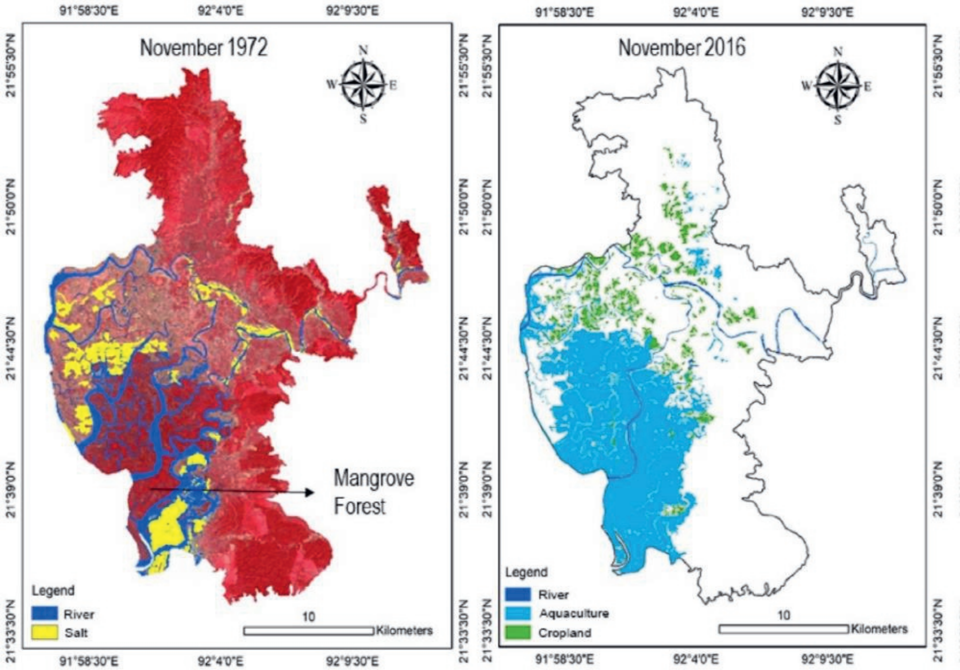

Fig. 1.2. Shows the base map (left) and recent spatial-temporal changes to major land use of the Chakaria sub-district. The mangrove forest is converted upon by aquaculture, crop land and salt production. (Source: Abdullah et al., 2021).

1.4. Shrimp aquaculture in Bangladesh

Bangladesh is one of the most suitable regions for fisheries and aquaculture in Asia with its vast wetlands, estuaries and alluvial soils. The fishery sector in Bangladesh is divided into four subsectors: (1) inland fisheries, (2) marine fisheries, (3) freshwater aquaculture and (4) coastal (brackish) aquaculture (DOF, 2016). Combined, freshwater and coastal aquaculture are referred to as 'inland aquaculture'. In particular, the production of inland aquaculture has increased greatly through the introduction of new technologies and species (DOF, 2021). Coastal aquaculture largely amounts to shrimp and prawn farming in ponds or aquatic enclosures (Shamsuzzaman et al., 2017).

Shrimp aquaculture, mostly practised in the south-western region of Bangladesh, plays an important role in the economy by generating a significant portion of foreign exchange earnings and serving as a source of employment (BBS, 2021). Bangladesh produces roughly 2.5% of the global shrimp aquaculture (Shamsuzzaman et al., 2017) and shrimp is the second largest export industry of the country. The country earned about USD 494 million by exporting fish and shrimp during 2019-2020, more than 80% of which was from the export of shrimp (DoF, 2021). Moreover, shrimp culture provides employment, income and food security to the rural people in the coastal areas where alternative livelihood options are very limited (Islam et al., 2016). More than 0.6 million people including women are engaged either directly or indirectly in this sector (DOF, 2021). Historically, shrimp production in Bangladesh has been export-driven, though in the recent past, the local consumer demands also expanded along with an increase in per capita income (BBS, 2021). However, shrimp farming is also associated with a number of persistent negative environmental and social impacts, which is very worrisome to the further sustainable development of the sector. Several authors have seriously questioned its continued viability (Troell et al., 2014; Naylor et al., 2021).

1.5. Switch to shrimp farming in coastal area

Bangladesh is characterized by a vast "coastal" area of 47,201 km^2, which amounts to 32% of the country surface area and 19 administrative districts out of the total of 64 administrative districts for the whole country (Ahmed, 2019). Typically, three main sectors of the coastal zone are distinguished; (1) the eastern zone; (2) the central zone and (3) the western zone (Fig. 1.3). The western zone, which includes the Sundarbans, is today the zone least disturbed and affected by human activity.

Over the last few decades, land use patterns in Bangladesh changed. Most important has been the construction of 139 polders covering almost half the coastal zone (12000 km^2 out of 28000 km^2). These were constructed along the coast to protect the coastal lands from tidal flooding as well as increasing rice production (BBS, 2010). This involved canalization and destruction of sediment-entrapping (mangrove) vegetation, and resulted in land subsidence and increased salt intrusion. In response to the ensuing water logging and salinization, many farmers have since switched from rice to export-oriented shrimp farming as an alternative livelihood strategy (Goswami and Ghosal, 2022). The rapid expansion of shrimp culture in the coastal folders encouraged shrimp farmers to clear the mangroves for shrimp pond construction, thereby undermining the broad ecosystem services (ES) provided by mangroves. This has resulted in a series of cascading negative environmental and socio-economic consequences which could have been avoided if attention had been given to the development of silvo-aquaculture, as broadly practiced in south and south-east Asia (Bosma et al., 2020). Silvo-aquaculture offers at least three advantages: (1) reduced conflict over mangrove deforestation and shrimp culture; (2) option to manage shrimp and mangroves jointly and allowing space for mangroves to continue fulfilling their broad ES (3) synergistic effects from mangroves associated with shrimp culture to improve water quality and reinforce shrimp production (Bosma et a., 2020; Ahmed et al., 2018; Islam et al., 2016).

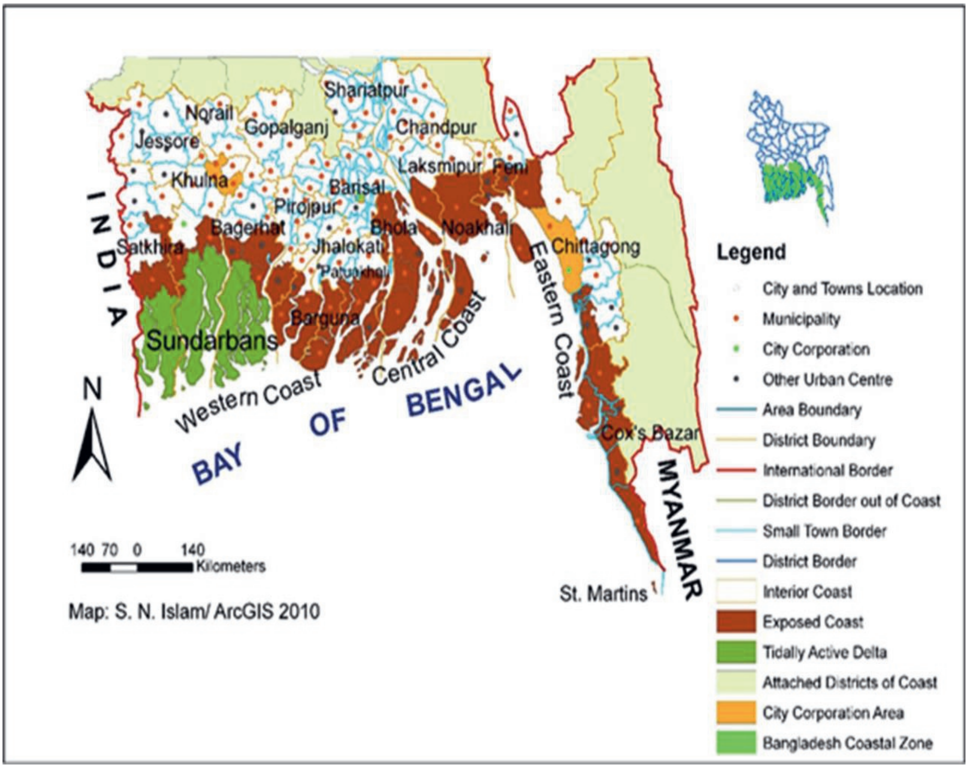

Fig. 1.3. Coastal zone of Bangladesh. (Source : Islam, 2010).

1.6. Shrimp culture intensification and use of formulated feed

Unsustainable shrimp aquaculture remains a profitable aquaculture business (at least on the short-term planning horizon) that continues to grow. In order to keep up with the demand, many shrimp farmers have intensified their production (Biao and Kaijin, 2007). As a result, shrimp culture has not only destroyed the mangrove ecosystem in some coastal countries but also expanded the use of expensive commercial feeds. Commercial feed is not only a costly input for small-scale farmers but also often leads to water pollution that increases shrimp vulnerability to disease (Islam and Bhuiyan, 2016; De Schryver et al., 2008; Tacon, 2002).

1.7. Mangroves as natural feed supplement for shrimp

The use of natural feed has been promoted as a way by which to minimize excess application of formulated feed (Porchas-Cornejo et al., 2012) and mangrove leaf litter has been identified as an inexpensive input stimulating in situ natural food production for shrimp (Nga et al., 2006; Tran et al., 2005). The decomposition of mangrove leaf litter enhances microbial activity and biofilm formation within decomposing leaf litter, which is a nutritive natural food for shrimp post larvae (Gatune et al., 2014, 2012). Such natural food can contribute up to 50–70% of the nutritional requirements of shrimp (Martinez-Cordova and Enriquez-Ocana, 2007; Enríquez, 2003; Tacon, 2002). Therefore, the introduction of mangrove in shrimp culture ponds might be a feasible, accessible and affordable way by which to reduce the input of costly and polluting pelleted feed.

1.8. Mangrove-shrimp co-management or Silvo-aquaculture

Silvo-aquaculture refers to the coupling of silviculture and shrimp culture and is a production system whereby mangrove trees are associated to different degrees with shrimp farming (Bosma et al., 2014). Integrating mangroves into shrimp culture in principle should allow the maintenance of a relatively high level of ecosystem integrity in the mangrove area while simultaneously allowing synergistic economic benefits to shrimp culture. In contrast to when mangroves are fully removed, when they are properly integrated into the shrimp culture landscape they can help maintain healthy nutrient fluxes through the landscape. Even in small amounts, when mangroves are present in shrimp culture areas, they can still serve as a source of nutrient input mainly in the form of leaf litter, and can also trap or take up both solid and dissolved nutrients. Mangrove litter may also play an important role in the production of organic shrimp which appeals to consumers for their higher quality (Paul and Vogl, 2012; Dhar et al., 2019). Hence, integrated shrimp mangrove farming is often referred to as “organic” aquaculture (Ahmed et al., 2018).

Some South-east Asian countries have introduced silvo-aquaculture as an eco-friendly shrimp farming system, whereby mangrove stands produce a large and high-quality litter input to aquatic systems (Nga et al., 2005). Silvo-aquaculture was first formally reported for Indonesia and then followed by Vietnam, Malaysia, the Philippines and Thailand (Primavera, 2000). Scholars from various countries already studied the role of specific mangrove species in mangrove silvo-aquaculture, describing practices and reporting benefits (Table 1.2).

In Vietnam, mangrove-based shrimp farming realized a net return three times higher than the net return obtained by extensive traditional shrimp farming, thanks to the higher shrimp yields and the larger-sized shrimp fetching a higher market price (Tran et al., 2013). Besides aquaculture products, mangroves in silvo-aquaculture systems provide timber and non-timber products (Debrot et al., 2020). A number of authors have also reported on bioactive molecules (Premanathan et al., 1999) having anti-diabetic (Revathi et al., 2014) and anti-viral activity (Bandaranayake, 2002, 1998; Sudheer et al., 2011).

Table 1.2

Silvo-aquaculture as practiced in several countries along with key research findings.

Country	Major findings	Reference
China	- Mangrove species *Aegiceras corniculatum* (L.) performed the best for planting in aquaculture ponds - High tolerance to long-term inundation - Effective in purifying aquaculture pond water	Peng et al., 2013
Indonesia	- Mangrove species *Avicennia alba, A. marina, A. officinalis, Bruguiera sexangula, Sonneratia aptala, S. caseolaris, Xylocarpus granatum, X. mekongensis* - Mostly practiced in the area of Demak, Central Java. - Mangrove coverage of 60% of the total area next to mono-culture shrimp ponds resulted in the highest income from shrimp aquaculture - Mussel culture contributes to profitability of silvo-aquaculture - Water exchange between a milk fish, seabass or tilapia reservoir pond and shrimp ponds helps to maintain water quality and reduces disease occurrence.	Bosma et al., 2020, Rejeki et al., 2019; Primavera, 1993.
Vietnam	- Mostly practiced in the Mekong Delta. - Dominant mangrove species in silvo-aquaculture is *Rhizophora apiculata*. - Shrimp culture with 30-50% mangrove coverages of the pond area gave the highest annual income. - Farmers practicing mangrove-shrimp aquaculture benefitted more than monoculture shrimp farmers.	Binh *et al.*, 1997
Hongkong	- Analysed litter input by *Kandelia candel* to shrimp ponds, showing leaf litter is the highest (40-69%) contributor. - Effective decomposition of mangrove litter, requires presence of macrofauna for shredding.	Lee, 1989

1.9. Selection of mangrove species and an initiative for silvo-aquaculture in Bangladesh

For a successful introduction of silvo-aquaculture, it is important to know and select mangrove species which have a proven positive impact on shrimp production. By experience, farmers largely know which species in their farming area are beneficial to shrimp culture. Therefore, the farmers choice and their experience might be valuable when it comes to selection of mangrove species. Rahman et al. (2020) analysed which mangrove species the shrimp farmers in the Sundarbans area prefer to plant on their shrimp pond dikes (Table 1.3).

Table 1.3

Ranking and percentage of preference of mangrove species among seven focused group discussions (FGDs in Bangladesh (modified from Rahman et al., 2020).

Preferred species (Scientific name)	The ranking by the seven FGDs *							Total	Preference order
	1	2	3	4	5	6	7		
S. apetala	6	3	7	6	5	3	6	36	**I**
S. caseolaris	3	3	4	4	2	3	3	22	**II**
A. officinalis	2	2	3	1	2	3	1	14	**III**
N. fruticans	2	3	0	1	2	0	1	9	**IV**
B. sexangula	1	1	0	0	1	1	2	6	**V**
H. fomes	0	2	1	0	1	1	1	6	**VI**
O. coarctata	1	0	0	1	2	1	0	5	**VII**

* The higher the mark the higher the preference of the 7 FGDs. Values range between 0 (lowest preference) and 6 (highest preference).

Based on the local availability and farmers choice the farmers surveyed by Rahman et al. (2020) preferred primarily *S. apetala*, *S. caseolaris*, *A. officinalis* and *B. sexangula* mangrove trees around their ponds.

1.10. Formulation of the problem statement and objectives

Research has shown that association of mangroves with shrimp farming may have positive effects but also negative effects, for example by introducing anti-nutrients into the culture system and thereby interfere with pond production (Bosma et al., 2020). Anti-nutritional substances can interfere with food utilisation and health, reducing pond production directly or indirectly through their metabolic products (Makkar, 1993). Important among these are protease inhibitors, phytates, saponins, phenolic compounds (gossypols, tannin), haemagglutinins, oligosaccharides and non-starch polysaccharides, alkaloids, antivitamins, and phorbol esters (Francis et al., 2001). Among the anti-nutritional compounds, tannins, saponins and phytates have been identified as the most important (Francis et al., 2001). Higher contents of tannins, saponins and phytates are harmful for both aquaculture and human consumption (Rout et al., 2015).

1.10.1. Positive effect of leaf litter

Nutrients released from decaying leaves may affect the shrimp through the following hypothetical path ways (Fig. 1.4):

- They supply nutrients (nitrogen and phosphorus) during decay, thus enhancing algal production (Roijackers and Nga, 2002). Leaves also function as a direct and indirect (the attached communities of algae, protozoa and micro-organisms) food source for all kinds of aquatic animals, including shrimp (Nga et al., 2006).
- Leaves may contain substrates or extracts which have prebiotic properties or may contain probiotic bacteria with anti-pathogenic properties (Wichienchot et al., 2011; Gonelimali et al., 2018).
- The leaves and crown of the mangrove tree influence shrimp performance by creating shelter from sun irradiation and heat (Clough et al., 2002).

1.10.2. Negative effect of leaf litter

For many plants, the anti-nutritional effects on aquaculture production has been studied, but not yet for the mangrove leaf litter. The anti-nutrients leached from the mangrove trees can impact the shrimp thorough the following pathways (Fig. 1.4):

- Tannin produces tannic acid which can be toxic to aquatic organism. Tannins affect digestion by binding to proteins or minerals. They decrease the feed intake, growth rate, feed efficiency and protein digestibility in experimental animals (Gemede et al., 2014).
- Saponins present in high concentrations in leaves and fruits of plant species, that leach into the aquatic environment affect food intake and the growth of aquatic animals due to its bitterness (Hajra et al., 2013).
- Phytates leached into the in aquatic environment affect the mineral utilization and reduce nutrient available from food. In addition, fish and shrimp do not produce enzymes to break down phytates (Gemede et al., 2014).

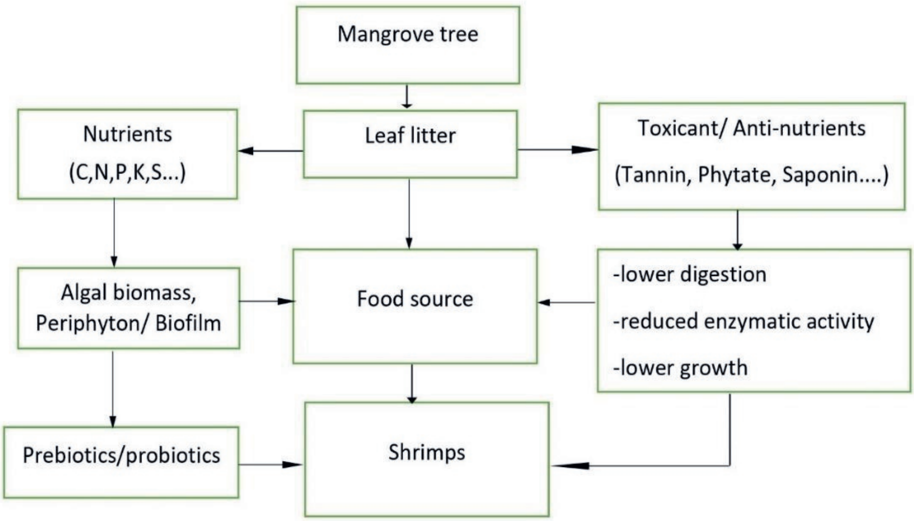

Fig. 1.4. A schematic (Flow) diagram to show the possible pathways of contribution of mangrove leaf litter to Shrimp.

1.10.3. Objectives

This study explores the effect of how mangrove leaf litter on shrimp production in fed and non-fed shrimp nursery systems. More specifically, the following research questions are addressed:

(1) What are the nutrient and anti-nutrient contents in preselected mangrove species and what is their impact on shrimp performance?

(2) What are the effects of leaf litter from single or mixed mangrove tree species and supplemental feed on shrimp performance?

(3) Are the positive effects of leaf litter mixtures from different mangrove tree species and supplemental feed similar in tanks and ponds?

(4) How does mangrove leaf litter affects shrimp quality criteria?

1.11. Outline of this thesis

This thesis comprises six chapters including the general introduction (Chapter 1) and the general discussion (Chapter 6). The outline of the chapters is given in a schematic diagram (Fig. 1.5). Chapter 2 analyses the nutrient and anti-nutrient content of four selected mangrove species and their impact on shrimp post larvae performance during a 4-week culture period. Subsequently, the effects on system performance of mangrove leaf litter on juvenile shrimp production in pelleted feed fed juvenile shrimp rearing systems was addressed in Chapter 3 for individual mangrove species and Chapter 4 for a mix of mangrove species. In addition, differences between fed shrimp nursery systems in tanks and ponds were explored in Chapter 4. In chapter 5, the effect of leaf litter on shrimp colour and product quality appreciation by farmers, local consumers and shrimp exporters was explored. Finally, in the General Discussion (Chapter 6), our research results are against the present practices and insights into mangrove-shrimp aquaculture systems.

Chapter 1 General introduction
Chapter 2 Identification of nutrients and anti-nutrients in mangrove leaf litter and its impact on shrimp post larvae performance (Objective 1)
Chapter 3 Synergistic effect of mangrove leaf litter and supplemental feed on shrimp post larvae performance (Objective 2)
Chapter 4 Effect of mixed mangrove leaf litter (Objective 2) on shrimp performance and differences in production between nursery tanks and mesocosm ponds (Objective 3)
Chapter 5 Effect of mangrove leaf litter on shrimp growth and color (objective 4)
Chapter 6 General discussion

Fig. 1.5. Schematic representation of the chapters of this thesis.

Chapter 2

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) postlarvae

This chapter has been published as:

Alam, M.I., Ahsan, M.N., Debrot, A.O., Verdegem, M.C.J., 2021. 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) postlarvae.

Aquaculture 542, 736865 . https://doi.org/10.1016/j.aquaculture.2021.736865.

Abstract

The release of nutrients and anti-nutrients from mangrove leaf litter plays an important role in the biogeochemical cycling in aquatic environments and directly or indirectly affects water quality and food availability to shrimp. In this study, we assessed nutrient and anti-nutrient loss during decomposition of leaf litter at a concentration of 1 g L^{-1} for four mangrove species (*Avicennia officinalis*, *Heritiera fomes*, *Sonneratia apetala*, *S. caseolaris*) to monitor water quality and to estimate how leaf litter influences shrimp postlarvae (PL) growth and survival. There were significant differences ($P < 0.05$) between the studied species in terms of mass loss of the leaf litter during the investigation period. There were also significant differences ($P < 0.05$) between the studied species in terms of loss of nutrients and anti-nutrients in the shrimp PL rearing tank during the four-week experimental period. Decomposing mangrove leaves stimulated availability of natural food for shrimp PLs. There was a strong positive correlation between mass loss and PL production. At the concentrations of leaf litter used, the anti-nutritional factors did not affect the PLs. PL survival with mangrove leaf litter was 75-82%, whereas all the PL died without any leaf litter. PL weight gain ranged from 0.83-3.33 mg d^{-1} where *S. apetala* leaf litter resulted in the highest PL growth rate, followed by *A. officinalis, S. caseolaris* and *H. fomes,* in that order ($P < 0.05$). Overall, mangrove leaf litter had a positive effect on shrimp performance in terms of growth and survival and this effect was highest for *S. apetala* leaf litter.

1. Introduction

Mangroves form a highly productive ecosystem, showing high primary and secondary productivity in intertidal coastal regions of the tropics and subtropics (Nagarajan et al., 2008). Mangrove roots and fallen leaf litter provide substrate for biofilm development and nutrients in the water column and stimulate fish production (Hutchison et al., 2014; Verweij et al., 2008; Nordhaus et al., 2006). Mangroves and aquaculture are not necessarily incompatible though commercial shrimp farming is identified as the main cause of mangrove loss (Hossain et al., 2001). Considering the ecological importance of mangroves as well as the economic value of shrimp culture, mangrove-based shrimp culture (silvo-aquaculture) is practiced in numerous countries, although not to the extend needed to conserve or restore mangrove biotopes. The first reports on silvo-aquaculture are from Indonesia (Schuster 1952 cited by Primavera, 1993), followed by Vietnam, Malaysia, the Philippines and Thailand (Primavera, 2000). In aquatic waterways the culture of seaweeds, molluscs (Rejeki et al., 2020) and fish in cages is possible adjacent to or between mangroves (Primavera, 1993) while in intertidal mangrove areas different types of silvo-aquaculture can be explored (Bosma et al., 2014; Primavera et al., 2007; Primavera, 2000). The ultimate goal of silvo-aquaculture is to increase the farmer’s income while improving environmental and economic resilience. However, from an aquaculture perspective, integration of mangroves with shrimp farming may either be detrimental or beneficial. As mangrove leaf litter is an important influencer of shrimp productivity in silvo-aquaculture, its net effect on shrimp production (either positive or negative) needs to be quantified.

Leaf litter input rate and composition affect water quality, survival and growth of shrimp. Leaching of nutrients and organic matter from mangrove litter may have positive effects on shrimp performance by supplying nutrients for algal production (Roijackers and Nga, 2002), and by stimulating the food web in shrimp ponds (Gatune et al., 2014; Nga et al., 2006; Hai and Yakupitiyage, 2005). On the other hand, leaf leachates include anti-nutritional substances among which especially tannins, saponins and phytates, and may deteriorate the water quality (Francis et al., 2001). High concentrations of these substances were found to have detrimental effects on shrimp survival and growth by affecting digestibility and hampering mineral utilization (Gemede and Ratta, 2014). Thus, analyses of the nutritional and anti-nutritional profiles of leaves and their decomposition rates in situ are important in determining whether particular mangrove species would be suitable for silvo-aquaculture. While a considerable body of knowledge exists on leaf litter production and decomposition rates in mangrove forests (Srisunont et al., 2017; Gladstone-Gallagher et al., 2014; Kamruzzaman et al., 2012; Imgraben and Dittmann, 2008; Khan et al., 2007; Silva et al., 2007; Bosire et al., 2005), little is known regarding the nutritional and anti-nutritional composition of leaf litter and their potential impacts on aquaculture production.

Different mangrove species might well have different impacts on shrimp production. Selection of the most suitable mangrove species is very important to the successful introduction of shrimp-based silvo-aquaculture. For Bangladesh, Rahman et al. (2020) identified 10 mangrove species potentially suitable for silvo-aquaculture. These were *Avicennia alba, A. officinalis, A. marina, Bruguiera sexangula, Kandelia candel, Sonneratia apetala, S. caseolaris, Heritiera fomes, Aegialitis rotundifolia*, and *Lumnitzera racemosa*. Among these, A. *officinalis*, *S. apetala, S. caseolaris* and *H. fomes* were selected for further analysis as these mangrove species are common in the mangrove forests and easily grow on the dykes of shrimp farms in the coastal region of the country. The local availability of propagules and seedlings, and farmer preference identified by Rahman et al. (2020) also supported the selection process.

The objectives of this study were to: (a) compare the nutritional and anti-nutritional contents of leaf litter from different mangrove species; (b) estimate the leaf litter mass loss over time; (c) assess the impact of the leaf litter on the water quality; and (d) measure and compare survival and growth of shrimp (*Penaeus monodon*, Fabricius, 1978) postlarvae (PL), in the presence or absence of mangrove leaf litter.

2. Methodology

2.1. Experimental design

This experiment was split in two parts. In the first part, nutrients and anti-nutrients in leaf litter of four selected mangrove species were analysed in the laboratory of Forestry and Wood Technology (FWT) and the laboratory of Pharmacy, Khulna University. In the second part, the impact of leaf litter on shrimp performances (in terms of survival and growth) and water quality was measured in tank experiments. The latter were carried out at a farm located in Debhata, Satkhira. The tank culture experiments took place under a tent of transparent plastic to prevent the effects of rain water intrusion, while providing ambient lighting. We used five treatments types, executed in triplicate in tanks stocked with PLs; four treatments involved the introduction of the four species of leaf litter while one treatment involved no leaf litter. We did not apply any formulated or supplemental feed as we expected the PLs to feed on the natural food produced based on decomposing leaf litter. A treatment without leaf litter served as control as the natural water source used may have provided an otherwise undocumented and uncontrolled source of nourishment.

In the tank experiment, shrimp were reared in fifteen fiber-enforced polyethylene tanks with a water volume of 1000-L. Natural water 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 tanks through a screen with 25 μm mesh-size net to keep predators and eggs/larvae of predators out. Each tank was aerated using one air stone (diameter 2 cm) connected to an electric air blower (RESUN, LP-100). Mangrove leaf litter collected from Sundarbans, Bangladesh (southern part) was directly added in the culture tanks at a concentration of 1 g L^{-1}. This loading rate was standardized following Hai and Yakupitiyage (2005). On the same day, 100 specific pathogens free (SPF) shrimp postlarvae (PL15; 0.01 g) obtained from a nearby hatchery (Desh Bangla Hatchery Limited, Khulna, Bangladesh) were stocked in each tank. The survival and growth experiment was conducted over four weeks.

2.2. Collection of leaf litter and sample preparation

Mangrove leaves which became yellowish before falling down naturally, referred to as "senescent" leaves, were collected. Leaves were collected by putting 30 litter traps (2mx2m) beneath the selected mangrove species during winter (November, 2018-January, 2019). At regular intervals, the fallen leaves were recovered from the traps and separated according to the species.

The collected leaves were air dried at room temperature for 48 hours. The leaves from each selected mangrove species were weighed (BH 300A, A & D Korea, Ltd.), mixed well and divided into two equal parts; one part was transferred to the shrimp culture tanks on the day of stocking the PLs, the other part was used for analysis of nutrients and anti-nutrients.

To identify the dry matter (DM), five gram of mixed leaves were considered as a sample and three samples (wet weight) of each species were dried in a vacuum drying oven (Vacuum Oven, OV-11, Korea) at 80°C until a constant weight (Hossain et al., 2011). This low drying temperature was used to minimize possible changes in leaf nutrient and anti-nutrient composition. The average weight was recorded as DM and expressed as g kg^{-1} wet weight. The sample for nutrient and anti-nutrient analysis was processed according to Allen (1989). A high speed grinder (Kent 16003) was used to finely grind the leaf sample. The powdered samples were packed into air-tight plastic bags and stored in the refrigerator (4 °C) until further analysis.

2.3. Quantification of nutrients

2.3.1. Determination of organic matter (OM), ash and ash free calorific value (AFCV)

The organic matter (OM) and ash content was measured according to Allen (1989) using a muffle furnace (Wise Therm Digital Muffle Furnace, FH-05) and the content was expressed as % DM. The gross caloric value (GCV, MJ kg^{-1} DM) in leaf litter was measured following the detailed protocol described by Fiori et al. (2015), using an Automatic Isoperibol Bomb Calorimeter (Parr 6400 Calorimeter). The ash-free calorific value (AFCV) was calculated based on the properties of calorific value and ash content. This was done using the equation described by Islam et al. (2019):

AFCV = GCV/ (1 – (Ash(g)/DM(g))).

The value is expressed as MJ kg^{-1} DM.

2.3.2. Determination of carbon, nitrogen and phosphorus

The total carbon content of the leaf samples was analysed directly by CHNS Elemental Analyzer Flash 2000 (Thermo scientific, USA). For total nitrogen and total phosphorus per mangrove species, leaf powder was acid-digested according to Allen (1989). Nitrogen (N) and phosphorus (P) concentrations in the sample were measured according to Weatherburm (1967) and Timothy et al. (1984), respectively, using an UV-Visible Recording Spectrophotometer (Shimadzu UV-160A, Japan). The content of C, N and P were expressed as % DM. The C: N ratio was calculated dividing total carbon by total nitrogen content.

2.3.3. Determination of crude fibre content

The crude fibre content of the leaf samples was determined according to Cunniff (1995). Powdered samples (1 g) were taken in a silica crucible and the extractives content was removed first through Soxhlet extraction with petroleum ether. The residue was digested with 1.25% H_2SO_4 and 1.25% NaOH solutions. The sample was then dried at 130 ^{0}C for 2 hr and ignited at 600 ^{0}C for 30 min. Crude fibre content was calculated by following formula:

Crude fibre (% DM) = $(W_1 – W_2)/W$ X100

Where, W= Weight of sample, W_1 = Weight of silica crucible with sample before ignition, W_2 = Weight of silica crucible with sample after ignition;

2.4. Quantification of Anti-nutrients

2.4.1. Determination of tannins

Tannin content in the samples was determined by the Folin-Denis method described by Saxena et al. (2013) with minor modification of the method of Schanderi (1970). Powdered samples (0.25 g) were extracted with 37.5 ml distilled water and heated in a flask gently and boiled for 30 min. Each sample was centrifuged at 2000 rpm for 20 min and the volume of the supernatant was brought up to 37.5 ml using distilled water in a 100 ml flask. An aliquot of 500 µl of the sample was treated with 1 ml of Folin-Denis reagent followed by 2 ml of sodium carbonate and allowed to stand for color development. The absorbance of the mixture was measured at 700 nm in a spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments). Tannic acid was used as standard. The tannin content was calculated based on spectrophotometer readings of sample concentrations and the standard (theoretical) concentration and expressed as % DM.

2.4.2. Determination of saponins

Saponin content in the samples was determined following the method described by Obadoni and Ochuko (2002). The powdered samples (ca.3 g) were dispersed in 30 ml of 20% aqueous ethanol. The suspension was stirred for 12 hrs with constant stirring at about 55 ^{0}C on a hotplate. The mixture was filtered (Whatman filter paper 1) and the residue was re-extracted with another 30 ml of 20% aqueous ethanol. The combined extracts (filtrates) were reduced to 15 ml over a water bath at 90 ^{0}C. The concentrated sample extract was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added and the sample was shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated twice. To the combined aqueous sample, 20 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was then heated in a water bath. After evaporation, the concentrated sample was dried in a drying bath to a constant weight and saponin content was calculated according to the formula:

Saponin (%) $=(W_2-W_1)/ W \times 100$

Where, W= Weight of sample, W_1= Weight of evaporating disc, W_2= Weight of disc + Sample

2.4.3. Determination of phytates

Phytate content was determined by the method described by Rout et al. (2015) using a minor modification of method of Wheeler and Ferrel (1971). A sample of 3 g was mixed in 25 ml of 10% trichloroacetic acid (TCA) in a 125 ml flask and shaken with mechanical shaker for 2 hrs. This sample then was centrifuged at 3000 rpm for 20 min. Ten (10) ml of the supernatant was mixed with 4 ml of $FeCl_3$ solution in a 50 ml centrifuge tube. The resulting solution was then heated in a boiling water bath for 45 min. To make the supernatant clear, one or two drops of 3% sodium sulphate in 10% TCA was added under continued heating. The supernatant was then centrifuged for 10-15 min at 3000 rpm and finally the clear supernatant was discarded. The precipitate so obtained was washed twice by dispersing it in 25 ml 10% TCA, after which it was heated again in boiling water for 10 min and centrifuged after cooling to room temperature. The precipitate was again dispersed in a few ml of water, followed by addition of 3 ml of 1.5 N NaOH, after which the volume was brought up to 30 ml with distilled water. After heating in boiling water for 30 min, the solution was filtered (Whatman No 2 paper); the precipitate was washed with 70 ml hot water and the filtrate was discarded. The precipitate on the filter paper was then dissolved with 40 ml hot HNO_3 (3.2 N) into a 100 ml volumetric flask. A 5 ml aliquot was taken and placed in a 100 ml volumetric flask and then diluted to 70 ml with distilled water, after which 20 ml of 1.5 M potassium thiocyanate (KSCN) was added. The pinkish-red colour obtained was measured immediately (within 1 min) at 480 nm in a spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments) using Ferric nitrate as the standard. The phytate content was calculated based on the spectrophotometer reading of sample concentration and standard (theoretical) concentration and expressed as percentage (%) DM.

2.5. Water quality monitoring

Temperature, salinity, pH, and dissolved oxygen (DO) in each tank were measured daily 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). Water samples were collected from the tank at a depth of 10-30 cm from the surface. Two BOD bottles (300 ml) for each replication of treatments were filled carefully with sample water without allowing air bubbles. In one bottle, DO was fixed following the Winkler method to measure initial DO while another bottle was left to incubate for 5 days. Both samples were analyzed in the Khulna University water quality laboratory following the method outlined in APHA (1998).

Chemical oxygen demand (COD) was measured bi-weekly. Samples were collected from the middle of the tank at a depth of 10-30 cm from the surface water 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 9.00–11.00 hr from three points in each tank and passed through a 45 µm mesh plankton net and combined. The concentrated samples were preserved in plastic bottles with 1ml 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 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 counted using a compound microscope (Lx 400; magnification-4x-100x, USA) and identified (where possible to genus level) using a 5.1M 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 tank water sample in liter.

2.7. Assessment of shrimp larval performances

The 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 papers 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:

$$SR\ (\%) = N_f/N_i \times 100$$

$$SGR\ (\%\ BW\ day^{-1}) = (\ln (BW_f) - \ln (BW_i))/D \times 100$$

Where 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 leaf mass loss, nutrient and anti-nutrient loss and decomposition rates

The leaf litter remaining in each tank at the end of the 4-week experiment was collected. The samples were prepared and the nutrients and anti-nutrients in the leaf residue also calculated as previously described. Mass loss was calculated on initial dry mass while the decomposition rate was calculated from mass loss divided by the duration of the incubation. The loss of nutrients and anti-nutrients from the leaves over a four-week incubation period was also calculated according to the mass loss during the decomposition process. All the values were expressed as % DM.

2.9. Statistical analysis

All measured values were expressed as mean ± standard deviation (SD). One-way ANOVA was conducted to compare the dependent variables for the four types of mangrove species. A comparison of growth rates between tanks with the mangrove litter and control tank without mangrove litter was not possible because all shrimp in the control tank died prematurely. For the significant differences, a post-hoc Tukey HSD test was used to determine pair-wise differences ($P < 0.05$). Correlations among the different variables were assessed using Pearson's correlation coefficient. Linear regression among selected variables were also done. Analyses were conducted using IBM SPSS statistical software (Version 26).

3.Results

3.1. Nutrients and anti-nutrients in leaf litter, decomposition and mass loss

The nutrients and anti-nutrients in leaf litter of four mangrove species (*H. fomes*, *A. officinalis*, *S. caseolaris* and *S. apetala*) were identified for both senescent leaves (Table 2.1) and the leaf litter residue after four weeks in the shrimp PL rearing tanks (Table 2.2). The loss of nutrients and anti-nutrients through mass loss was also calculated during the incubation of leaf litter over a four week period in the shrimp PL rearing tanks (Table 2.3).

No significant differences ($P > 0.05$) between freshly fallen senescent leaves of mangrove species were found for ash free caloric value (MJ kg^{-1} DM), tannin or phytate content (% DM) but there were significant differences ($P < 0.05$) among the species in terms of crude fiber, ash, organic matter (OM), carbon (C), nitrogen (N), phosphorus (P) and saponin content (% DM) (Table 2.1).

Table 2.1

Nutrients and anti-nutrients contents in senescent leaves of four selected mangrove species.

Nutrients/anti-nutrients (% DM; unless specified within brackets)	Mangrove species				S.E.M.	*P*-value
	H. fomes	*A. officinalis*	*S. caseolaris*	*S. apetala*		
Energy content (MJ AFDM Kg^{-1})	18.7	19.6	18.7	18.8	0.2	ns
Crude fibre	33.4^{d}	27.7^{c}	22.5^{b}	18.4^{a}	1.7	***
Ash	4.1^{a}	10.8^{b}	11.1^{b}	11.9^{b}	1.0	***
Organic Matter	95.9^{b}	89.0^{a}	88.7^{a}	87.7^{a}	1.0	***
Carbon	48.3^{c}	44.8^{b}	44.7^{b}	44.1^{a}	0.5	***
Nitrogen	1.35^{a}	2.01^{b}	2.79^{c}	1.98^{b}	0.2	***
Phosphorus	0.02^{b}	0.01^{a}	0.03^{d}	0.02^{c}	0.0	***
Tannin	1.84	1.73	1.80	1.79	0.0	ns
Phytate	0.33	0.43	0.36	0.38	0.0	ns
Saponin	1.58^{b}	1.16^{a}	1.23^{a}	1.29^{a}	0.1	***
C:N	35.8^{c}	22.3^{b}	16.1^{a}	22.3^{b}	2.2	**

Small letter used as superscript to indicate significant differences, 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, $P > 0.05$).

Table 2.2

Nutrients and anti-nutrients contents (%DM) in leaves of four selected mangrove species after four weeks of incubation in shrimp PL rearing tanks.

Nutrients/anti-nutrients (% DM)	**Mangrove species**				**S.E.M.**	***P*-value**
	H. fomes	*A. officinalis*	*S. caseolaris*	*S. apetala*		
Organic Matter	95.5^{d}	81.4^{b}	84.5^{c}	77.3^{a}	2.0	***
Carbon	47.9^{d}	40.8^{b}	42.6^{c}	38.4^{a}	1.1	***
Nitrogen	1.16^{a}	1.83^{c}	2.30^{d}	1.67^{b}	0.1	***
Phosphorus	0.02^{ab}	0.01^{a}	0.02^{b}	0.01^{a}	0.0	*
Tannin	1.64^{b}	1.28^{a}	1.18^{a}	1.33^{ab}	0.1	*
Phytate	0.24	0.33	0.24	0.33	0.0	ns
Saponin	1.29^{b}	0.73^{a}	0.80^{a}	1.00^{ab}	0.1	*

Small letter used as superscript to indicate significant differences, 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, $P > 0.05$).

Table 2.3

Mass loss (% DM), nutrient and anti-nutrient loss (% DM) of leaves of four selected mangrove species over a four-week period in shrimp PL tanks.

Loss on Initial weight of leaves (%DM; unless specified within brackets)	**Mangrove species**				**S.E.M.**	***P*-value**
	H. fomes	*A. officinalis*	*S. caseolaris*	*S. apetala*		
Mass Loss	23.0^{a}	45.8^{c}	39.4^{b}	50.7^{c}	3.2	***
Decomposition rate (% day^{-1})	0.83^{a}	1.6^{c}	1.4^{b}	1.8^{c}	0.1	***
Organic Matter	23.3^{a}	50.3^{c}	$42.3^{\mathbf{b}}$	56.5^{d}	3.8	***
Carbon	23.6^{a}	50.6^{c}	42.3^{b}	57.1^{d}	3.8	***
Nitrogen	33.7^{a}	50.5^{b}	50.1^{b}	58.3^{c}	2.8	***
Phosphorus	36.8^{a}	63.5^{bc}	55.8^{b}	73.4^{c}	4.4	**
Tannin	31.4^{a}	60.2^{b}	60.3^{b}	63.5^{b}	4.0	***
Phytate	44.8^{a}	$58.2^{\mathbf{b}}$	60.1^{b}	56.3^{b}	2.0	**
Saponin	37.5^{a}	66.0^{b}	$60.5^{\mathbf{b}}$	62.0^{b}	3.7	**

Small letter used as superscript to indicate significant differences, according to Tukey HSD test ($P<0.05$). P value is expressed as a symbol ($P < 0.001$: ***; $P < 0.01$: **; $P<0.05$: *;)

There were also significant ($P < 0.01$) differences between species in terms of C:N ratios. The highest C:N ratio was found in *H. fomes* (36) followed by *S. apetala* (22), *A. officinalis* (22) and *S. caseolaris* (16) (Table 2.1). However, for the decomposed leaf litter residues, there were significant differences ($P < 0.05$) among the species for all types of nutrients and anti-nutrients, except phytate (Table 2.2). *Heritiera fomes* leaf litter was the highest in crude fibre (33% DM), OM (96% DM), C (48% DM), tannin (1.8% DM) and saponin (1.6% DM) content, whereas *S. apetala* was lowest for all those parameters except for tannin. Among the other species, *S. caseolaris* was the highest in N content (2.8% DM), *A. officinalis* was lowest in P (.01% DM) and saponin (1.2% DM) content, the latter being similar to saponin contents in *S. caseolaris* and *S. apetala* (Table 2.1).

There were also significant differences ($P < 0.001$) in decomposition rate among the species after four-week incubation in the shrimp rearing tanks (Table 2.3). The highest decomposition rates (1.8% DM d^{-1}) were found for *S. apetala* and the lowest were for *H. fomes*. Accordingly, the highest percentages of OM (57%), C (57%), N (58%), P (73%) and tannin (64%) losses occurred from *S. apetala* leaves. *Heritiera fomes* leaves showed the lowest loss in percentages. For *S. apetala*, degraded leaves had the lowest OM (77%), C (38%) and P (0.01%) content. *Heritiera fomes* had the highest OM (96%), C (48%), P (0.02%), tannin (1.64%) and saponin (1.29%) content. *Avicennia officinalis* was found to be higher in decomposition rate and mass loss than *S. caseolaris*. As a result, *A. officinalis* was found with higher nutrient and anti-nutrient loss than *S. caseolaris* except for saponin.

3.2. Impact of leaf litter decomposition on water quality

No differences ($P > 0.05$) in water quality parameters were found between the tanks treated with the different mangrove species except for temperature, BOD and phytoplankton concentration (Table 2.4).

Table 2.4

Average water quality parameter values observed in shrimp PL rearing tanks during a four-week incubation period, with leaf litter from four different mangrove species.

Water quality parameter	**Mangrove species**				**S.E.M.**	***P*-value**
	H. fomes	*A. officinalis*	*S. caseolaris*	*S. apetala*		
Temperature (^{0}C)	28.01^b	27.99^b	27.98^b	27.93^a	0.01	***
pH	7.87	7.89	7.91	7.94	0.01	ns
DO (mg L^{-1})	5.36	5.43	5.38	5.38	0.10	ns
BOD (mg L^{-1})	1.98^a	2.34^c	2.13^b	2.41^d	0.05	***
COD (mg L^{-1})	40.0	48.9	45.6	46.7	1.71	ns
TAN (ppm)	0.13	0.1	0.1	0.1	0.01	ns
NO_2-N (ppm)	0.07	0.27	0.17	0.23	0.03	ns
Phytoplankton (cells ml^{-1})	2.50^a	5.83^b	4.17^{ab}	9.17^c	0.80	***
Zooplankton (cells ml^{-1})	2.50	5.0	3.33	5.0	0.57	ns

Small letter on the superscript indicate significant differences, 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, $P > 0.05$).

The temperature of tank water ranged from 27.9-28.0 ^{0}C where the temperature in the tank waters incubated with *S. apetala* was slightly but significantly different (*P* < 0.001) from the other three species of mangrove leaf litter. The highest BOD was measured in the tanks with *S. apetala* leaves (2.41 mg L^{-1}) and the lowest was measured in tanks with *H. fomes* litter (1.98 mg L^{-1}). The BOD in the tanks with *H. fomes* litter was lower (*P* < 0.001) than for the other mangrove species. There were also significant differences (*P* < 0.001) in phytoplankton concentrations between tanks treated with different mangrove species. The highest concentration of phytoplankton was found with *S. apetala* (9.2 cells ml^{-1}) and the lowest number with *H. fomes* (2.50 cells ml^{-1}). In general, tank waters with *H. fomes* had lower concentrations for leachates, except for TAN. The highest pH was measured in the tanks with *S. apetala* leaves (7.94) and the lowest was measured in tanks with *H. fomes* litter (7.87). DO levels also showed no significant differences (*P* > 0.05) between tanks incubated with the different leaf litter species. The COD was the highest in tanks with *A. officinalis* (49 mg L^{-1}) and the lowest with *H. fomes* (40 mg L^{-1}) but no significant difference could be demonstrated (*P* > 0.05). The TAN concentrations were higher in tanks with *H. fomes* litter (0.13 ppm) but lower for those with *S. apetala* (0.1 ppm). For neither TAN nor NO_2-N concentrations were there significant differences (*P* > 0.05) in concentration among the mangrove species. There were also no significant differences in zooplankton concentrations between the different mangrove treatments (*P* > 0.05).

3.3. Impact of decomposing leaf litter on PL survival, weight gain and specific growth rate (SGR)

The survival rate in the tanks with leaf litter ranged between 75% and 82% (Fig. 2.1a) and did not differ significantly between mangrove leaf treatments (*P* > 0.05). In contrast, the shrimp PL in the control treatment without any leaf litter started to die on day 3 and on day 8 all the shrimp PL had died (Fig. 2.1b).

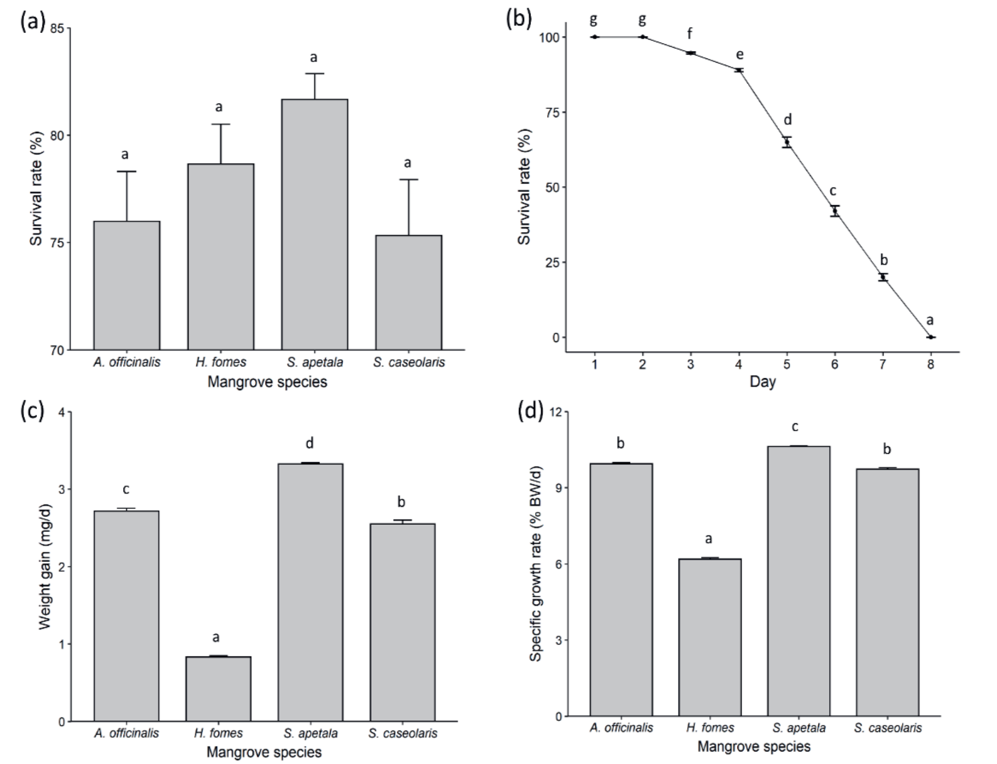

Fig. 2.1. (a-d): Impact of leaf litter of four selected mangrove species on shrimp performances: (a) Survival rate (%) of Shrimp PL with four types of leaf litter, (b) Survival rate (%) decreased with time (day 01 to day 08) in the control (without any leaf litter), (c) Weight gain (mg d^{-1}) of shrimp PL with four types of leaf litter, (d) SGR (%BW d^{-1}) of shrimp PL with four types of leaf litter; Values are means (± SD) of three replicate tanks per treatment. Different letters above data points indicate significant differences.

The growth rates of shrimp PL did differ significantly (P < 0.05) depending on the mangrove species used in the tanks. The average daily growth rate was highest for the larvae incubated with *S. apetala* leaf litter and the lowest for those incubated with *H. fomes* litter (Fig. 2.1c). The SGR was highest for larvae reared with *S. apetala* (10.6) leaf litter and lowest in larvae reared with *H. fomes* (6.2) (Fig. 1d) (P < 0.05). This concurred with the highest final shrimp size reached in *S. apetala* tanks and lowest in *H. fomes* tanks.

Significant correlations ($P < 0.05$) were identified between different pairs of variables. We found a negative correlation between crude fiber content and decomposition rate of leaf litter, and positive correlations between decomposition rate of leaf litter and BOD, mass loss of leaf litter and phytoplankton concentration, and PL weight gain and phytoplankton concentration (Fig. 2.2).

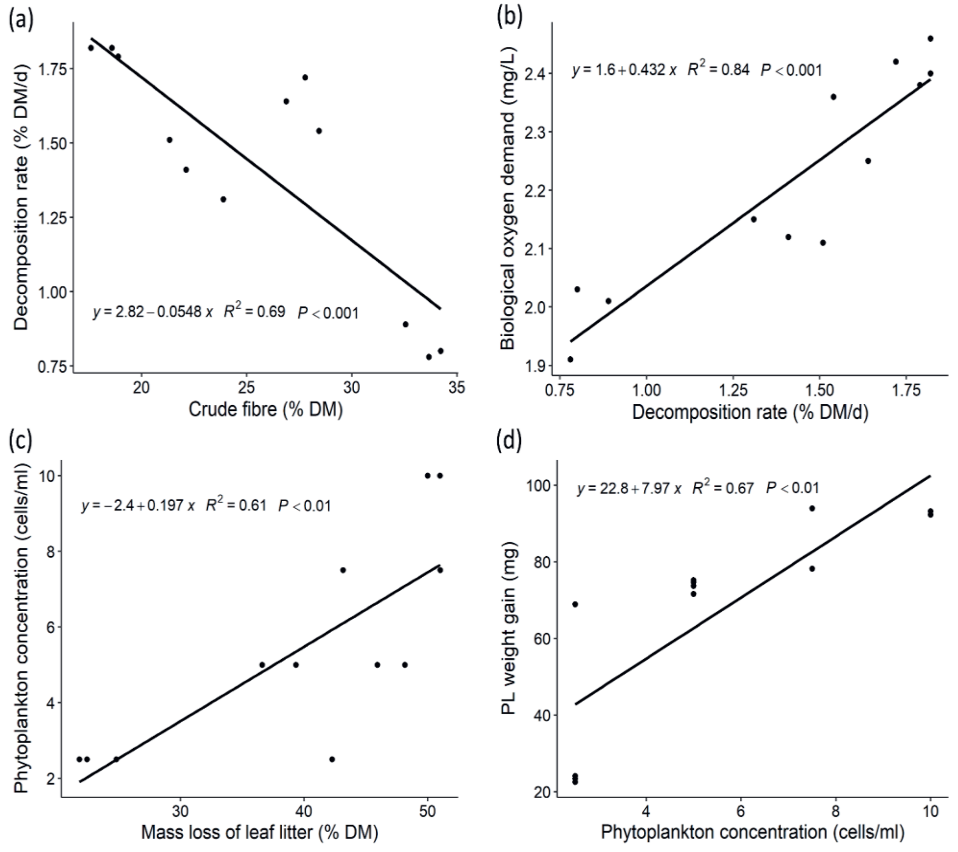

Fig. 2.2. (a-d): Linear regression of (a) Crude fiber content in leaf litter and decomposition rate, (b) Decomposition rate and biological oxygen demand, (c) Mass loss of leaf litter and phytoplankton concentration, (d) Phytoplankton concentration and PL weight gain.

4. Discussion

4.1. Decomposition, mass loss and biochemical changes (nutrient and anti-nutrient composition) in leaf litter

In this study we found differences in the biochemical composition of freshly fallen leaf litter of different mangrove species (Table 2.1). Mangrove leaves vary in their organic and inorganic constituents according to species, age, season and physical or morphological characteristics of the leaves (Hossain et al., 2011; Basak et al., 1998, 1996; Tam et al., 1998). The leaves of the mangroves species studied differed in their tendencies to lose mass and release biochemical components during decomposition (Table 2.3). Rajendran and Kathiresan (2000) previously studied biochemical changes in decomposing leaves of two mangrove species, *Rhizophora apiculata* and *Avicennia marina,* and found that different rates of leaf decomposition between species led to different rates of mass loss of the decomposing leaves. This was in part due to the rapid leaching of water-soluble organic and inorganic substances during the initial stages of the decomposition process (Hossain et al., 2009; Ashton et al., 1999) and to microbial breakdown (Hossain et al., 2014). We observed lower mass loss for *H. fomes* among the four studied species. Hossain et al. (2014) observed the similar tendency of mass loss for *H. fomes* in comparison to three other mangrove species *Excoecaria agallocha, Ceriops decandraand* and *Xylocarpus mekongensis* from the Sundarbans. The variation in crude fibre contents (% DM) might be a determinant of variation in decomposition rate. In our study, the crude fibre content was highest in *H. fomes* (33%) and lowest for *S. apetala* (18%), and negatively correlated (Fig. 2.2a) to the decomposition rate of the different species of mangrove litter, as also previously reported by Du et al. (2020) and Ibrahima et al. (2008).

4.2. Impact of dry matter, carbon, nitrogen and phosphorous loss from mangrove leaf litter on water quality and shrimp performance

Mangrove leaf litter is an important source of organic matter in tropical and subtropical aquatic environments, supporting the microbial-based food web and providing natural food to PLs (Gatune et al., 2014; Nga et al., 2006). Considering the efficiency (30-36%) of microbial conversion of the portion of mangrove leaves lost to decomposition, it appears that a significant percentages of mangrove detritus is relatively rapidly assimilated into microbial biomass and thus potentially available to the aquatic food web (Benner et al., 1986). Mangrove litter releases nutrients and supports periphytic biofilm growth, a good food source for PLs (Gatune et al., 2012). In our study, the mangrove species with higher decomposition rates contributed more nutrients through mass loss in the shrimp culture tank (Table 2.3). Faster weight loss by the leaves meant that more organic and inorganic compounds became available for microbiota development (Wetzel, 1995), resulting in better PL growth, and illustrated by the positive correlation between leaf litter mass loss and PL weight gain (Fig. 2.2c). The results clearly showed that the mangrove litter supplied to the tanks served as a needed food source for the PL. Decomposing mangrove leaf litter stimulates natural food production (Rejeki et al., 2019; Nga et al., 2006). Natural food can contribute up to 50-70% of nutritional requirements of shrimp held in culture ponds (Martinez-Cordova and Enriquez-Ocana, 2007; Enriquez, 2003; Tacon, 2002). Thus the natural food produced from decomposed leaf litter helped the PLs to survive and gain weight. It cannot be excluded that the difference in survival rate between treatment with leaf litter and the controls without leaf litter could have partially been due to the leaf litter serving as shelter and reducing cannibalism (Hai and Yakupitiyage, 2005)

We found no significant differences ($P > 0.05$) in water quality parameters between the types of leaf litter, except for biological oxygen demand (BOD mg L^{1}) and algal biomass (cells ml^{-1}). A higher BOD indicated more decomposition and conversion of litter into phytoplankton as shown by significant correlations between leaf litter mass loss and phytoplankton concentration, and between PL weight gain and phytoplankton concentration (Fig 2.2 (b-d)). In our study large quantities of leaf litter were available in comparison to the PL biomass, supporting PL production, while the water quality remained good. Clearly, in cases with much higher litter stocking densities or lower levels of aeration, the PLs could just as well have experienced detrimental conditions, leading to higher mortality by sudden depletion of DO (Rejeki et al., 2019; Nga et al., 2006; Hai and Yakupitiyage, 2005). Hence, while our results show a positive effect of mangrove litter, the outcome of leaf litter addition is situation-specific, so the results cannot be generalized. In our experiment, aeration kept the water volume in rearing tanks aerobic. The PLs prefer a well oxygenated environment, which is found in estuaries. The shallow water in estuaries ensure increase of DO concentration in water through constant wave action (Bozkurt and Kabdasli, 2013). An estuary on a mangrove coast provides a lot of food, substrate and protection to young penaeids (Vance et al., 1990; Zimmerman and Minello, 1984). In our results, higher BOD and algal biomass, were found in tanks treated with *S. apetala* litter followed by *A. officinalis, S. caseolaris* and *H. fomes*. This differences might be due to the quality of organic matter in the water as influenced by decomposition of leaf litter, as indicated by the BOD:COD ratio (Rojas-Tirado et al., 2017). There was a strong positive correlation ($P < 0.01$; r=0.820) between OM and BOD. As the water quality was not affected by mangrove species, no significant difference in survival rate of the shrimp was observed.

One limitation of our experiments is that the duration (4 weeks) was short, allowing only limited time to develop any potential negative effects of organic matter decomposition. Sustainable accumulation and decomposition of organic matter might lead to a decline of water quality, cause stress, reduce growth and increase the susceptibility to disease and mortality of fish and shrimp (Jackson et al., 2003). Therefore, additional studies are needed to look at the longer-term effects of prolonged accumulation of organic load so as to develop insight into how to benefit from mangrove leaf decomposition without experiencing its potentially negative effects at higher leaf densities and for longer periods of exposure. Considering the positive effect of *Sonneratia apetala* leaf litter, it is recommended to perform a leaf litter dose-response follow-up experiment for this mangrove species.

4.3. Impact of tannin, saponin and phytate from mangrove leaf litter on water quality and shrimp performance

Along with nutrients, tannin, phytate and saponin were released in the shrimp PL rearing tank through decomposition of the leaves. Fitzgerald (1999) reported that higher concentrations of tannin might be toxic to shrimp in silvo-aquaculture systems. Hai and Yakupitiyage (2005) identified higher amounts of tannin (ranged 8.2-28.7 mg L^{-1}) in the water column leached from leaves of *R. apiculata*, *A. officinalis*, *Excoecaria agallocha* and *Acacia auriculiformis* in shrimp experimental tanks and their effects on shrimp growth and survival depending on the loading rate of leaves and leaf concentrations of tannin. However, some researchers also stated that anti-nutrients sometimes act as non-nutritive compounds with positive effects. For instance, Sudheer et al. (2011) found the mangrove species *Ceriops tagal* to be effective against white spot syndrome virus (WSSV) disease of shrimp. Thus, the resistance properties of tannins to microbial degradation and their anti-bacterial, antiviral, antifungal activity (Krzyzowska et al., 2017) might be interesting topics for further research. As we found considerable concentrations of tannin in leaf litter of all four species in comparison to other nutrients (N, P) and anti-nutrients (phytate, saponin) it might also be interesting to have a challenge test to study how tannin help protect against shrimp diseases. Though anti-nutrients have been found to impact the water quality and shrimp performance in other studies (Rejeki et al., 2019), we found no significant impact based on the (lower) litter densities used, the time frame of the growth experiment and the level of aeration used in our study.

Other work also suggests that phytate affect PL performance by affecting the mineral utilization and reducing enzymatic activities in postlarvae (Gemede and Ratta, 2014). On the other hand, phytate sometimes plays a positive role by supplying available P through breakdown of phytate-P (Kumar et al., 2012). Though termed anti-nutrients, saponins also sometimes play a positive role (Freeland et al., 1985). Saponins increase digestibility of carbohydrate-rich food because of their detergent-like activity by reducing viscosity and preventing obstruction of movement of digesta in fish intestines (Hajra et al., 2013) and possible also for shrimp PLs. Our results show a positive correlation ($P > 0.05$; r=0.016) between phytates and weight gain. This suggests but does not prove a causal relationship. The correlation ($P > 0.05$; r = -0.086 (survival), r=-0.319 (weight gain)) between saponin and shrimp performances (survival and weight gain) was negative but insignificant. As a result, there was no mentionable negative impact of phytate and saponin contents on the shrimp performances.

Considering the overall impact of nutrients and anti-nutrients of mangrove leaf litter on shrimp PL performance in this study, it appears that the leaf litter of selected mangrove species can be of use to enhance shrimp survival and growth performances.

5. Conclusions and recommendations

There is an urgent need to develop more sustainable and ecologically and socio-economically resilient approaches to food production. This is particularly the case for vulnerable tropical muddy coastlines where mangrove vegetation has been cleared in the past for large-scale shrimp pond culture. A case in point are the Sundarbans-associated muddy mangrove coasts of Bangladesh, the country that has worldwide been shown to be most vulnerable to climate change risks (World Bank, 2018). In this study we demonstrate positive effects of different species of mangrove leaves on water quality, shrimp growth and survival rate under controlled conditions at a concentration of 1 g (fresh leaves) L^{-1}. *Sonneratia apetala* was found to perform better in terms of nutrients return to the aquatic environment through mass loss during decomposition and gave the most positive effect on shrimp growth rate. *Heritiera fomes* showed positive effects on survival but (compared to control tanks without mangroves) but growth was the lowest of all four species tested. *Avicennia officinalis* and *S. caseolaris* showed similar and intermediate growth performance of shrimp PL. Finally, to introduce silvo-aquaculture using the studied species we recommend further research on:

1. How to optimize growth performance by combining supplementary feeding with leaf litter addition;
2. The performances of PL using mixed mangrove species leaf litter;
3. The effect of mangrove litter in ponds as compared to tanks.

Acknowledgements

This study was funded by NWO-GCP-WOTRO-ARF program and SOLIDARIDAD NETWORK ASIA. Thanks are due to Roel H Bosma, Project manager aquaculture & Assistant professor sustainable production systems (retired), Aquaculture and Fisheries Group, Wageningen University and Research for his initial support in organizing the project on which this study is based. Thanks are also due to the Ministry of Fisheries and Livestock, the Government of the People's Republic of Bangladesh for allowing the lead author leave from work to pursue his PhD research. Finally, thanks are also due to the laboratories of Forestry and Wood Technology, Pharmacy, Fisheries and Marine Resource Technology discipline in Khulna University for allowing to use their facilities.

Chapter 3

Synergistic effects of mangrove leaf litter and supplemental feed on water quality, growth and survival of shrimp (*Penaeus monodon*, Fabricius, 1798) postlarvae

This chapter has been published as:

Alam, M.I., Debrot, A.O., Ahmed, M.U., Ahsan, M.N., Verdegem, M.C.J., 2021. Synergistic effects of mangrove leaf litter and supplemental feed on water quality, growth and survival of shrimp (*Penaeus monodon*, Fabricius, 1798) postlarvae.

Aquaculture 545, 736865 . https://doi.org/10.1016/j.aquaculture.2021.737237.

Abstract

Shrimp based mangrove-aquaculture (silvo-aquaculture) is practiced in many countries of the world and leaf litter of different mangrove tree species is a potential nutrient source in these systems. The present study evaluated the effects of mangrove leaf litter from four mangrove species (*Sonneratia apetala*, *S. caseolaris*, *Avicennia officinalis* and *Heritiera fomes*) on the production of juvenile shrimp (*Penaeus monodon*) with and without supplemental feed. Fifteen-day-old postlarvae (PL_{15}) with an average weight of 0.01g were reared in 1100 L fibre-reinforced polyethylene tanks containing 1000L of 10 ppt saline water and a water depth of 0.9 m. Leaf litter with or without supplemental feed was applied to the tanks according to a 4×2 factorial design. The PLs were stocked at a density of 100 per tank and the experiment was conducted for 4 weeks without any exchange of water. Both mangrove species and feed application affected shrimp performance and water quality parameters except dissolved oxygen (DO), chemical oxygen demand (COD) and zoo-plankton concentration. The average survival rate of juvenile shrimp ranged from 86-94% in the treatments with both leaf litter and feed, from 75-82% in the treatments with only leaf litter and 88% in the treatment with only feed. However, 100% mortality was observed in the treatment without any leaf litter or supplemental feed. Combined, leaf litter and feed resulted in 21 to 33% higher weight gain of shrimp PL than based on the combined contributions of leaf litter only or feed only, indicating synergism. Among the different mangrove species, *S. apetala* (23.1%) contributed the highest to total weight gain followed by *A. officinalis* (21.6%), *S. caseolaris* (21.6%) and *H. fomes* (10%). The lower feed conversion ratio (FCR) (0.18-0.27) in the treatments combining leaf litter and supplemental feed as compared to the feed-only treatment (0.41) indicated that leaf litter (directly or by stimulating natural food production) contributed to supplemental feeding. The growth of phytoplankton also appeared to contribute in low FCR as evidenced by a positive correlation ($P < 0.001$, r=0.681**) between phytoplankton concentration and shrimp weight gain. The synergistic effect between leaf litter and supplemental feed can help the farmer to minimize the shrimp production cost by lowering the feed input and enhancing mangrove three coverage on pond dikes as an inexpensive source of natural food.

1. Introduction

Mangroves are highly productive ecosystems in terms of primary and secondary productivity in the coastal waterbodies of the tropics and subtropics. Mangrove roots and fallen leaf litter provide substrate for biofilm production, and provide nutrients to the water column which stimulate productivity (Gatune et al., 2014, 2012; Reef et al., 2010; Verweij et al., 2008; Nordhaus et al., 2006). The leaves of mangrove trees enter the detritus pathway and substantially contribute to aquatic food webs supporting fisheries production (Hutchison et al., 2014). However, for leaves to contribute to productivity, the litter needs to go through various decomposition steps (Hutchison et al., 2014) that starts with the leaching of soluble compounds accompanied by microbial decomposition. The entire process is accelerated by shredders like crabs and other animals that feed directly on leaf litter, making it more accessible to adjacent fish communities (Kamruzzaman et al., 2019). Because mangroves support aquatic production, mangrove-based aquaculture systems, alternatively termed silvo-aquaculture have been developed in many Asian countries such as the Philippines (Aypa and Bagonguis, 2000), Indonesia (Sukardjo, 2000), Vietnam (Binh et al., 1997; Johnston et al., 2000), Thailand (Tanan and Tansutapanich, 2000), Myanmar (Win, 2000) and Malaysia (Sze and Ahmad, 2000). As aquaculture is considered as one of the main causes of the destruction of mangroves, silvo-aquaculture systems represent a more integrated approach to pond culture and may simultaneously help to conserve mangrove resources and enhance economic benefits to coastal communities where aquaculture is important (Fitzgerald, 2000). Different systems of silvo-aquaculture are practiced (Bosma et al., 2014; Primavera et al., 2007; Primavera, 2000). Most silvo-aquaculture systems are extensive, mainly relying on natural food produced from fallen mangrove leaves (Rejeki et al., 2020, 2019; Nga et al., 2006; Nga and Roijackers, 2002). In contrast, formulated feed is the most energy demanding and costly input used to enhance shrimp production. However, apart from being too costly for small-scale farmers, use of formulated feed has been associated with water pollution due to excess use of feed (Islam and Bhuiyan, 2016; De Schryver et al., 2008; Tacon, 2002). Some strategies have been evaluated worldwide to minimize the problem, one of which is the promotion and contribution of natural food (Porchas-Cornejo et al., 2010). Mangrove leaf litter is a natural food for shrimp (Gatune et al., 2014, 2012; Nga et al., 2006; Hai and Yakupitiyage, 2005). In addition, litter can function as a shelter against predation (Nga et al., 2006, Hai and Yakupitiyage, 2005). Therefore, the combination of natural food and formulated feed should have a positive effect on all the production parameters of shrimp as observed by Porchas-Cornejo et al. (2012). The combined effect, or synergy can be identified by measuring the individual and combined effects of leaf litter and formulated feed. A positive synergistic effect, if any, would make shrimp aquaculture more productive in an environmentally friendly way. Such synergy could also help to align interests of farmers and mangrove restoration, and could be an effective way to minimize the conflicts between shrimp culture and mangrove loss (Ahmed et al., 2017; Bosma et al., 2014; Primavera, 2000). While there are potential benefits of mangrove leaf litter there are also potential detrimental effects, including the release of anti-nutrients from the leaves during decomposition, decreased oxygen levels and increased Biological oxygen demand (BOD), Chemical oxygen demand (COD) and nitrite and total ammonium nitrogen (TAN) concentrations (Nga et al., 2006; Hai and Yakupitiyage, 2005; Nga and Roijackers, 2002). However, the positive and negative effects of mangrove leaf litter, as well as any synergistic effects, might differ depending on the species of mangrove.

Considering the above, the present research investigated the effect of leaf litter from different mangrove species and formulated feed on shrimp production and water quality.

2. Methodology

2.1. Experimental design

The experiments were carried out at a farm located in Debhata, Satkhira, on the northern rim of the Sundarbans mangrove area in Bangladesh. They took place under ambient conditions, with rearing tanks covered with a transparent plastic roofing that allowed avoidance of 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 4x2 factorial design with mangrove tree species (*Avecennia officinalis*, *Sonneratia apetala, S. caseolaris* and *Heritiera* fomes) as source of leaf litter serving as the first factor and food (with or without formulated feed) as the second factor (Table 3.1). In addition, there were two control treatments, one receiving only formulated feed and another receiving neither feed nor leaf litter. All treatments were executed in triplicate. The eight leaf litter treatments were analysed as a factorial experiment. The two additional treatments, were used to explore synergy between formulated feed and leaf litter addition as well as to assess the effect of mangrove leaf litter on shrimp performance.

Table 3.1

Design of experiment with treatment type.

Tanks with mangrove leaves				
Feeding type	Mangrove species			
	S. apetala	*S. caseolaris*	*A. officinalis*	*H. fomes*
Feed	Sa-F	Sc-F	Ao-F	Hf-F
No Feed	Sa-nF	Sc-nF	Ao-nF	Hf-nF
Tanks without mangrove leaves				
F				
nF				

Sa-F=*S. apetala* leaf litter and feed*,* Sc-F=*S. caseolaris* leaf litter and feed*,* Ao-F=*A. officinalis* leaf litter and feed*,* Hf-F= *H. fomes* leaf litter and feed*,* Sa-nF=*S. apetala* leaf litter and no feed*,* Sc-nF=*S. caseolaris* leaf litter and no feed*,* Ao-nF=*A. officinalis* leaf litter and no feed*,* Hf-nF= *H. fomes* leaf litter and no feed F= Feed only, nF= no feed;

The shrimp were reared in 1100 L fibre-reinforced polyethylene tanks containing 1000 L of brackish water (salinity of 10 ppt) with a water depth of 0.9 m. Brackish water collected from a nearby canal was stocked in a pond and left to settle for one week. The top layer of water from this pond was transferred to the experimental tanks through a screen with 25 µm mesh size net to keep predators and their eggs and larvae out. Each tank was aerated using a single air stone (diameter 2 cm) connected to an air blower (RESUN, LP-100). Mangrove leaf litter was directly added in the culture tanks at a concentration of 1g L^{-1} (wet weight). This loading rate was standardized following Hai and Yakupitiyage (2005). On the same day, 100 specific pathogens free (SPF) shrimp postlarvae (PL) of 15 days old with an average weight of 0.01 g obtained from Desh Bangla Shrimp Hatchery, Batiaghata, Khulna, were stocked at a rate of 1 PL /10 L of water in each tank. The experiment assessing growth and survival was conducted over a four-week period and the water was not exchanged during the experiment. The survival and growth indices were calculated only at the end of the experiment.

2.2. Selection of mangrove species and collection of leaf litter

Selection of mangrove species was done following Rahman et al. (2020). Senescent leaves that fell down naturally, after changing color from greenish to yellowish, were collected from the selected mangrove species in the Sundarbans mangrove forest. The traps were 2 by 2 m, and installed beneath the selected mangrove species during winter (November 2018-January, 2019). At regular intervals, the fallen leaves were recovered from the traps, separated by species and prepared for use in the experiment. The decomposition rates (% day^{-1}) of the selected mangrove species (*A. officinalis*= 1.6; *S. apetala*= 1.8; *S. caseolaris*= 1.4; *H. fomes*= 0.8) as identified by Alam et al. (2021a), were expected to affect shrimp growth in fed and non-fed systems.

2.3. Feeding the shrimp PL and calculation of FCR

Shrimp growth was monitored weekly in the control treatment that received only feed at 5% body weight per day, and these data were used to adjust the feeding rate for all treatments. Feed "Titas Tiger" from Bismillah Feed Mills Limited, Mollahat, Bagerhat, with 12% of moisture, 36% of protein, 10% of lipid, 7% of fibre, 18% of ash, 1.9% of calcium and 1.7% of phosphorus, was fed once daily at 5% BW d^{-1}. After harvest, FCR was calculated as the total feed given divided by total shrimp biomass gain.

2.4. Water quality monitoring

Temperature, salinity, pH, and dissolved oxygen (DO) in each tank were measured daily using, respectively, a Hanna (Taiwan) digital thermometer, an Atago (Japan) hand refractometer, a (Eutech) pH meter (Singapore), 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.

Biological oxygen demand (BOD_5) was measured weekly. For this, two water samples were collected from each tank at a depth of 10-30 cm from the surface in 300 ml BOD bottles without collecting air bubbles. In one bottle, DO was fixed following the Winkler procedure to measure initial DO while other bottle was set to incubate for 5 days. Both sample types were analyzed at the Khulna University water quality laboratory. The BOD_5 was calculated by following the method outlined in APHA (1998).

Chemical oxygen demand (COD) was measured bi-weekly. Samples were collected from the middle of the tank at a depth of 10-30 cm from the water surface. The analysis was done following the Open Reflux (OR) method outlined in APHA (1998) at Khulna University.

2.5. Sampling and analysis of plankton

Phytoplankton and zooplankton samples were collected on day 1 and 28. Samples (15 L per sample) were collected at 9.00–11.00 hr from 3 points in each tank and passed through a 45 µm mesh plankton net and combined. The concentrated samples were preserved in plastic bottles with 1ml of Lugol's solution. The abundance estimations of plankton (individual. L^{-1}) were done using a 1 ml Sedgewick-Rafter (S-R) counting chamber. One ml of sample was poured into 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-4x-100x, USA) and identified (where possible to genus level) using 5.1M C-Mount CMOS Camera- Aptina MT9P001 CMOS (Color). Plankton were identified using keys 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 organisms per liter, P = the number of plankton counted in 10 fields, C = the volume of concentrated sample (ml) and V= the volume (in L) of water in the sample.

2.6. Assessment of shrimp postlarvae performances:

Growth and survival indices were calculated at the end of the four-week period using the formula described by Busacker et al. (1990). After harvesting the shrimp juveniles were counted, placed on tissue paper to remove excess water and bulk weighed to calculate the average weight at harvest. Weight gain was calculated by deduction of initial weight from the final weight. Daily weight gain was calculated from final weight gain divided by the number of culture days. The formulae for calculation of feed conversion rate (FCR), survival rate (SR) and specific growth rate (SGR) were:

$$\mathrm{FCR}\ (\mathrm{g\,g^{-1}}) = \frac{\mathrm{Feed_{Tot}}}{\mathrm{WG_{Tot}}}$$

$$\mathrm{SR}\ (\%) = N_f/N_i \times 100$$

$$\mathrm{SGR}\ (\%\ \mathrm{BW\ day^{-1}}) = \frac{\ln(\mathrm{BWf}) - \ln(\mathrm{BWi})}{\mathrm{D}} \times 100$$

where $\mathrm{Feed_{Tot}}$ (g) is the total amount of feed; $\mathrm{WG_{Tot}}$ (g) is the total weight gain between stocking and harvesting; N_f is the number of juvenile shrimp collected at final harvest; N_i is the number of PLs stocked; BW_f is the final average body weight (g); BW_i is the initial average body weight (g); and D is the duration of the experiment (day).

2.7. Calculation of synergy between feed and leaf litter

The calculation of individual and synergistic contributions of leaf litter and feed was done based on total weight gain in shrimp juveniles. The calculation was done as follows:

$$\text{Contribution of leaf litter (\%)} = \frac{\text{Total weight gain with leaf litter (g)}}{\text{Total weight gain with leaf litter and feed(g)}} \times 100$$

$$\text{Contribution of feed (\%)} = \frac{\text{Total weight gain with feed (g)}}{\text{Total weight gain with leaf litter and feed (g)}} \times 100$$

3

Synergistic effect (%) = 100 - (contribution of leaf litter + contribution of feed).

2.8. 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 mangrove species used. A factorial analysis was carried out, with the main factors feed and mangrove leaf litter species and the sampling date as a repeated measure factor using the general linear model (GLM). For the significant differences, a post-hoc Tukey HSD test was used to determine pair-wise differences ($P < 0.05$). Correlations among the different variables were assessed using Pearson's correlation coefficients. Principal component analysis (PCA) was performed using PRIMER 6, to help assess the relationships between environmental parameters based on a reduced number of composite variables. These composite variables are assumed to explain the covariation among the environmental parameters. The first principal component is a linear combination of the environmental parameters which explains as much as possible of the variation between samples. The second principal component, explains as much possible of the remaining variation, and so on. The different principal components are independent, unitless and normalized with a mean equal to 0 and a variance equal to 1. The meaning of each component was interpreted based on the relative size and sign of the coefficients of the regressions indicating the importance of each variable. The effect of the environmental parameters on PL performance (weight gain and survival) was analyzed with distance based linear models (DistLM) in the PRIMER 6 package.

3. Results:

The experimental results showed the significance of leaf litter of mangrove species as source of natural food for the shrimp PL. A positive effect of both mangrove species ($P < 0.05$) and feed ($P < 0.001$) was observed for survival rate, but there was no interaction effect ($P > 0.05$) between the two factors. The survival rate ranged from 76-94% where the highest survival rate was observed for Sa-F and the lowest was for Sc-nF (Fig. 3.1a). Though there was a higher survival (85-94%) for leaf litter and supplemental feed combined, there was also good survival (75-81%) in the treatments with only leaf litter (Fig. 3.1a). In the treatment with feed only, the survival was 88% but in the treatment without leaf litter or feed all the shrimp died before day 8. For individual weight gain and SGR, there was a significant interaction between mangrove species and feed ($P < 0.001$). Among the treatments, the highest (0.37 g) average individual body weight gain was recorded in treatment Sa-F and the lowest (0.03gm) in treatment Hf-nF (Fig. 3.1b). The same was observed for SGR (Fig. 3.1c). The average individual weight gain in the treatment with feed only was 0.17 g. When looking at FCR, Sa-F showed the best performances of all treatments (Fig 3.1d). The highest FCR (0.41) was found for the treatment with formulated feed only whereas the lowest (0.18) was found for treatment Sa-F.

The total weight gain based on feed only was 15.3 g and the total weight gain based on leaf litter ranged from 2.4 to 8.2 g ($P < 0.05$; Table 3.2). The contribution of leaf litter to total weight gain ranged between 10 and 23%. The contribution of feed ranged between 43 and 64%. Combined, leaf litter and feed resulted in 21 to 33% higher weight gain than based on the combined contribution of leaf litter alone or feed alone. Among the different mangrove species, Sa contributed most to total weight gain, Hf the least while Sc and Ao at intermediate level (Table 3.2).

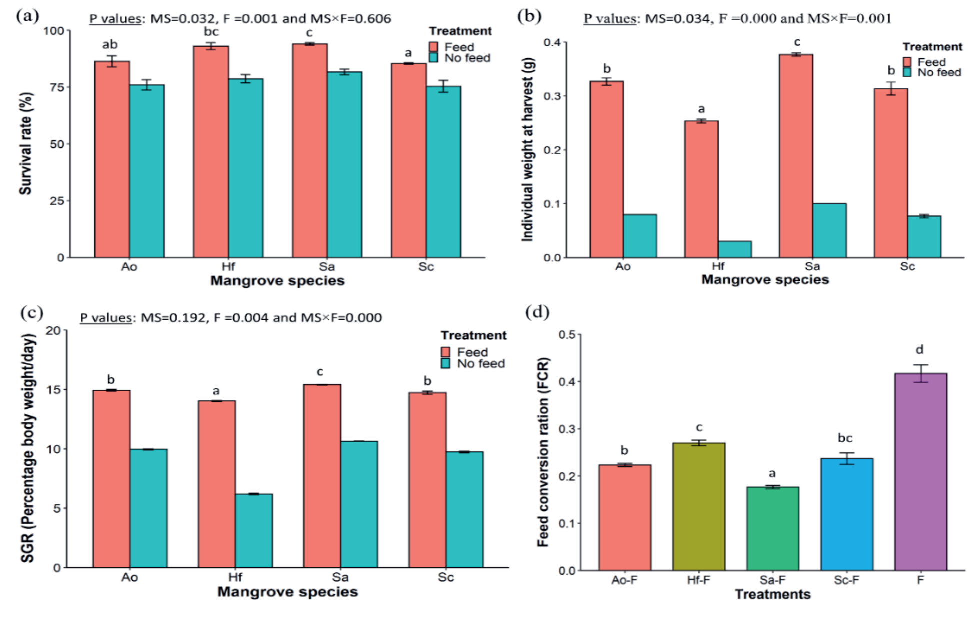

Fig. 3.1. (a-d): The performance of shrimp PL in different treatments with four types of mangrove leaf litter with and without supplemental feed: (a) Survival (%) (b) individual weight (g) at harvest, (c) Specific growth rate (SGR; % BW d^{-1}) and (d) Feed conversion ratio (FCR) in fed treatments, Letter above bars in graphs indicate statisitical differences between leaf litter types (main factor) ($P < 0.05$). The abbreviation to express *P* values used as MS for mangorve species, F for feed and MS x S as interaction term.

Significant main effects were observed for all the water quality parameters except DO, COD and zooplankton concentration (Table 3.3). A significant interaction ($P < 0.05$) between mangrove species and feed was found for BOD_5, while all water quality parameters, except DO, changed over time ($P < 0.05$). The average pH in different treatments ranged from 7.87-7.93 and differed significantly ($P < 0.01$) between mangrove species. The lowest pH was observed in the feed only treatment (Table 3.3). The pH in different treatments was affected ($P < 0.01$) by mangrove species but not by feeding ($P > 0.05$). The pH decreased over time in all treatments ($P < 0.001$) (Table 3.3).

Table 3.2

Contribution of leaf and feed in individual weight gain during nursery from PL_{15} to juvenile shrimp for 04-week.

Considered factors	**Mangrove species**				***P*-value**
	Sa	Sc	Ao	Hf	
Total weight gain with leaf and feed (g)	35.4 ± 0.89^{c}	26.7 ± 1.91^{ab}	28.2 ± 0.96^{b}	23.6 ± 0.98^{a}	***
Total weight gain with leaf litter only (g)	8.2 ± 0.21^{c}	5.8 ± 0.58^{b}	6.1 ± 0.32^{b}	2.4 ± 0.10^{a}	***
Total weight gain with feed only (g)	15.3 ± 1.28				n.a.
Contribution of leaf litter (%) to weight gain	23.1 ± 0.56^{b}	21.6 ± 1.78^{b}	21.6 ± 1.88^{b}	10 ± 0.75^{a}	***
Contribution of feed (%) to weight gain	43.1 ± 3.33^{a}	57.1 ± 2.92^{bc}	54.1 ± 3.16^{b}	64.8 ± 5.36^{c}	**
Synergistic effect (%)	33.8 ± 3.81^{b}	21.3 ± 1.34^{a}	24.2 ± 2.61^{ab}	25.1 ± 5.84^{ab}	*

Presented values are the mean ± SD. Small letter on the superscript indicate significant differences, according to Tukey HSD test (P<0.05). P value is expressed as a symbol (P < 0.001: ***; P < 0.01: **; P < 0.05: *;).

For BOD_5, there were effects of mangrove species and feed (P < 0.05) as well as their interaction (P < 0.001). Among mangrove species, the highest BOD_5 (2.54 mg L^{-1}) was observed for Sa and the lowest (1.92 mg L^{-1}) for Hf (Table 3.3). Overall, the BOD_5 increased with time (P < 0.001), and different mangrove species affected the BOD_5 differently (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 did not (P > 0.05). The TAN concentration increased over time (P < 0.001), and the increase was more with feed than without feed (FxT, P < 0.05) (Table 3.3).

Table 3.3

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						
	Sa	Sc	Ao	Hf	Yes	No	MS	F	MSXF	Time (T)	MSXT	FXT	MSXFXT
pH	7.93 ± 0.02^{b}	7.91 ± 0.01^{ab}	7.89 ± 0.02^{a}	7.87 ± 0.03^{a}	7.87 ± 0.06	7.90 ± 0.03	**	ns	ns	***	ns	ns	ns
DO(mg L^{-1})	5.37 ± 0.03	5.34 ± 0.03	5.35 ± 0.04	5.34 ± 0.03	5.35 ± 0.02	5.35 ± 0.04	ns	ns	ns	ns	ns	ns	ns
BOD_5 (mg L^{-1})	2.54 ± 0.14^{c}	2.14 ± 0.03^{b}	2.36 ± 0.06^{bc}	1.92 ± 0.08^{a}	2.28 ± 0.33^{b}	2.20 ± 0.19^{a}	***	*	***	***	***	ns	*
COD (mg L^{-1})	49.5 ± 3.27	45.0 ± 8.88	48.4 ± 6.58	41.1 ± 5.02	43.5 ± 9.47	45.3 ± 5.94	ns	ns	ns	***	ns	ns	ns
TAN (mg L^{-1})	0.15 ± 0.12	0.11 ± 0.09	0.15 ± 0.09	0.19 ± 0.15	0.24 ± 0.11^{b}	0.08 ± 0.07^{a}	ns	**	ns	***	ns	**	ns
NO_2-N (mg L^{-1})	0.27 ± 0.09^{c}	0.19 ± 0.10^{b}	0.25 ± 0.08^{c}	0.17 ± 0.08^{a}	0.22 ± 0.12^{b}	0.20 ± 0.08^{a}	*	**	ns	***	ns	**	ns
Phytoplankton (inds. ml^{-1})	23.3 ± 6.64^{c}	13.8 ± 6.66^{ab}	18.0 ± 7.81^{bc}	9.2 ± 2.58^{a}	17.2 ± 9.81^{b}	11.7 ± 4.92^{a}	***	***	ns	***	***	***	ns
Zooplankton (inds. ml^{-1})	4.59 ± 1.88	2.92 ± 1.02	4.17 ± 2.04	2.50 ± 0.00	3.00 ± 1.04	3.96 ± 1.98	ns	ns	ns	***	ns	ns	ns

Presented values are the mean ± SD. Small letter used as superscript indicate significant differences 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, $P > 0.05$).

3

Table 3.4

Pearson's correlations among different important variables. The following parameters from 9 treatments, with 3 replicates each (n =27), were included in the analysis: pH, DO, BOD_5, COD, TAN, NO_2-N, phytoplankton, zooplankton, weight gain and survival rate. The parameters DO, zooplankton and survival rate are not shown because they did not show any significant correlations.

		pH	BOD_5	COD	NO_2-N	Weight gain
BOD_5	Pearson Correlation	0.646**				0.335
	Sig. (2-tailed)	0.000				0.088
COD	Pearson Correlation	0.576**	0.650**			0.212
	Sig. (2-tailed)	0.002	0.000			0.289
TAN	Pearson Correlation	-0.473*	-0.258	-0.301	0.024	0.484*
	Sig. (2-tailed)	0.013	0.194	0.127	0.904	0.010
NO_2-N	Pearson Correlation	0.425*	0.641**	0.238		0.616**
	Sig. (2-tailed)	0.027	0.000	0.233		0.001
Phyto plankton	Pearson Correlation	0.475*	0.795**	0.503**	0.729**	0.681**
	Sig. (2-tailed)	0.012	0.000	0.007	0.000	0.000

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

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^{-1}, never reaching toxic levels. Among the mangrove species, Sa and Ao as source of leaf litter resulted in higher NO_2-N concentrations than Sc and Hf ($P > 0.05$) (Table 3.3).

The three most abundant phytoplankton species were *Cladophora nitellopsis, Closterium tumidium and Pediastrum tetras.* The variation in zooplankton were less and the most abundant species was *Acartia tonsa.* The factors mangrove leaf litter and feeding both affected the phytoplankton concentration ($P < 0.05$). The highest phytoplankton concentrations were observed with Sa leaf litter (23.3 inds. ml^{-1}) and the lowest with Hf leaf litter (9.2 inds. ml^{-1}). Phytoplankton concentrations increased over time, with both leaf litter and feeding causing a faster increase in phytoplankton concentration at the end of the experiment (MS x T and F x T; $P < 0.001$).

3

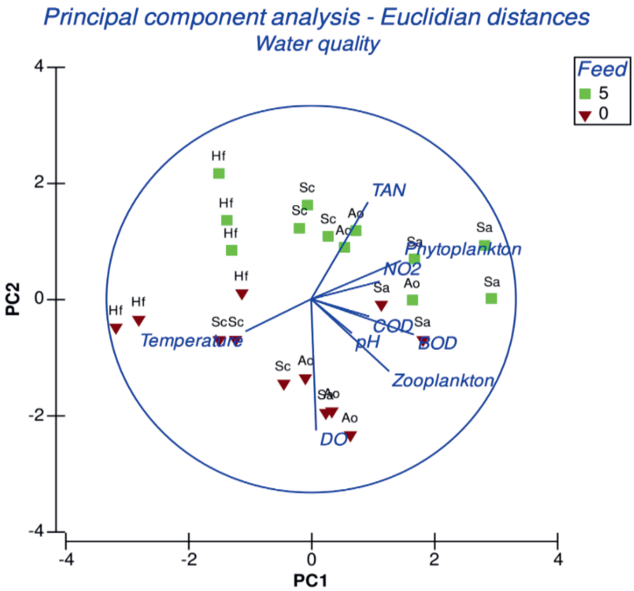

Fig. 3.2. Principal components analysis (PCA) of environmental parameters based on Euclidian distances. PC1 and PC2 = principal component axis 1 and 2, showing effects of Feed (5% bw d^{-1} feed; no feed) and Mangrove Species leaf litter (Sa, *Sonneratia apetala*; Ao, *Avicennia officinalis*; Sc, *Sonneratia casiolaris*; Hf, *Heritiera fomes*).

Pearson correlation analysis among different parameters showed that the majority of variables were correlated (Table 3.4), the nature of which was further analyzed with principal component analysis.

Principal component analysis showed that environmental parameters (Fig. 3.2, Table 3.5) were influenced by both the factors 'feeding' and 'mangrove leaf litter species'. Leaf litter from different mangrove species and feed both provided nutrients which led to a higher density of phytoplankton, and higher BOD_5 in the water column (PC1, Table 3.5). The latter correlated with plankton density causing turbidity and reduced sunlight incidence and hence also reduced water temperature. Differences between mangrove species were responsible for 77% of the variation among treatments for PC1, with Sa and Ao leaf litter resulting in higher phytoplankton concentrations. Feeding and leaf litter input reduced the dissolved oxygen concentration while they increased the TAN concentration in the water column (PC2, Fig. 3.2, Table 3.5), although the average concentration stayed below 0.25 mg TAN L^{-1} (Table 3). The results also show that the effect of feed addition was intermediate between leaf litter addition and leaf litter combined with feed.

Environmental parameters individually accounting for more than 40% of the variation in shrimp performance were phytoplankton abundance, TAN and NO_2 concentrations and temperature (DistLM). Combined, environmental parameters explained 89% of the total variation seen in shrimp performance in terms of average weight gain and survival.

Table 3.5

ANOVA of principal components 1 and 2 for factors Feed and Mangrove Species and multi comparisons (Tukey test).

Principal component	**PC1**		**PC2**	
Temperature	*-0.321*		0.167	
pH	0.198		0.176	
Dissolved oxygen	0.024		**0.679**	
BOD_5	**0.501**		0.184	
COD	0.284		0.089	
Total ammonia N (TAN)	0.277		**-0.502**	
Nitrite (NO_2)	*0.334*		-0.091	
Phytoplankton	**0.438**		-0.199	
Zooplankton	*0.381*		0.373	
Interpretation	Higher plankton biomass contributing to turbidity and biological oxygen demand		Increased oxygen consumption and TAN release due to nutrient inputs	
ANOVA model significance	***		***	
r^2	0.89		0.85	
Variance source	Sign.	%SS	Sign.	%SS
Mangrove species	**	77.0	***	22.2
Feed	***	11.2	*	68.4
Mean multi-comparisons by Feeding				
Feed	a		b	
No feed	b		a	
Mean multi-comparisons by Mangrove Species				
Sonneratia apetala (Sa)	a		ab	
Avicennia officinalis (Ao)	b		a	
Sonneratia caseolaris (Sc)	c		ab	
Heritiera fomes (Hf)	d		b	

4. Discussions

4.1 Synergistic effect of mangrove leaf litter and supplemental feed on shrimp performance

In all treatment combinations of our experiments, survival was above 75%, with on average a 10% higher survival observed in fed treatments (Fig. 3.1a). Using the same concentration (1 g L^{-1}) of mangrove *(Rhizophora apiculata* and *Avicennia officinalis*) leaf litter and 10% BWd^{-1} supplemental feed, Hai and Yakupitiyage (2005) observed 80% survival. The high survival (75-81%) of PL with only leaf litter in our experiments demonstrates that litter directly or indirectly via the food web contributes to the nutrition of the shrimp during their nursery period. Decomposing mangrove leaf litter releases nutrients supporting natural food production (Nga et al., 2006) and microbial biofilm development which in turn is of nutritive value to penaeid shrimp postlarvae (Gatune et al.,2014, 2012). We observed 10-23% contributions by only leaf litter to weight gain, whereby the effect of various mangrove species differed ($P < 0.001$) (Table 3.2). The differences in decomposition rate of organic matter among the species might be the cause of differences in the contributions of leaf litter to weight gain. Mangrove leaf litter with a higher decomposition rate results in more decomposing organic matter or detritus in the system (Alam et al., 2021a). In turn, from this detritus more nutrients are released for algae production (Fazi and Rossi, 2000) which serves as a direct or indirect source of food to heterotrophs (Verweij et al., 2008; Nordhaus et al., 2006; Roijackers and Nga, 2002). Alam et al. (2021a) identified that *S. apetala* (Sa) leaf litter had the highest decomposition rate from among the mangrove species and contributed to the highest shrimp weight gain, as was also found in this experiment. As a consequence, Sa leaf litter in combination with supplemental feed led to more phytoplankton and more synergy. Zooplankton, phytoplankton and bacteria are natural foods for shrimp PL and juveniles (Porchas-Cornejo et al., 2012) that contribute up to 50-70% of the nutritional requirements of shrimp (Martinez-Cordova and Enriquez-Ocana, 2007; Enriquez, 2003; Tacon, 2002). Phytoplankton has been found to be nourishing and even vital to shrimp nutrition during the postlarvae stages (Thong, 2017). Not surprisingly, in our experiment, there was a significant positive correlation between phytoplankton concentration and shrimp production (Table 4). The provision of 1g L^{-1} leaf litter combined with 5% BWd^{-1} supplemental feed in this experiment led to better shrimp growth than in an experiment with *R. apiculata, A. officinalis* and *Excoecaria agallocha* leaf litter and 10% BWd^{-1} supplemental feed conducted by Hai and Yakupitiyage (2005). It should be mentioned, however, that an empirical comparison in this regard is difficult as environmental conditions in both experiments were different.

Commercially-formulated feed was clearly a more complete nutrient source for the PL than mangrove leaf litter and a higher growth was realized based on feed than based on leaf litter (Table 3.2). However, when combined, mangrove leaf litter and supplemental feed resulted in a higher growth rate than expected presumably because of the cumulative effects of leaf litter and feed (Table 3.2). This resulted in a lower FCR in the treatment with leaf litter and feed than the treatment with only feed. Martinez-Cordova et al. (2011) similarly identified that utilization of natural food contributes to the lowering of FCR in shrimp culture. We also found that, in the treatments with leaf litter, lower FCRs were observed in those treatments where more plankton was present.

When feed is applied in excess, it can be detrimental to shrimp production performance by deteriorating water quality (Chainark and Boyd, 2010; Pandit and Nakamura, 2010). However, in our study, in all treatments the water quality stayed within the safe limits though both leaf litter and feeding affected water quality during the four-week period of our experiments and the effects became most pronounced towards the end of the experiment.

4.2 Effect on water quality and PL performance

A dissolved oxygen level lower than 2 mg L^{-1} reduces the growth rates of *P. vannamei* (Seidman and Lawrence, 1985). Allan and Maguire (1991) estimated the lethal level (96 h LC_{50}) of DO for juvenile *P. monodon* is 0.9 mg L^{-1}. The DO level in our study was similar between treatments for survival and growth. With a similar concentration of mangrove leaf litter Hai and Yakupitiyage (2005) observed that DO levels ranged from 4.9-5.0 mg L^{-1} with an aeration regime whereas Nga et al. (2006) observed DO levels to decrease (4.0-0 mg L^{-1}) and mangrove leaf litter leachate concentrations (0-10 g L^{-1}) to increase over time. In our study, all the tanks were aerated, so the outcome of this experiment is relevant to well-managed pond settings with sufficient oxygen.

Leaf litter application affects pH and is in turn affected by mangrove species (Marschner and Noble, 2000; Deano and Robinson, 1985). In our study, the pH differences between treatments were small but significant ($P < 0.05$). The positive correlation between pH and BOD_5 and the slightly higher pH observed in treatments with leaf litter suggest that differences in decomposition rates of the different species of leaf litter caused the observed differences in pH as found previously by Alam et al. (2021a). The pH values observed in our study were within the optimum range (7.5-9.0) for shrimp production (FAO, 1986) and, therefore had little influence on PL performances.

Decomposition of mangrove leaf litter or feed led to significantly differing levels of BOD in the tanks. Decomposition of organic matter not only enhances the microbial loads (Little et al., 2008) but facilitates biofilm development on the decomposing leaf litter (Gatune et al.,2014; 2012)**.** In our study, the BOD_5 was higher in the treatments with leaf litter than in those with supplemental feed only. We found a positive correlation ($r= .795^{**}$; $P > 0.05$) between the BOD_5 and phytoplankton abundance which in turn also positively correlated ($r=.681^{**}$; $P > 0.01$) with shrimp performance (Table 3.5). The mangrove species Sa with the higher BOD_5 concurred with the highest observed shrimp growth while Hf had the lowest BOD_5 and resulted in the lowest growth performance. The differences in decomposition rates of different mangrove species caused the differences in BOD_5 among the treatments (Alam et al., 2021a). Previously, Alam et al. (2021a) found that the BOD_5 and decomposition rate of leaf litter were positively correlated while another work shows that this depends on how refractive the leaves are to biological breakdown (Rojas-Tirado et al.,2017). The higher the BOD, the more rapidly oxygen will be depleted (Banrie, 2012) which might cause stress in non-aerated system (Boyd, 2018). The BOD_5 levels observed in our study were below 25 mg L^{-1}, as recommended by Kasnir et al. (2014).

Both leaf litter and feed are a source of nitrite (NO_2-N) and total ammonium nitrogen (TAN) (Dutra and Ballester, 2017). 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 (Hari et al., 2004). The latter is an intermediate product resulting from microbial nitrification and denitrification (Wickins, 1976). In our study, the positive correlation between pH and BOD_5 suggests that more biodegradable organic matter (OM i.e., BOD) in the water column concurred with a (slightly) higher pH. More TAN correlated with a lower pH and more biodegradable OM (BOD) in the water column as well as with lower nitrite concentrations (Table 3.4). We interpret this as being indicative of better conditions for nitrification and denitrification. Chen and Lei (1990) identified the safe value of TAN and NO_2-N for *P. monodon* juvenile to be 3.7 mg L^{-1} and 3.8 mg L^{-1}, respectively, whereas Banrie (2012) advised to maintain concentrations of nitrite and ammonia below 1 mg L^{-1} and 0.5 mg L^{-1}, respectively, as was the case in our study.

5. Conclusions and recommendations

The present study showed that, when feed and leaf litter are combined, extra growth and survival can be realized by the synergistic effect between leaf litter and feed. Of the four mangrove species tested, *S. apetala* appeared to be the best, followed by *A. officinalis* and *S. caseolaris.* The least effective mangrove species was *H. fomes.* Our results also show how the use of a mangrove species may help increase shrimp pond productivity by providing valuable food input into the pond. Thus, we think that planting mangroves along the margins of shrimp ponds not only serve as an inexpensive source of food but that their presence will provide a higher pond productivity and lower FCR than in fed ponds with no mangrove trees in or adjacent to the pond. However, additional research questions need to be addressed to justify changes in customary practice of coastal shrimp farming in favour of silvo-aquaculture including: (i) what maximum leaf litter concentrations are possible before a high oxygen demand will negatively impact shrimp yields? (ii) what are the long-term impacts of leaf litter on water quality and shrimp performance both under mesocosm conditions and in large culture ponds? Answering these questions will provide practical guidelines for mangrove based silvo-aquaculture to farmers.

Acknowledgements

This study was supported by the Bangladesh Mangrove Polders for Shrimp Aquatic Productivity (Mangrove Polders) Project which received funding from NWO-WOTRO (Contract: W 08.270.347). Thanks are due to Roel H Bosma, Project manager aquaculture & Assistant professor sustainable production systems (retired), Aquaculture and Fisheries Group, Wageningen University and Research for his initial support in organizing the project on which this study is based. Thanks are also due to the Ministry of Fisheries and Livestock, the Government of the People’s Republic of Bangladesh for allowing the lead author leave from work to pursue his PhD research. Finally, thanks are also due to the laboratories of Forestry and Wood Technology, Pharmacy, Fisheries and Marine Resource Technology discipline in Khulna University for allowing to use their facilities.

Chapter 4

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

This chapter has been published as:

Alam, M.I., Ahmed, M.U., Yeasmin, S., Debrot, A.O., Ahsan, M.N., Verdegem, M.C.J., 2022. Effects of mixed leaf litter of four mangrove on shrimp postlarvae (*Penaeus monodon*, Fabricius, 1798) performance in tank and mesocosm condition in Bangladesh.

Aquaculture 551, 737968 . https://doi.org/10.1016/j.aquaculture.2022.737968.

Abstract

Mangrove leaf litter is a potential source of nutrients for shrimp postlarvae. 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 postlarvae 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 postlarvae 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) and SaScAoHf (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 shrimp in 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 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, 2012; Hutchison et al., 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; Nagaranjan 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 change 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 postlarvae 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 the production of juvenile shrimp 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 the 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 4x2 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 4.1). 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^{-1} (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 postlarvae (PL_{15}) were stocked at a rate of 1 PL/10 L of water in each tank. The postlarvae 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 experiment only included fed treatments, hence feed was no longer a factor for comparison (Table 4.1). 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 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 ponds 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 were 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 postlarvae (PL_{15}) obtained from the Desh Bangla Shrimp Hatchery, were stocked in each pond. The experiment was run for a four-week period in dry season (January-April, 2020) without any exchange of water.

Table 4.1

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;					

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, with a 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 hr 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-4x-100x, USA) and identified (where possible to genus level) using 5.1M 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:

$$SR\ (\%) = N_f/N_i \times 100$$

$$SGR\ (\%\ BW\ day^{-1}) = (\ln(BW_f) - \ln(BW_i))/D \times 100$$

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); BW_i 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 80-89% while the highest survival rate was observed for SaAoHf and the lowest for SaScAoHf (Table 4.2). The average survival without application of supplemental feed was 84% (Table 4.2). In the treatment with feed only, the survival 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 4.2). The same was observed for SGR (Table 4.2). 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 4.2). 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. 4.1). 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 4.3). 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.

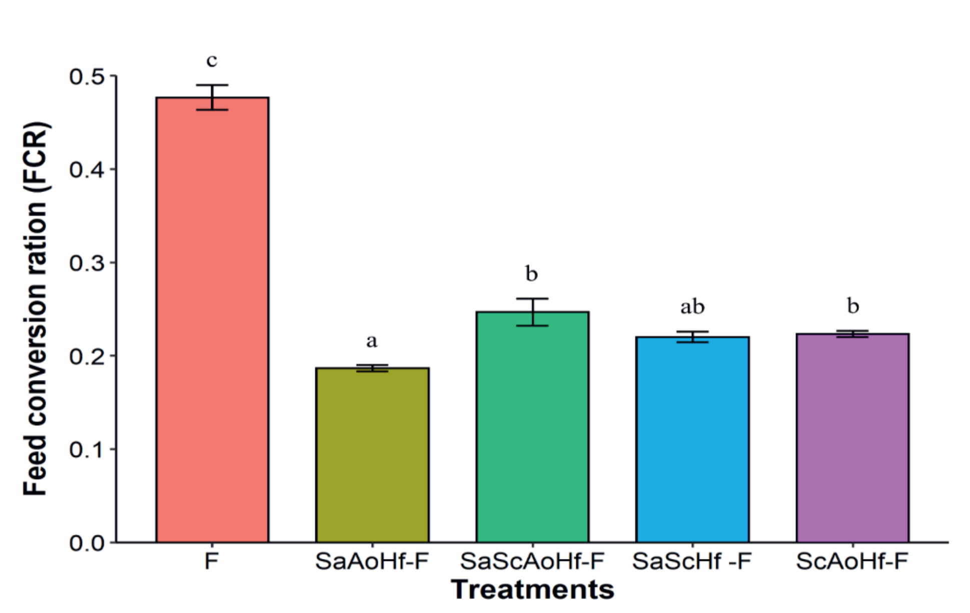

Fig. 4.1. : Feed conversion ratio (FCR) in fed treatments in tank experiment. Letters above bars in graphs indicate statistical differences ($P < 0.05$) between treatments.

Though there were no significant differences ($P > 0.05$) between mangrove species in their contribution (%) to shrimp juvenile weight gain (Table 4.3), the different combinations of mixed leaf litter did give significant difference. SaAoHf contributed the most to total weight gain, ScAoHf the least, with SaScAoHf and SaScHf at intermediate levels (Table 4.3).

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 4.4). 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 4.4) and was affected ($P < 0.01$) by both mangrove species and feeding.

Table 4.2

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^{-1})	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 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, $P > 0.05$). Sa = *Sonneratia apetala* leaf litter; Sc = *S. caseolaris* leaf litter; Ao = *Avicennia officinalis* leaf litter; Hf = *Heritiera fomes* leaf litter; F = Feed only.

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^{-1}) was observed for SaAoHf and the lowest (2.34 mg L^{-1}) for SaScAoHf (Table 4.4). Overall, the BOD_5 increased with time ($P < 0.001$), and different combination of mangrove species affected the BOD_5 differently over time (MS x T, $P < 0.001$), while this was not the case with feeding (F x T, $P > 0.05$).

4

Table 4.3

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^{a}	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, $P > 0.05$). Sa = *Sonneratia apetala* leaf litter; Sc = *S. caseolaris* leaf litter; Ao = *Avicennia officinalis* leaf litter; Hf = *Heritiera fomes* leaf litter;

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 4.4).

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^{-1}, thus never reaching toxic levels. Among the mangrove leaf litter combinations, SaScAoHf was higher in NO_2-N concentrations, followed by SaAoHf, SaScHf and ScAoHf ($P > 0.05$), in that order (Table 4.4).

Table 4.4

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^{-1})	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^{-1})	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^{-1})	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^{-1})	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^{-1})	0.31 ± 0.13[b]	0.29 ± 0.19[ab]	0.25 ± 0.14[a]	0.33 ± 0.13[b]	0.33 ± 0.15[b]	0.20 ± 0.11[a]	*	**	ns	***	ns	**	ns
Phytoplankton (inds. ml^{-1})	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. ml^{-1})	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, $P > 0.05$).). Sa = *Sonneratia apetala* leaf litter; Sc = *S. caseolaris* leaf litter; Ao = *Avicennia officinalis* leaf litter; Hf = *Heritiera fomes* leaf litter; F= only Feed.

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^{-1}) and the lowest with SaScAoHf leaf litter (20 inds. ml^{-1}). 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 experiment

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 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 (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 (72%) was found in the ponds with the mixture of *S. apetala, A. officinalis* and *H. fomes* leaf litter (Table 4.5). 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.

Table 4.5

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^{-1})	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 ($P < 0.001$: ***; $P < 0.01$: **; $P < 0.05$: *). Sa = *Sonneratia apetala* leaf litter; Sc = *S. caseolaris* leaf litter; Ao = *Avicennia officinalis* leaf litter; Hf = *Heritiera fomes* leaf litter; F= only Feed.

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 4.5). 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 (Table 4.5). 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 4.5).

The ponds with leaf litter showed better FCR performances than the ponds applied with feed only (Table 4.5). 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 zooplankton concentration (Table 4.6). 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^{-1}) was measured in the ponds applied with *S. casiolaris, A. officinalis* and *H. fomes* leaf litter and lowest (1.29 mg L^{-1}) 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 4.6). 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^{-1}) were found in the ponds with *S. apetala, A. officinalis* and *H. fomes* leaf litter and the lowest (77 inds. ml^{-1}) 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^{-1}) concentrations were observed in ponds with *S. casiolaris, A. officinalis* and *H. fomes* while the lowest (37 inds. ml^{-1}) 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 4.6).

Table 4.6

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 (^{0}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^{-1})	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^{-1})	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^{-1})	34.17 ± 3.82	23.33 ± 2.89	28.33 ± 2.89	23.33 ± 9.46	26.67 ± 2.89	ns
TAN (mg L^{-1})	-	-	-	-	-	-
NO_2-N (mg L^{-1})	-	-	-	-	-	-
Phytoplankton (inds. ml^{-1})	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^{-1})	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). Sa = *Sonneratia apetala* leaf litter; Sc = *S. caseolaris* leaf litter; Ao = *Avicennia officinalis* leaf litter; Hf = *Heritiera fomes* leaf litter; F= only Feed.

3.3 Soil quality:

The soil quality was quantified before the start of the pond experiment (Table 4.7) and after four weeks when the experiment was terminated (Table 4.8). Before start the experiment, the soil quality of the experimental ponds did not show any significant differences ($P > 0.05$) between ponds (Table 4.7).

Table 4.7

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 are not significantly different according to Tukey HSD test ($P > 0.05$). ns : not significant, $P > 0.05$. Sa = *Sonneratia apetala* leaf litter; Sc = *S. caseolaris* leaf litter; Ao = *Avicennia officinalis* leaf litter; Hf = *Heritiera fomes* leaf litter; F= only Feed.

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 4.8).

Table 4.8

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, $P > 0.05$). Sa = *Sonneratia apetala* leaf litter; Sc = *S. caseolaris* leaf litter; Ao = *Avicennia officinalis* leaf litter; Hf = *Heritiera fomes* leaf litter; F= only Feed.

Pearson correlation analysis among different parameters showed that the majority of variables were correlated (Table 4.9). 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. 4.2).

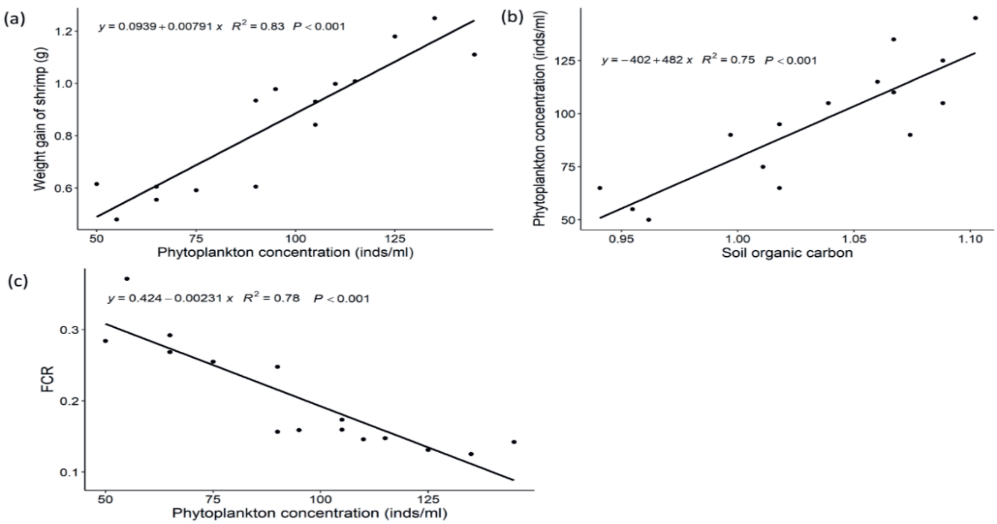

Fig. 4.2. (a-c) : Linear regression of (a) Soil organic carbon and phytoplankton concentration, (b) Phytoplankton concentration and individual weight gain of shrimp, and (c) Phytoplankton concentration and Feed conversion ratio.

Table 4.9:

Pearson's correlations among different important variables (*n* = 15).

	Weight gain	Survival	pH	BOD_5	COD	Phyto plankton	Zoo plankton
Weight gain	1						
Survival	0.428	1					
pH	-0.004	**-0.517***	1				
BOD_5	**0.538***	**0.901****	-0.457	1			
COD	0.502	-0.180	0.000	0.100	1		
Phyto plankton	**0.910****	**0.526***	-0.114	**0.684***	0.450	1	
Zoo plankton	**0.535***	**0.601***	-0.346	**0.653***	0.307	.512	1

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

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 4.2) 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 4.5). Such higher survival 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 4.3). 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; Enriquez, 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 4.2, Table 4.5). 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 4.9). 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* as providing 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 farmers to start using of mangroves in shrimp pond settings (Bosma et al. 2016).

In our results we observed higher growth performances under pond conditions than in culture tanks (Table 4.2 and Table 4.5). 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. 4.2b). 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. 4.2a) and the negative correlation between FCR and phytoplankton concentration (Fig. 4.2c) suggest that the growth of phytoplankton correlated to the weight gain of shrimp juvenile and the lowering of the FCR. Phytoplankton has been found vital to shrimp nutrition during the postlarvae stages (Thong, 2017).

4.2 Effect of leaf litter on water quality

The average DO level in our tank experiment (Table 4.4) was significantly higher than the DO levels measured in the pond experiments (Table 4.6). While we used an 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 were observed in shrimp rearing tanks (Table 4.4) both with and without supplemental feed, no effects of mangrove species composition on pH were found in the pond setting (Table 4.6). This is similar to the results by Marschner and Noble (2000), Deano and Robinson (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 mixture in leaf litter affected pH (Table 4.3). This might be due to the differences in decomposition rates of the different leaf litter type 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 tanks (Table 4.3) but not in ponds (Table 4.6). 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^{-1}) 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 have 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 was no difference ($P > 0.05$) in soil parameters whereas at the end of the experiment, there were 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 postlarvae 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 full life cycle of farmed shrimp or is or is it best applied in shrimp nursery systems?; (iv) what is the length of time needed before newly planted mangrove can contribute to the pond environment via the provision of leaf litter?

Acknowledgements

This study was supported by the Bangladesh Mangrove Polders for Shrimp Aquatic Productivity (Mangrove Polders) Project which received funding from NWO-WOTRO (Contract: W 08.270.347). Thanks are due to Roel H Bosma, Project manager aquaculture & Assistant professor sustainable production systems (retired), Aquaculture and Fisheries Group, Wageningen University and Research for his initial support in organizing the project on which this study is based. Thanks are also due to the Ministry of Fisheries and Livestock, the Government of the People’s Republic of Bangladesh for allowing the lead author leave from work to pursue his PhD research. Finally, thanks are also due to the laboratories of Forestry and Wood Technology, Soil Water and Environment, Fisheries and Marine Resource Technology discipline in Khulna University for allowing to use their facilities.

Chapter 5

Effect of mangrove leaf litter on shrimp (*Penaeus monodon*, Fabricius, 1798) growth and color

This chapter has been submitted as:

Alam, M.I., Yeasmin, S., Khatun, M.M., Rahman, M.M., Ahmed, M.U., Debrot, A.O., Ahsan, M.N., Verdegem, M.C.J., 2022. Effect of mangrove leaf litter on shrimp (*Penaeus monodon*, Fabricius, 1798) growth and color.

Aquaculture Reports

Abstract

Color, texture and flavor are all important determinants of seafood product quality and value. For shrimp, *Penaeus monodon*, one of the highest-priced shrimp species of the SE Asia shrimp industry, especially the dark tiger-striped coloration is a key product quality criterion. We here evaluated the effects of leaf litter of the mangrove species *Avicennia officinalis* (Ao), *Heritiera fomes* (Hf), *Sonneratia apetala* (Sa) and *S .caseolaris* (Sc) on length, weight and body color of shrimp (*Penaeus monodon*) postlarvae (PL). Fifteen-day-old PL (weight: 0.01g) were reared in 1100-L fibre-reinforced polyethylene tanks. Five treatments having three replications were conducted for four weeks. Four treatments received leaf litter of the four mangrove species and supplemental feeds and one control treatment was provided with supplemental feed only (FO). The pH, biological oxygen demand (BOD_5), phytoplankton and zooplankton biomass were lower, while total ammonia nitrogen (TAN) concentration were significantly higher in the FO treatment than in treatments including leaf litter. PL survival averaged 89% and only differed significantly between the Sa and Sc leaf litter treatments ($P < 0.05$). After 4 weeks of culture, larvae in the FO treatment had significantly shorter body and carapace length, lower body weight and a lower specific growth rate than in larvae in the other treatments, while Sa-reared larvae attained significantly greater length and weight than in the other leaf litter treatments. Shrimp body coloration and pattern with leaf litter treatments were significantly different from the FO treatment. Sa-reared larvae at harvest were significantly darker in body color than larvae reared with other mangrove species. Shrimp value chain stakeholders preferred dark shrimp as an indicator of organic production and higher price. Mangrove leaf litter was found to enhance the production and color quality of black tiger shrimp and this technique can be applied in commercial shrimp aquaculture to improve product quality.

1. Introduction

Shrimp farming is widely practiced in coastal areas of tropical and subtropical countries (Viet Nguyen et al., 2020). It is one of the fastest growing economic activities and sources of foreign-exchange earnings for several developing countries in Asia and Latin America (He et al., 2021). Bangladesh is well known as a shrimp producing country in South-East Asia. The country earned about US$ 494 million by exporting fish and shrimp in 2019-2020, more than 80% of which was from the export of shrimp (DoF, 2021). Moreover, shrimp culture provides employment, income and food security to the rural people in the coastal areas where alternative livelihood options are very limited (Karim and Mustari, 2015; Al-Mamun et al., 2021). Shrimp culture systems are diverse in Bangladesh, ranging from integrated rice−shrimp systems to semi−intensive systems (FAO, 2022). Semi−intensive and extensive systems are different in terms of management, production and disease occurrence and control (Islam et al., 2016). Extensive shrimp farming is commonly practiced in shallow ponds in the coastal zone, stocking shrimp postlarvae (PL) at low densities with no complementary feeding. To fertilize these ponds, some low cost inputs such as compost produced from agriculture and household plant and animal waste may be applied (Ariyati et al., 2019). Alam et al. (2021a, b, 2022), Rejeki et al. (2019) and Ariyati et al. (2019) observed that mangrove leaf litter positively affects shrimp growth performance. Addition of mangrove leaf litter enhances natural food production and improves the use efficiency of supplemental and commercial feed (Alam et al., 2021a, b, 2022). These results suggest that mangrove litter could play an important role in the production of organic shrimp which appeals to consumers for their high quality (Paul and Vogl, 2012; Dhar et al., 2019). Integrated shrimp mangrove farming is often referred to as organic aquaculture (Ahmed et al., 2018).

Growth rate is a key metric in fisheries and aquaculture. Individual growth performance is of great interest because of its importance to yield (Crane et al., 2019). Growth performance is also influenced by environmental conditions such as water temperature, diet, turbidity and water chemistry (Tacon et al., 2002; Shoup et al., 2007; Shoup and Lane, 2015; Crane and Einhouse, 2016). Like growth, the color pattern of shrimp is also important as it can be influenced by the type of production system and generates a price premium in some export markets (Wade et al., 2013; Rodriguez et al., 2017). The same also holds for fish species (Uribe et al., 2018). Considering consumer preferences, Parisenti et al. (2011) observed that a dark color in shrimp was more preferred by consumers than a pale color. Although the effect of mangrove restoration on shrimp performance is well documented, little information is available on the effects of leaf litter on growth characteristics (e.g., total length, body length and carapace length) and body color.

The shrimp sector produces primarily for export markets, and to a lesser extent for domestic markets. If consumers, domestic and foreign, would recognize colored shrimp as organic, healthy and good quality, this can help to promote mangrove shrimp aquaculture. Therefore, the objective of this study was to assess the effects of leaf litter on these characteristics in shrimp PL culture and to explore color preference by various stakeholders in the shrimp sector.

2. Methodology

2.1. Sample collection: mangrove leaf litter and shrimp PL

The mangrove species providing the leaf litter types used in this experiment were selected following Alam et al. (2021a, 2022) and Rahman et al. (2020). These were the species *Avicennia officinalis* (Ao), *Heritiera fomes* (Hf), *Sonneratia apetala* (Sa) and *S. caseolaris* (Sc). Naturally-fallen yellowish senescent leaves of these species were collected from the Sundarbans mangrove forest. Leaf traps were 2 by 2 m and installed beneath the trees during winter (November 2018-January, 2019). At regular intervals, the fallen leaves were recovered from the traps, separated according to species and prepared for the use in this experiment.

Specific pathogens free (SPF) shrimp PL of 15 days old with an average weight of 0.01g were obtained from the Desh-Bangla Shrimp Hatchery, Batiaghata, Khulna, Bangladesh.

These were brought to the experimental site in oxygenated plastic bags. After arrival at the experimental site, the shrimp PLs were acclimatized by gradually adjusting the salinity and temperature to the conditions in the experimental rearing tanks, before releasing them.

2.2. Experimental design

The culture experiment was carried out at a farm located in Debhata, Satkhira at the northern rim of the Sundarbans mangrove area in Bangladesh. Culture was conducted under ambient conditions in which the rearing tanks were covered with a translucent plastic roofing that prevented large fluctuations in salinity due to heavy rain, while still maintaining the natural diurnal variation in light incidence. The experiment included five treatments, all receiving commercial feed (F) at 5% body weight per day. Four treatments received leaf litter of four different mangrove species (*Avicennia officinalis:* Ao, *Heritiera fomes*: Hf, *Sonneratia apetala:* Sa and *S. caseolaris:* Sc) and the 5th treatment was a control provided with feed only (FO). All treatments were executed in triplicate, and the treatments were randomly assigned to the experimental rearing tanks.

The shrimp were reared in 1100-L fibre-reinforced polyethylene tanks containing 1000 L of brackish water (10 ppt) with a water depth of 0.9 m. Brackish water collected from a nearby canal was stocked in a pond and left to settle for one week. The top layer of that settled water was transferred to the experimental tanks through a 25 µm mesh size net to keep out large debris and predators, including their eggs and larvae, from the experimental tanks. Each tank was aerated using a single air stone (diameter 2 cm) connected to an air blower (RESUN, LP-100). Mangrove leaf litter was directly added in the culture tanks on day one at a concentration of 1 g L^{-1} (wet weight). This loading rate was standardized following Hai and Yakupitiyage (2005). On the same day, 100 shrimp PLs were stocked at a rate of 1 PL/ 10L of water in each tank. The experiment was conducted for a four-week period and the water was not exchanged during this experimental period. The average shrimp body weight in the control treatment tanks was checked weekly to adjust the amount of feed administrated during the next week. The experiment was terminated after four weeks, when all the shrimp were collected.

Total biomass and number of shrimp per experimental tank were recorded at stocking and harvest. Shrimp survival, growth and color were assessed, recorded, and analyzed at the end of the experiment. Finally, a survey among shrimp value chain stakeholders was conducted to identify the shrimp color preference by the shrimp sector in Bangladesh.

2.3. Feeding the shrimp PL and FCR

The commercial "Titas Tiger" feed named "starter" was purchased from the Bismillah Feed Mills Limited, Mollahat, Bagerhat, Bangladesh. This feed is labelled as containing 12% moisture, 36% protein, 10% lipid, 7% fibre, 18% ash, 1.9% calcium and 1.7% phosphorus. It was applied in the tank at 5% BW of shrimp PL per day. The feeding table for all tanks was prepared weekly based on the average size and survival rate recorded in the control treatment tanks. The FCR per tank was calculated after harvesting as the total feed given (g wet weight) divided by total shrimp biomass gain (g wet weight) as described by Alam et al. (2022).

2.4. Data collection

Temperature, salinity, pH and dissolved oxygen (DO) in each tank were measured daily and biological oxygen demand (BOD_5) was measured weekly. Procedures for sample collection, preparation and analysis were following the methods described by Alam et al. (2022).

Phytoplankton and zooplankton samples were collected on day 1 and 28. Samples (15 L per sample) were collected at 9.00–11.00 hr from 3 points in each tank. Procedures for sample collection, preparation and analysis were following the method described by Alam et al. (2022).

The survivability of PL was monitored twice every day. The shrimp PLs were harvested after 28 days. The survival rate, individual body weight gain (IWG) and specific growth rate (SGR) were calculated following Busacker et al. (1990) as described by Alam et al. (2022).

At the same time, 15 Pls from each experiment unit were anaesthetized in an ice bath and a photograph of each shrimp was made by using a digital camera (Canon DS126621) after placing it on a laminated graph paper. All photos were taken under day-light condition at the same place and time. From the raw images (Fig. 5.1), total length (TL), body length (BL), and carapace length (CL) were measured using the ImageJ software (version 1.50i). TL (from tip of the rostrum to tip of the telson), BL (from post orbital border of the carapace to the tip of telson) and CL (from posterior margin of orbit to posterior edge of carapace) were measured following Rebello et al. (2014).

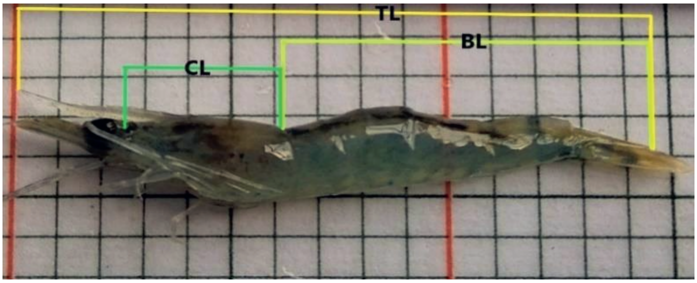

Fig. 5.1. Different types of length measured in P. monodon; TL: Tip of the rostrum to tip of the telson ; BL: Post orbital border of the carapace to tip of the telson: CL: Post orbital border of the carapace to posterior edge of carapace.

Fig. 5.2. Categories of different color patterns found in experimental shrimp PL such as dark, grey-blue and pale.

Color patterns of individual PL from different treatments was recorded from the captured raw images. The shrimp displayed three different distinct colorations. These were referred to as Dark (dark greenish-blue), Grey–blue and Pale (light grey/transparent) (Fig. 5.2). The percentage occurrence of animals displaying these three color variants was determined for each tank and used for comparison between treatments.

Finally, a survey on color preference was done among shrimp producers, local consumers and exporters. The producers were in turn divided into two groups: mangrove farmers (who have naturally grown mangroves in their farms) and non-mangrove farmers, making for a total of 4 groups. For each stakeholder categories, 25 persons were randomly selected for the survey. They were asked to indicate their shrimp color preference and the reason of their color preference as well as their opinion if color of fresh shrimp did or did not influence their client's product appreciation, and if so, for which reason.

2.5. Statistical analyses

All analyses were done using R version 4.0.2 (R Development Core Team, 2020). R-packages used include onewaytests, car, ggplot2, multcomp, FSA, gmodels, rcompanion, vcdExtra and PerformanceAnalytics. The assumptions of normality and homogeneity were tested using Shapiro–Wilk test and Levene's test (Dag et al., 2019). A univariate analysis of variance (ANOVA) model was applied when a variable followed the assumption of normality (John Fox and Sanford Weisberg, 2019), followed by the Tukey multiple comparison test (Hothorn et al., 2008). The Kruskal-Wallis (K-W) test was applied to a variable when transformations failed to give a normal distribution, followed by the Dunn multiple comparisons test (Ogle et al., 2020). In both models, 'feeding types' were included as the independent variable, while an individual growth metric (except color pattern) was incorporated as the dependent variable. Since 'color pattern' is a categorical variable, Pearson's chi-squared was applied (Warnes et al., 2018; Mangiafico, 2020) to explore the differences in body color among different feeding groups. A mosaic plot was made to display the color variation among different feeding groups (Frinedly, 2017). In order to assess the relationship between growth performance and plankton biomass (both phytoplankton and zooplankton), a correlation test was performed (Peterson and Carl, 2020).

3.Results

3.1 Water quality

The analysis revealed significant differences in water quality parameters between treatments except for the parameters DO and NO_2-N (Table 5.1). Tanks receiving only feed had significantly lower pH, BOD_5, phytoplankton and zooplankton biomass, and higher TAN concentrations than the treatments that also received leaf litter (Table 5.1). Among the leaf litter treatments, the highest BOD_5, phytoplankton and zooplankton biomass was observed with Sa leaf litter, while these parameters were lowest with HF leaf litter. Results with the Ao and Sc leaf litter treatments were not significantly different for any of the water quality parameters except BOD_5 (Table 5.1). The temperature for all treatments ranged between 27.8 ^{0}C to 28.1 ^{0}C and did not differ significantly between treatments.

It was not possible for us to identify phytoplankton to species level except for the green algae *Closterium tumidium*. Most of the zooplankton could also only be identified to genus level, except for the copepod *Acartia tonsa*. These two plankton species were the numerically most abundant species of phytoplankton and zooplankton, respectively.

Table 5.1

Average water quality parameter values measured in shrimp PL rearing tanks during a four-week culture period provided with leaf litter from four different mangrove species and supplemental feed.

Water parameters	Feeding types					S.E.M	*P*-value
	Ao-F	Hf-F	Sa-F	Sc-F	FO		
DO (mg L^{-1})	5.36	5.34	5.35	5.34	5.38	0.01	ns
pH	7.87^{b}	7.87^{b}	7.92^{b}	7.90^{b}	7.77^{a}	0.01	**
BOD_5 (mg L^{-1})	2.37^{c}	1.86^{a}	2.67^{d}	2.15^{b}	1.82^{a}	0.09	**
TAN (mg L^{-1})	0.23^{a}	0.27^{ab}	0.17^{a}	0.20^{a}	0.50^{b}	0.04	**
NO_2-N (mg L^{-1})	0.47	0.3	0.53	0.5	0.23	0.04	ns
Phytoplankton (inds. ml^{-1})	21.7^{ab}	6.7^{ab}	25.0^{b}	16.7^{ab}	4.8^{a}	2.39	*
Zooplankton (inds. ml^{-1})	6.7^{ab}	5.0^{ab}	8.3^{b}	5.0^{ab}	4.8^{a}	0.53	*

Values are presented as means. Treatments with no letter in common are significantly different ($P < 0.05$). *P* value is expressed as a symbol ($P < 0.01$: **; $P < 0.05$: *; ns: not significant). Ao-F = *Avicennia officinalis* leaf litter and feed; Hf-F = *Heritiera fomes* leaf litter and feed; Sa = *Sonneratia apetala* leaf litter and feed; Sc = *Sonneratia caseolaris* leaf litter and feed; FO= Feed Only.

3.2. Survival and growth of shrimp PL

At the end of the experimental period (28 days) there were significant differences ($P < 0.05$) in percentage survival between treatments where Sa-F had the highest survival rate and Sc-F the lowest (Table 5.2). In terms of exoskeletal and growth related parameters, analysis showed that PL in the FO treatment realized a smaller TL, BL, CL, IWG and SGR than in other treatments. Among the leaf litter treatments, shrimp cultured with Sa leaf litter performed best (Table 5.2).Though the TL reached by the PL was similar for the treatment with Hf and Sc, the IWG and SGR were higher in the Sc-F treatment than in the HF-F treatment (Table 5.2).

Table 5.2

Shrimp performance observed in controlled condition in tanks with different leaf litter mangrove species and supplemental feed.

Parameters	Feeding types					S.E.M	*P*-value
	Ao-F	Hf-F	Sa-F	Sc-F	FO		
Survival (%)	86[ab]	93[ab]	94[b]	85[a]	88[ab]	1.15	*
Total length (TL; cm)	2.77[bc]	2.63[b]	2.92[c]	2.71[b]	2.23[a]	0.03	**
Body length (BL; cm)	1.86[bc]	1.76[b]	1.97[b]	1.80[b]	1.48[a]	0.02	**
Carapace length (CL; cm)	0.90[bc]	0.86[b]	0.95[c]	0.91[bc]	0.73[a]	0.01	**
Individual weight gain (IWG; g)	0.32[c]	0.25[b]	0.37[d]	0.30[c]	0.17[a]	0.01	**
Specific growth rate (g bw d^{-1})	14.9[c]	14.0[b]	15.4[d]	14.7[c]	12.7[a]	0.09	**

Presented values are presented as means. Means in each rows sharing different superscript letter are significantly different according to Tukey HSD test ($P > 0.05$). P value is expressed as a symbol ($P < 0.001$: **; $P < 0.05$: *). Ao-F = *Avicennia officinalis* leaf litter and feed; Hf-F = *Heritiera fomes* leaf litter and feed; Sa = *Sonneratia apetala* leaf litter and feed; Sc = *Sonneratia caseolaris* leaf litter and feed; FO= Feed Only.

The FCR in all treatments was very low, ranging between 0.20 and 0.46, and significantly different between treatments. Shrimp in rearing tanks with leaf litter realized a better FCR than animals in the FO treatment (Fig. 5.3).

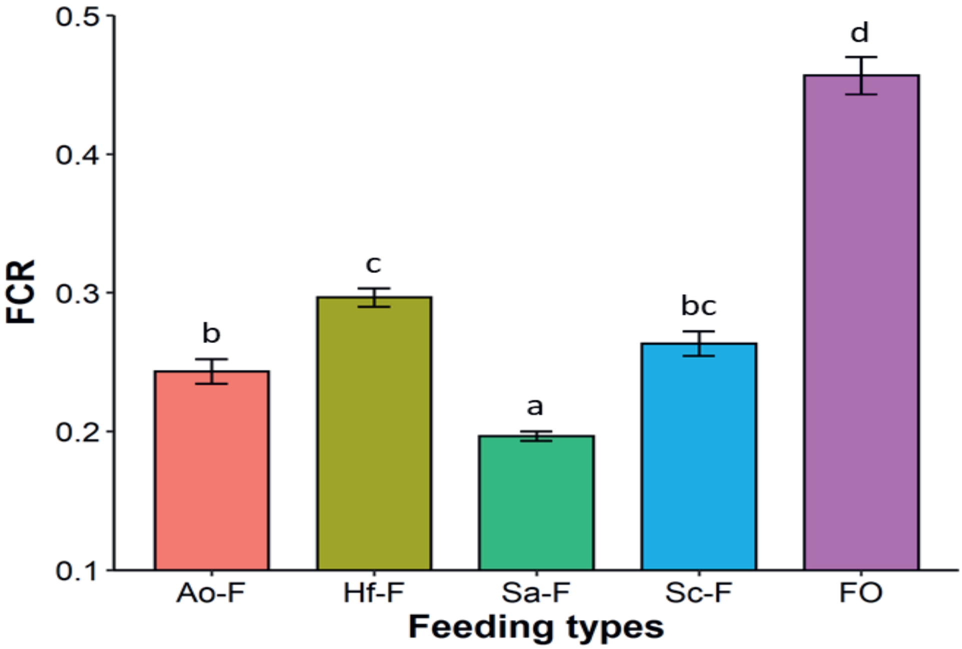

Fig. 5.3. Feed conversion ratio (FCR) in different fed treatments. Values are means (± SE) of three replicate. The bars in graph sharing the same superscript letter or absence of superscripts are not significantly different ($P > 0.05$). Ao-F = *Avicennia officinalis* leaf litter and feed*;* Hf-F = *Heritiera fomes* leaf litter and feed; Sa-F = *Sonneratia apetala* leaf litter and feed; Sc-F = *Sonneratia caseolaris* leaf litter and feed; FO= Feed Only.

3.3. Coloration

The relative frequencies of the three different body color categories differed between treatments (Pearson's chi-squared test: χ^2=95.46, $P < 0.001$). After the experimental period, 100% of the shrimp in the FO treatment were pale colored which was a higher percentage ($P < 0.001$) than for shrimp in the Hf-F (56%), Sc-F (47%), Sa-F (31%) or Ao-F (22%) treatments (Fig. 5.4a). On the other hand, shrimp in the Ao-F treatment were 67% grey-blue colored (Fig. 5.4a) which was higher ($P < 0.001$) than the percentage of grey-blue shrimp in the Hf-F (29%), Sa-F (29%) and FO (0%) treatments, and similar to shrimp in the Sc-F treatment (49%), ($P = 0.05$). In the Sa-F treatment, 40% of shrimp had the dark body color, which was higher ($P < 0.001$) than the percentage of dark body colored shrimp in the Hf-F (16%), Ao-F (11%), Sc-F (4%) and FO (0%) treatments (Fig. 5.4a). The most striking overall difference was between shrimp cultured with mangrove and those without mangrove (FO) as the latter were uniformly pale in color (Fig. 5.4b).

(a) (b)

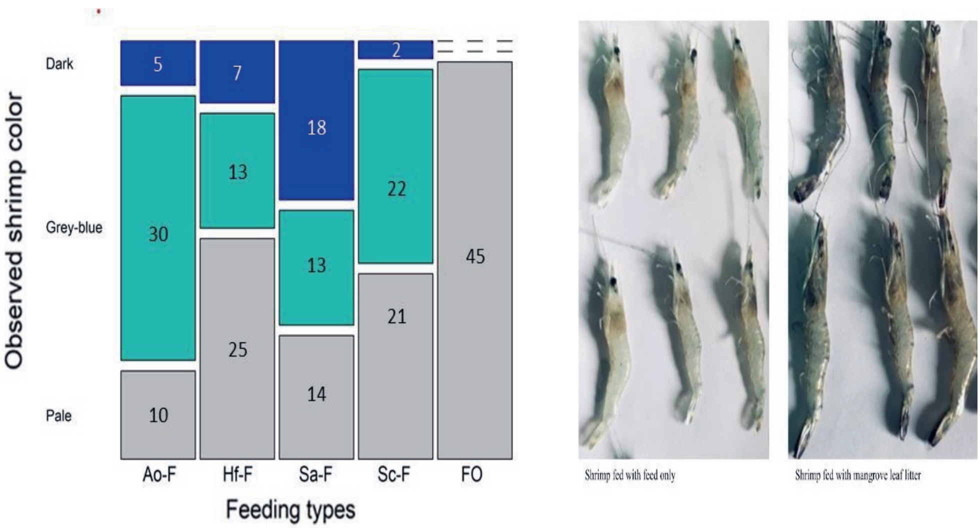

Fig. 5.4. (a-b): (a) The mosaic plot showing overall color variation among different treatments. Numbers within each treatment bar within each color category indicate the number of shrimp that were found in that particular color category for that treatment. (b) Comparison of overall color of harvested juvenile shrimp reared with feed and mangrove leaf litter.

3.4. Correlations between phenotypic characteristics and primary productivity

There were significant positive correlations among different physical and life history parameters (Fig. 5.5). TL and WG were significantly and positively correlated with both zooplankton and phytoplankton (Fig. 5.5).

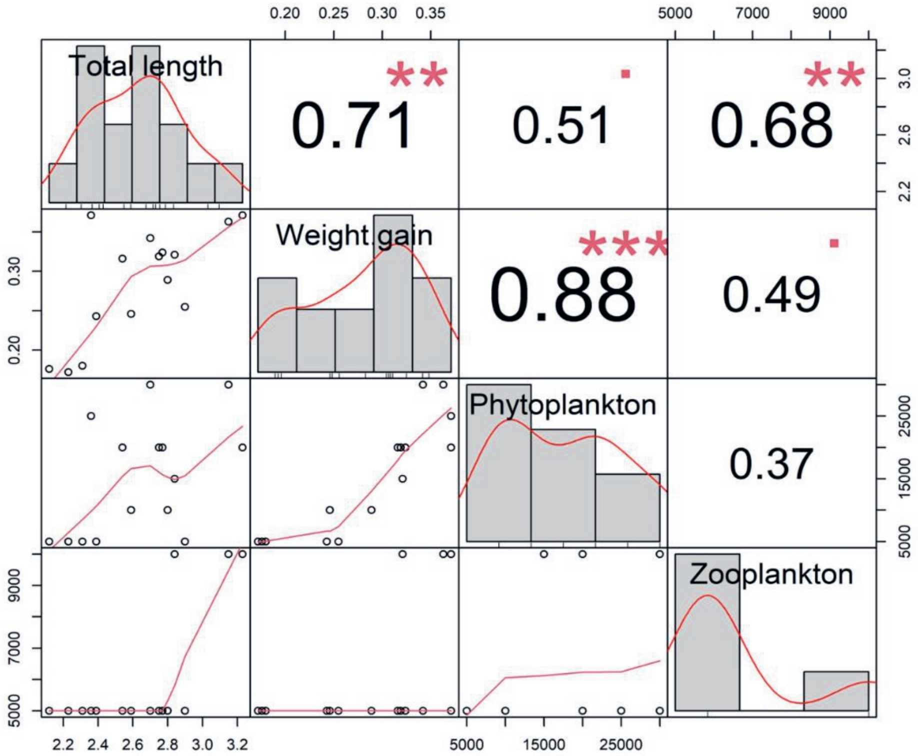

Fig. 5.5. Correlations between shrimp growth characteristics and the primary productivity during the experiment. Here, the variables' full names are: Total length (cm); Weight gain (g); Phytoplankton (inds. ml^{-1}). and Zooplankton (inds. ml^{-1}) with the values given on the x and y axes. Correlation coefficients (r) are indicated with numeric values, while significance levels (*P*) are denoted by asterisks (*<0.05, **<0.01, ***<0.001).

3.5. Color preference by the stake-holders

The highest percentages of persons within each stakeholders category preferred dark colored shrimp followed by grey-blue colored shrimp. No stakeholders gave preference to pale colored shrimp (Table 5.3).

Table 3

Outcomes of Pearson's chi-squared test to explore the preference of different stakeholders for various fresh shrimp tissue color variations.

Stakeholders	**Percent of stake-holders preferred shrimp color**			χ^2	***P***
	Dark	Grey-blue	Pale		
MF	76	24	0	19.82	***<0.001***
NMF	80	20	0	19.14	***<0.001***
LC	52	48	0	21.15	***<0.001***
Exporter	64	36	0	20.85	***<0.001***
The values are presented as percentage means. MF: mangrove farmer; NMF : Non-mangrove farmer; LC : Local consumer.					

5

All of the queried exporters (100 %) indicated that shrimp color had an important effect on selling price even though they did not give an indication of the magnitude of the price difference between colored and pale shrimp. High percentages of mangrove farmers (36 %) and local consumers (60%) also indicated that color influences price. In contrast, 100% of non-mangrove farmers, 64% of mangrove-farmers and 40% of local consumer said that color does not influence shrimp selling price (Fig. 5.6).

When asking how color could affect selling price, the majority of farmers did not answer. However, all categories of stakeholders mentiond colored shrimp as a criteria of freshness. The majority exporters associated darker colored shrimp to freshness, while others considered dark color an indicator of organicly grown or higher buyer’s demand (Fig. 5.7).

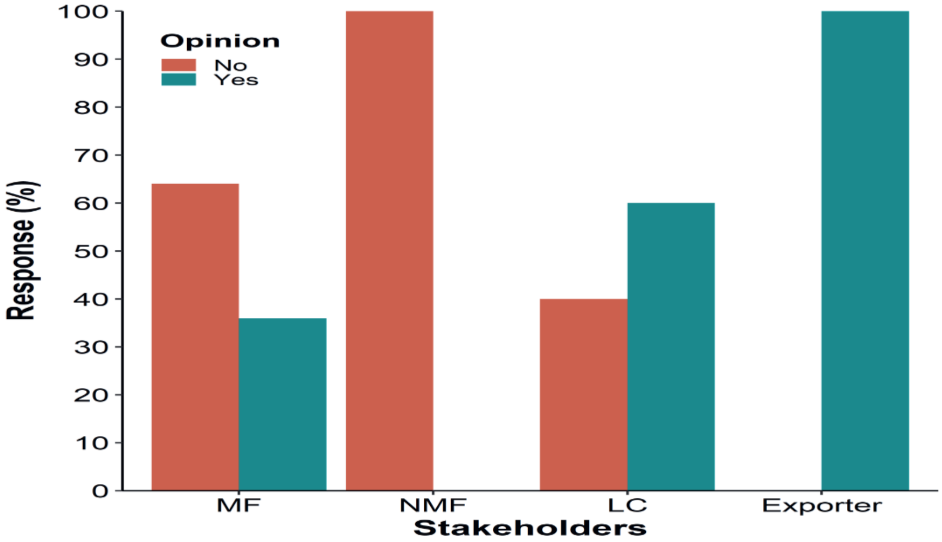

Fig. 5.6. The perception of different stakeholder groups regrading the effect of fresh shrimp color on product selling price. MF : Mangrove farmer; NMF : Non-mangorve farmer; LC: Local consumer.

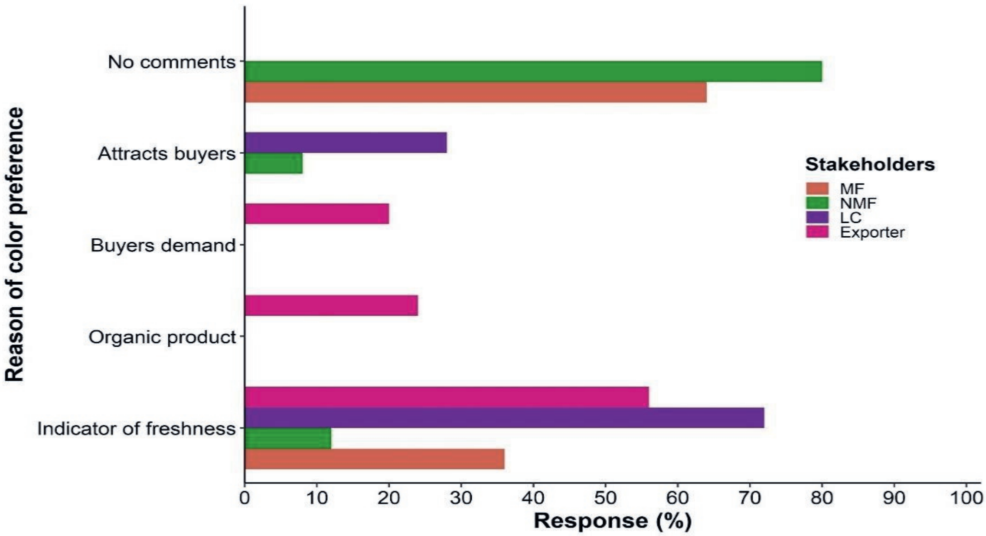

Fig. 5.7. The responses by stake-holders regarding their reason of preference of darker colored shrimp above pale shrimp.

4. Discussion

4.1. Water quality

The experiment found significant differences between treatments for water quality parameters. DO is one of the most important limiting factors for shrimp PL reared with mangrove leaf litter (Nga et al., 2006). Mangrove leaf litter loading at excessive concentrations depleted DO in the water of shrimp culture tanks (Hai and Yakupitiyage, 2005; Nga et al., 2006). However, mangrove leaf litter with aeration and at a loading rate of 1 g L^{-1} did maintain a good DO level as observed by others (Alam et al., 2021a, b; Hai and Yakupitiyage, 2005), as well as in this experiment.

The pH concentration in our experiment did not differ significantly between the various leaf litter treatments, but was significantly lower in the FO treatment (Table 5.1). This finding is consistent with some other studies (Deano and Robinson, 1985; Marschner and Noble, 2000; Hai and Yakupitiage, 2005; Alam et al., 2021a, b, 2022) that demonstrated that leaf litter can lower pH in shrimp culture. However, in all cases, the pH ranged between 7.5 and 8.0 and did not adversely affect shrimp performance as it was within the known safe operational range of 7.5-9.0 for shrimp PL (FAO 1986).

In all treatment tanks, both TAN and NO_2-N remained within acceptable limits (TAN: 1 mg /L; NO_2-N: 0.5 mg L^{-1}) as described by Banrie et al., (2012). The N-input load was higher in leaf litter supplemented tanks than in FO tanks, which can be expected to cause a higher release of TAN in leaf litter tanks. However, contrary to expectations, the TAN concentration was higher in the FO treatment. We speculate that this may have been due to the leaf litter stimulating the sequestration of ammonia into natural food (plankton), thus lowering the TAN concentration in the water column (Ebeling et al., 2006).

The higher nutrient loading in the leaf litter treatment tanks than in the FO treatment tanks, supported the production of new biomass, likely by means of biofilm formation as described by Gatune et al. (2012, 2014) and affected BOD_5, pH, TAN and NO_2-N concentrations in the tanks (Alam et al., 2021a).

The nutrients released from decomposing leaf litter enhanced the shrimp culture food web, as documented by presence of higher numbers of phytoplankton and zooplankton in leaf litter treatment tanks than in FO treatment tanks (Alam et al., 2021a, b). Similar to Alam et al. (2021a), tanks receiving Sa leaf litter had the highest concentrations of both phytoplankton and zooplankton, while Hf leaf litter resulted in the lowest concentrations of the four mangrove species studied (Table 5.1). The most abundant phytoplankton species *Closterium tumidium* and zooplankton specie *Acartia tonsa* identified in leaf litter treatments in this study were also identified by Alam et al. (2021b) in a study with leaf litter of the same mangrove species. *Closterium tumidium* and *Acartia tonsa* are further also known as common species in mangrove estuaries (Schwamborn et al., 2002; Satpati et al., 2013; Magalhaes et al., 2015)

4.2. Shrimp PL survival, growth and coloration

Decomposing mangrove leaf litter is an important direct or indirect food to shrimp PL, but an excessive loading rate can become detrimental (Hai and Yakupitiyage, 2005; Nga et al., 2006). In our experiment, mangrove leaf litter at a loading rate of 1 g L^{-1} did not affect shrimp survival (see also Alam et al., 2021a).

The growth performance of shrimp PL in terms of IWG, SGR, TL, BL and CL was much better with leaf litter than without leaf litter (Table 5.2), benefiting from synergy between leaf litter and supplemental feed (Alam et al., 2021b). In the leaf litter treatments, the highest performance in terms of TL, BL, CL, IWG and SGR was observed in Sa-F treatment and the lowest in the Hf-F treatment. This has previously been suggested to be associated with the higher BOD_5 and rate of decomposition of Sa leaf litter (Alam et al., 2021a), as microorganisms responsible for decomposition and biofilm formation are an important natural food for shrimp PL (Gatune et al., 2012, 2014). The higher number of phytoplankton and zooplankton in tanks with leaf litter and feed than in FO tanks also supported the higher shrimp growth performance (Fig. 5.5) as these are known as nutritive natural foods in shrimp nursery system (Prochas-Corenjo et al., 2010; Thong, 2017).

While similar to our results, the positive effects of mangrove leaf litter on shrimp have been described before, most significant was that the present study revealed major shrimp color differences between treatments. In our experiments, mangrove leaf litter influence shrimp coloration besides growth performnace, whereas pelleted food alone yielded only pale shrimp (Fig. 5.4 a, b). Possibly, carotenoids, especially astaxanthin, and chlorophyll in chloroplasts of mangrove leaf litters (Dhankhar et al., 2012; Banerjee et al., 2017; Cadiz et al., 2021) influenced the body color in shrimp (Wade, 2013; Rodriguez et al., 2017). When consumed by larger zooplankton, like copepods, which in turn are eaten by the shrimp, this enhanced shrimp color pigmentation (Ananthi et al., 2011; de Carvalho and Caramujo, 2017). In contrast, the much lower levels of natural phytoplankton and zooplankton developing in culture tanks fed only pelleted feed gave little or no color to shrimp.

Among all different stakeholders interviewed, their response indicating shrimp with dark color as evidence of organic and fresh shrimp and stimulating buyer’s demand might add special value to promote the reintroduction of mangrove into the shrimp culture systems. Shrimp labelled as organic are preferred by consumers (Paul and Vogl, 2012; Dhar et al., 2019), and a majority of consumers link vivid color to freshness and paleness to lower quality (Parisenti et al., 2011). This means that mangrove leaf litter not only enhances natural food availability to shrimp culture but will also results in higher percentage of dark colored shrimp, which will be in higher demand because they are more appealing to consumers. This can be used as additional incentive to help promote mangrove-shrimp aquaculture.

5. Conclusion

Color, texture and flavor are all important determinants of seafood product quality and value. Shrimp is a valuable export item and its growth performance and quality are important. For the black tiger shrimp, *P. monodon*, one of the highest-priced shrimp species of the SE Asia shrimp industry, dark tiger-striping are a key product quality criterion. At the same time, mangroves remain underutilized while leaf litter represents a major opportunity for shrimp aquaculture (Alam et al. 2021a, b, 2022). Therefore, the present study underlines the importance of mangrove shrimp aquaculture co-management, not only from an ecological point of view but also from an economic point of view. Finally, with proper planning and controlled management, inclusion of mangrove back into the culture system can help transform shrimp aquaculture towards sustainability and help reconcile mangrove restoration with shrimp aquaculture.

Chapter 6

General discussion

6. General Discussion

Mangrove leaf litter is a known potential source of nutrients for shrimp postlarvae (PL). The release of nutrients and anti-nutrients from mangrove leaf litter plays an important role in the bio-geochemical cycling in aquatic the environment and directly or indirectly affects water quality and food availability in shrimp nursery systems. The nutrient contribution of leaf litter depends on several factors, such as the mangrove species concerned, the amount of nutrients and anti-nutrients contained in the litter, the decomposition rate of the litter and finally the amount and way in which the litter is applied in the shrimp nursery system.

6.1. Nutrient and anti-nutrient content in leaf litter of four studied mangrove species relevant to mangrove-shrimp aquaculture

Mangrove leaves vary in organic and inorganic composition according to species, age and environment (Basak et al.,1996, 1998; Gody et al., 1997 Tam et al., 1998; cited by Hai et al., 2005). We found large and significant differences ($P < 0.05$) in the content of C, N, P, tannins, phytate and saponin in leaf litter between the four tested mangrove species (Chapter 2, Table 2.1). Chanda et al. (2016) identified the carbon and nitrogen content (% DM) in leaf litter of the same species we studied (Chapter 2). Between the two studies, the observed values were quite similar, be it with minor differences in rankings (Table 6.1). In both studies, the highest carbon content was in *H. fomes* and the lowest in *S. apetala*, whereas the highest N-content was in *S. caseolaris* and the lowest in *H. fomes.* The ranking of the two intermediate-ranked mangrove species had a different order in the two studies, but for these species, differences in carbon and nitrogen content in leaf litter were small, making their rankings easily inter-changeable. Also the C:N ratio followed a similar ranking pattern in the two studies, with the highest C:N ratio in leaf litter of *H. fomes* (average C:N ratio 38) and the lowest in *S. caseolaris* (C:N 18). The C:N ratio of the intermediate-ranked species *A. officinalis* and *S. apetala,* were again very similar. Although the study locations are far apart, both are located in the Sundarbans, where the climatological, geological, physical, biological and ecological conditions that are quite similar. More studies would be necessary to evaluate if results will be similar with mangrove trees grown under highly different environmental and bio-physical conditions.

In numerous aquaculture studies it has been suggested that a nutrient input with a high C:N ratio is preferred, because it improves water quality and supports production (Avnimelech, 1999; Kabir et al., 2020; Tinh et al., 2021). However, with mangrove leaf litter this seems to not be the case. For instance, in Chapter 3 we found the highest synergy between feeding and leaf litter application with *S. apetala*, a mangrove species with an intermediate C:N ratio (Table 2.1). However, in the same experiment, the lowest synergy was found for *H. fomes*, the mangrove species with leaf litter with the highest C:N ratio. One possible explanation why the high C:N ratio of *H. fomes* stimulated system performance less than leaf litter of mangrove species with a low C:N ratio, might be the short 4-week duration of the experiment. With a longer duration of the experiment and more time for decomposition (Chapter 2, 3), maybe *H. fomes* might have been better able to stimulate the system's performance.

Information in literature on anti-nutrient content in mangrove leaf litter has been very limited. In Chapter 2, we report anti-nutrient concentrations showing higher concentrations for tannin in comparison to saponin and phytate (Table 2.1). The tannin content in leaf letter varied between species (1.7-1.8 % DM) which was lower than the findings of Cundell et al. (1979) and Kristensen et al. (2008) who documented 6% tannin on dry matter content basis in *Rhizophora mangle* leaf litter. This demonstrates that tannin contents varies depending on the species. The phytate contents of all mangrove species was studied in Chapter 2 (Table 2.1) and was also found to be lower than the 1.10% phytate content documented for *Laguncularia racemosa* leaves by Yahaya et al. (2018).

Rout et al. (2015) studied tannin, phytate and saponin contents in the edible mangrove fruits of *Bruguiera gymnorrhiza, Rhizophora apiculata, Kandelia candel and Xylocarpus granatum*, and the values ranged respectively between 6-9 % DM, 0.005-0.006 % DM

Table 6.1

Nutrients content in the leaf litter of four mangrove species in two different studies (Alam et al., 2021a and Chanda et al., 2016).

Leaf litter nutrient content	**Mangrove species**							
	H. fomes		*A. officinalis*		*S. caseolaris*		*S. apetala*	
	Chanda	Alam	Chanda	Alam	Chanda	Alam	Chanda	Alam
Carbon (% DM)	54	48	47	44.8	50	44.7	42	44.1
Ranking* C% Alam	1		3		2		4	
Ranking C% Chanda		1		2		3		4
Nitrogen (% DM)	1.3	1.4	1.91	2.01	2.5	2.8	1.87	1.98
Ranking N% Alam	4		2		1		3	
Ranking N% Chanda		4		3		1		2
C:N ratio	41	36	25	22.3	20	16	23	22.3
Ranking C:N Alam	1		2		4		3	
Ranking C:N Chanda		1		2		4		2

* Ranking from high (rank 1) to low values (rank 4).

and 2-3 % DM. The findings thus show that, fruits and propagules of some mangrove species contain much higher amounts of tannin than leaf litter. In addition, the tannin content in fruits depended on how ripe the fruit was (Kyraleou et al., 2017). Nevertheless, the tannin content in mangrove leaves as well as litter fall rates and decomposition rates need to be taken into consideration before selecting which species and how many mangrove trees will be planted around a pond. Decomposing mangrove leaf litter of *Avecennia marina* and *Rhizopora apiculata* caused mortality in *P. monodon* culture tanks with no water exchange (Hai and Yakupitiyage, 2005; Nga et al., 2006; Rejeki et al., 2019), suggesting that it is important to properly control the amount of mangrove leaf litter that can fall into ponds or is added to tanks. In this thesis, following Hai and Yakupitiyage (2005) we provided leaf litter at the rate of 1 g L^{-1} and found that it greatly improved the production in juvenile shrimp in tanks (Chapter 2 and 3).

6.2. Contribution of nutrients and anti-nutrients within decomposition period of four weeks

It is important to know how fast mangrove leaf litter decomposes and nutrients and anti-nutrients are consequently released into the culture medium or pond waters. Decomposing organic matter provides organic and inorganic nutrients supporting microbial activity (Wetzel, 1995). In Chapter 2, we observed that the decomposition rate of leaf litter affects both the release of nutrients and anti-nutrients in the shrimp culture tanks. The mangrove species with higher decomposition rates contributed higher amounts of nutrients and anti-nutrients to the culture medium or pond waters (Table 2.3). In addition, the composition of leaf litter has a strong influence on its degradability and decomposition rate (Benner and Hodson, 1985). Moreover, the decomposition rate and mass loss also differs between mangrove species, and within species based on age, season and environment (Mishra and Kumar, 2016; Chanda, et al., 2016; Hossain et al., 2014, 2011).

The fibre content in leaf litter was found to be negatively correlated to its decomposition rate (Fig. 2.2). Therefore, the leaf litter species *H. fomes* with higher fibre content was found with lower decomposition rate and contributed lower amount of organic matter through mass loss in the PLs culture tanks (Table 2.1 and Table 2.3). In addition, others have found that a lower crude fibre content in leaf litter, correlates with a higher rate of mass loss during the initial stage of decomposition (Hossain et al., 2011; Ibrahim et al., 2010, 2008).

In our experiments there was a small drop in the N content of the leaf litter after a 4-week decomposition period for all four mangrove species studied, in comparison to freshly collected leaf litter (Table 2.1 and Table 2.2). This did not concur with Chanda et al. (2016), who observed that the percentage of N increased slightly in the decomposed leaf litter biomass remaining after six weeks in the culture tank. Hossain et al. (2014) who observed that the N content in leaf litter initially decreases and increases during later stages. Whereas, initially nitrogen is released from decomposing organic matter as our results indicate, at a later stage new growth of bacterial biomass immobilizes nitrogen in the decomposing litter causing the N content to increase (Hossain et al., 2014, 2011).

Little information is available on the accumulation of anti-nutrients to or their release from mangrove leaf litter. Hai and Yakupitiyage (2005) identified higher amounts of tannin (ranged 8.2-28.7 mg L^{-1}) in the water of leaf litter leachates of *Rhizophora apiculata*, *Avicennia officinalis*, *Excoecaria agallocha* and *Acacia auriculiformis* in experimental shrimp culture tanks, showing that it is important to control the input of mangrove leaf litter in aquaculture rearing systems.

6.3. Effect of leaf litter on water quality

6.3.1 Effect of leaf litter on DO

Maintaining water quality needed for healthy growth and survival of culture organisms in aquaculture systems receiving high nutrient inputs is challenging. To a large extent this is because decomposition of excess food/feed input reduces oxygen content. To avoid such problems in addition to possibly excess tannins, we kept the application rate low at 1 g leaf litter L^{-1} rearing volume applied at the start of our experiments. To avoid oxygen depletion by bacteria decomposing the leaf litter, all experimental tanks were aerated. At the applied loading rate of leaf litter and with oxygenation, the water quality was found to remain suitable to culture during the tank experiments (Chapter 2, 3, 4 and 5). Hai and Yakupitiyage (2005) observed that with a leaf litter loading above 0.5 g L^{-1} the dissolved oxygen (DO) concentration in non-aerated rearing tanks was significantly reduced, and negatively affected shrimp survival and growth. Nga et al. (2006) also observed that the DO concentration dropped with a high input of mangrove leaf litter to aerated or to non-aerated culture tanks, causing higher shrimp PL mortality. Ariyati et al. (2019) and Rejeki et al. (2019) did not observe any significant change in the DO concentration with leaf litter (minced leaves and leachates) addition in aerated experimental shrimp culture tanks (Table 6.2). Hence, mangrove leaf litter can be used in shrimp culture systems to contribute to natural food production and possibly to improve resilience to disease, but it is important to manage litter input rates and to monitor the DO concentrations so as to be ready to intervene should DO concentrations drop.

6.3.2 Effect of leaf litter on pH

When leaf litter is the only nutrient input during PL culture, we found the pH to remain stable, and to not depend on the mangrove species used (Chapter 2, Table 2.4). However, when leaf litter and feed were applied together, the pH decreased over time, but the decrease in pH was not affected by which mangrove species was being used. A similar gradual decline in pH was observed when only feed was applied (Chapter 3, Table 3.3). However, when the leaf litter applied to fed tanks originated from a mix of different mangrove species, the mangrove species mix did affect the pH (Chapter 4, Table 4.4). While considering the others works, Hai and Yakupitiyage (2005) and Rejeki et al. (2019) showed aeration and the loading rate of leaf litter interact in affecting the pH, especially when the oxygen demand of the rearing system approaches the limits of oxygen input into the system. The tendency for the pH to drop is (logically) more pronounced in non-aerated systems. Even so, Ariyati et al. (2019) did not observe any significant change in pH level in experimental water added with mangrove leaf litter. This means that other factors, such as C:N ratio and CO_2 generation (Tinh et al., 2021), nutrient accumulation over time or diurnal variations (Gobler et al., 2017), all can affect organic matter decomposition rates and pH in aquatic systems.

6.3.3. Effect of leaf litter on BOD in water

BOD is commonly used as a proxy measure of the amount of bio-degradable material in the water column. In all of our experiments we observed changes in BOD level over time, especially in the treatments with leaf litter input. The positive correlation between BOD and decomposition of leaf litter as we found in our experiments (Fig. 2.2b) was fully to be expected (Chapter 2, 3 and 4). In addition, as the nutrient loading due to feeding increased over time, the BOD in the water column also increased as is common to aquaculture systems (Boyd, 2020).

6.3.4. Effect of leaf litter on TAN and NO_2-N in water

During culture, the TAN concentration in fed treatments normally increases due to ammonia release during digestion and decomposition of uneaten feed and faeces (Dauda et al., 2019). Sometimes, a small amount of ammonia is also released during decomposition of mangrove leaf litter which can raise the TAN concentration in the rearing environment, especially in systems without water exchange (Rejeki et al., 2019; Ariyati et al., 2019). However, to the contrary, as found in our results, when the sole nutrient input was mangrove leaf litter (Chapter 2), the TAN concentration declined faster when the decomposition rate of mangrove leaf litter was highest. This decline in TAN concentration might have been due to a combination of three processes. The first process is the conversion of TAN into NO_2 and subsequently into NO_3 by autotrophic bacteria. When this occurs the NO_3 concentration rises and the pH gradually drops over time. Unfortunately, NO_3 concentrations could not be measured during our experiments, but during culture, the NO_2 concentration (which was measured) in all our aerated tank experiments did increase (Eding et al., 2006). This concurred under high DO concentrations maintained by aeration. Nevertheless, nitrite is also formed during denitrification, but requires anoxic conditions (Nga et al., 2006). Such conditions can still be found in aerated systems below the surface in suspended organic particles. Therefore, the conversion of NO_3 into NO_2 due to denitrification (Wickins, 1976), although most likely minor, might have been a second explanation for the increase in NO_2 concentration as documented in our experiments (Lindholm-Lehto et al., 2020). A third process, complicating the relation between nitrification and denitrification by reducing the NO_2 concentration in aquatic systems is the process of anaerobic ammonium oxidation (anammox), in which nitrite and ammonium ions are directly converted in diatomic nitrogen and water (Grossart et al., 2020; Tal et al., 2009).

6.3.5. Effect of leaf litter on the production of natural planktonic food

A fast decomposition rate of leaf litter concurred with a high phytoplankton concentration in PL rearing tanks and pond water of our experiments (Fig. 2.2c). The latter was due to the release of inorganic nutrients that stimulated phytoplankton production and which directly contributed to PL nutrition. In natural aquatic systems, phytoplankton is a high-quality food driving the algae-based food web which may support more than 93% of the fish production, with bacteria and detritus driving the rest of the production (Brett et al., 2017). In addition, particulate organic matter in the water column may also provide additional micronutrients. An example of this would be "biofloc" which is a mixture of live, senescent and dead microorganisms held together in a polysaccharide matrix (Deng et al., 2021). Obviously, not all microorganisms enhance growth. For example, dinoflagellates in juvenile shrimp rearing systems receiving mangrove leaf litter and vegetable compost, were found to decrease shrimp growth and survival (Ariyati et al., 2019).

6.4. The effect of leaf litter on shrimp PL performance

While mangrove leaf litter has demonstrable positive effects on shrimp culture, high concentration of leaf litter in juvenile shrimp rearing systems should be avoided as it might cause hypoxia or a high concentration of anti-nutrients (e.g. tannin) in the culture water (Hai and Yakupitiyage, 2005; Nga et al., 2006). By determining the amount of mangrove leaf litter that can be safely added to PL rearing systems prior to the start of our experiments while also monitoring water quality, we were able to concentrate on the benefits of mangrove leaf litter. Some studies providing insight into which mangrove species and what concentrations of mangrove leaf litter can be conductive to growth and PL survival are summarized in Table 6.2

Table 6.2

A summary of different studies showing the effects of different concentrations of mangrove leaf litter on shrimp growth and survival.

Reference	Mangrove species	Leaf litter concentration; rearing period	Rearing systems	Main findings
Hai and Yakupitiyage, 2005	*Rhizophora apiculata, Avicennia officinalis, Excoecaria agallocha*	0.125, 0.25, 0.5, 1 g L^{-1}; 8 weeks	Tanks with and without aeration	- High leaf litter loading rates reduces DO and survival of shrimp in non-aerated treatments - Leaf litter loading rate increased the tannin concentration - Mangrove leaf litter at a loading rate of 1g L^{-1} positively influences survival and growth of shrimp
Nga et al., 2006	*Rhizopora apiculata*	2.5 - 5.0 g fresh leaves L^{-1}, 10 g and 15 g leachate per tank leachates; 8 weeks	Tanks with and without aeration	- Shrimp survival and biomass decreased with higher loading rate of leaves and leachates - Moderate amount of leaves or their leachates supported shrimp performance
Rejeki et al., 2017	*Avicennia marina, Rhizopora apiculata.*	0.125, 0.25, 0.125 g minced leaves L^{-1} and 0.125 g leachates of minced leaves L^{-1}; 37 days	Tank with and without aeration	- Leaf concentration had no effect on DO, tannin, NH_3-N and H_2S concentrations - The tannin concentration did not correlate with other water quality parameters, survival rate and shrimp growth - Decomposing mangroves leaves in tanks without water exchange increased TAN concentration to toxic level
Ariyati et al., 2019	*Avicennia marina*	0.125 g chopped and whole leaves L^{-1}; 50days	Tanks	- Growth was better with chopped than with whole leaves - during the experiment the water quality remained favourable and conductive to survival and growth

For all of our experiments, the concentration of plankton biomass based on different mangrove species or mangrove species combinations is shown in Fig. 6.1.

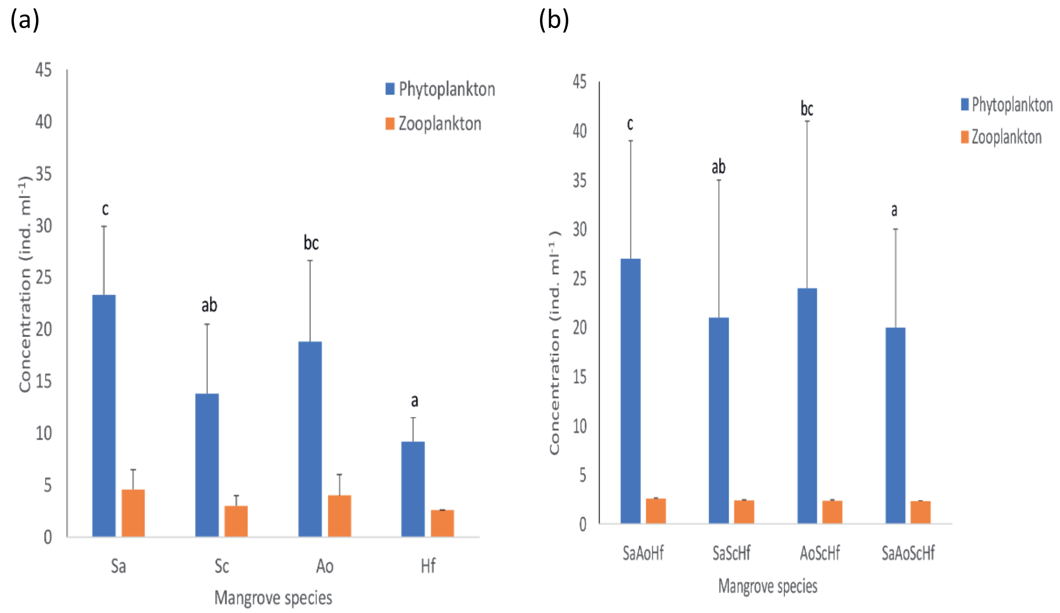

Fig. 6.1. (a-b): Plankton concentration in leaf litter treatment (1 g L^{-1}) tanks : (a) single species of mangrove leaf litter and (b) combined species of mangrove leaf litter. Letter above bar in graphs indicate significant differences (P < 0.05) among leaf litter types. Sa = *Sonneratia apetala*; Sc= *Sonneratia caseolaris*; Ao= *Avicennia officinalis ;* Hf = *Heritiera fomes*.

The higher the decomposition rate of leaf litter the faster the shrimp grew (Chapter 2, Fig. 2.2 c, d). Especially phytoplankton is very nourishing and vital to PL nutrition (Thong, 2017). This was also evidenced in our experiments with the positive correlation between the phytoplankton and shrimp weight gain (Chapter 2, 3, 4 and 5; Ariyati et al., 2019).

6.5 Synergistic effect of leaf litter on shrimp PL weight gain

In our experiments, the survival rate without any food or leaf litter (0%) and survival rate with only leaf litter (75-82%) demonstrated the utility of leaf litter as a source of food. However, growth performance with only leaf litter was not satisfactory. Commercially-formulated feed was a clearly more complete nutrient source for the PL than mangrove leaf litter and a higher growth rate was realized based on the feed than based on leaf litter alone (Table 3.2). However, the highest growth rate was realized using a combined application of commercially formulated feed and leaf litter. Apparently, natural food produced from the decomposition of leaf litter complemented formulated feed synergistically and enhanced growth performances for shrimp postlarvae in terms of weight gain (Table 3.2). The synergism in all the conducted experiments was higher for the treatments with leaf litter demonstrating a higher decomposition rate. This suggested that higher decomposition of leaf litter produced higher amount of natural food which in turn supported higher growth performances to the shrimp larvae. The decomposition of leaf litter and feed wastes enhanced the release of inorganic nutrients (TAN, NO_2, NO_3, NH_3, PO_4, etc) nourishing the algae. The algae are the food for zooplankton and both phytoplankton and zooplankton are nutritive foods for shrimp PLs (Thong, 2017).

6.6. Mangrove leaf litter and FCR

We observed significant differences in FCR among the treatments in all four experiments (chapter 3, 4 and 5) depending on the application of leaf litter. Due to the enhancement of the food web with mangrove leaf litter, shrimp weight gain due to the resulting natural food lowered the FCR in all treatments. These findings signify that the application of expensive formulated feed can be reduced in silvo-aquaculture thanks to the input of mangrove leaf litter. Hence, the wise use of mangroves is shown to be an efficient way to not only to save expenses for formulated feed but to also minimize water quality problems caused by the decomposition of excess feed.

6.7. Necessity of proper silvo-aquaculture planning

Similar to many other studies, we were also able to demonstrate the positive impact that mangrove leaf litter can have on shrimp performance. However, to achieve positive effects, it is also clear that excessive leaf litter loading rates need to be avoided and might cause hypoxia for shrimp (Nga et al., 2006; Hai and Yakupitiyage, 2005). Hence, proper planning and monitoring of water quality is needed when using mangrove based silvo-aquaculture. According to Bosma et al., (2016; 2020), the nature-based model of silvo-aquaculture will be more effective when the water replenishment in the pond and the availability of natural food produced from the leaf litter input into the pond are carefully monitored and regulated.

6.8. Recommendations for future research

Thesis outcomes can contribute to finetune silvo-aquaculture culture practices, by choosing the right mangrove species and controlling the input of leaf litter in culture systems. Additional research is needed to introduce silvo-aquaculture successfully in terms of both ecological and economical points of view, some of which are:

(i) to identify the number of mangrove trees and the mangrove species coverage proportion in shrimp culture ponds . This also need a clear guideline how to minimize the load of organic matter entering the tank or pond;

(ii) to examine the long term effect of leaf litter during the full life cycle of shrimp so that a clear description of the footprint can be made based on research findings;

(iii) to identify the length of time needed before newly planted mangrove can contribute to shrimp production;

(iv) to identify the position of mangrove trees on the dike, on the central platform in ponds or in a combination of both locations to get maximum benefit for shrimp;

(v) to integrate the research outcomes into a sustainable management strategy for mangrove-shrimp aquaculture;

6.9. Conclusion

Selection of mangrove species is very important to introduce mangrove-shrimp co-management practices. Shrimp farmers might select *Sonneratia apetala* as a first choice to introduce in their shrimp culture ponds following *Avicennia officinalis*, *S. caseolaris* and *Heritiera fomes* in that order. While combining the four mangrove species, the combination of *S. apetala*, *A. officinalis* and *H. fomes* was found with best performances than other combinations. The leaf litter even with lower concentration and without aeration showed 3 times higher growth performances in mesocosm condition in ponds. We did not find major negative effects of fresh leaf litter input at a concentration of 1 g L^{-1} (wet weight) on water quality in aeration tanks, but shrimp nursery in ponds looks more promising, especially when developing aerated systems, allowing farmers to increase production and raise income in a sustainable way.

References

A

Abdullah, H.M., Ahmed, S.M., Khan, B.M., Mohana, N.T., Ahmed, T., Islam, I., 2021. Agricultural and fisheries production in a regional blending and dynamic fresh and saline water system in the coastal area of Bangladesh. Env. Chal. 4, 100089. https://doi.org/10.1016/j.envc.2021.100089.

Ahmed, H., 2019. Bangladesh coastal zone management status and future trends. J. of Coas. Zon. Manag.

Ahmed, N., Thompson, S., Glaser, M., 2018. Integrated mangrove-shrimp cultivation: Potential for blue carbon sequestration. Ambio 47, 441-452.

Ahmed, N., Cheung, W.W.L., Thompson, S., Glaser, M., 2017. Solutions to blue carbon emissions: Shrimp cultivation, mangrove deforestation and climate change in coastal Bangladesh. Mar. Pol. 82: 68-75. https://doi.org/10.1016/j.marpol.2017.05.007.

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) postlarvae. 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) postlarvae. Aquaculture 545, 737237. https://doi.org/10.1016/j.aquaculture.2021.737237.

Alam, M.I., Ahmed, M.U., Yeasmin, S., Debrot, A.O., Ahsan, M.N., Verdegem, M.C.J., 2022. Effects of mixed leaf litter of four mangrove on shrimp postlarvae (*Penaeus monodon*, Fabricius, 1798) performance in tank and mesocosm conditions in Bangladesh. Aquaculture 551, 737968. https://doi.org/10.1016/j.aquaculture.2022.737968.

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.

Allen, S.E., 1989. Chemical analysis of ecological material, 2nd edition. Blackwell Scientific Publications, Oxford.

Al-Mamun, A., Murray, F.J., Sprague, M., McAdam, B.J., Roos, N., de Roos, B., Pounds, A., Little, D.C., 2021. Export-driven, extensive coastal can benefit nutritionally vulnerable people. Frontiers in Sustainable Food Systems . https://doi.org/10.3389/fsufs.2021.713140.

Ananthi, P., Santhanam, P., Nandakumar, R., Ananth, S., Jothiraj, K., Kumar, S.D., Prasath, B.B., Jayalakshmi, T., 2011. Production and utilization of marine copepods as live feed for larval rearing of tiger shrimp *Penaeus monodon* with special emphasis on Astaxanthin enhancement. Ind. J. of Nat. sci. 11 (8).

APHA: American Public Health Association, 1998. Standard methods for the examination of water and wastewater. APHA-AWWA-WEF, Washington D. C.

Ariyati, R.W., Rejeki, S., Widowati, L.L., Elfitasari, T., Bosma, R.H., 2019. Effect of three types of liquid compost combined with Avicennia marina leaves on growth and survival of tiger prawn (Penaeus monodon). Int. Aquat. Resear. 11, 323-324. https://doi.org/10.1007/s40071-019-00239-X.

Ashton, E.C., Hogarth, P.J., Ormond, R., 1999. Breakdown of mangrove leaf litter in a managed mangrove forest in Peninsular Malaysia. Hydrobiologia 413, 77-88. https://doi.org/10.1023/A:1003842910811.

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.

Avnimelech, Y., 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176, 227–235. https://doi.org/10.1016/S0044-8486(99)00085-x.

Aypa, S.M., Bagonguis, S.R., 2000. Philippines: Mangrove-friendly aquaculture. In: Primavera, J.H., Garcia, L.Ma.B., Castranos, M.T., Surtida, M.B. (Eds.), Mangrove-Friendly Aquaculture. SEAFDEC, pp. 41– 56.

Aziz, A., Paul, A. R., 2015. Bangladesh Sundarbans: present status of the environment and biota. Diversity, 7(3), 242-269. https://doi.org/10.3390/d7030242.

B

Banerjee, K., Chowdhury, G.R., Mitra, A., 2017. Astaxanthin level of dominant mangrove floral species in Indian Sundarbans. MOJ Biogg. Org. Chem. 1(2):25-28. https://doi.org/10.15406/mojboc.2017.01.00008.

Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl. Ecol. Manag. 10, 421–452. https://doi.org/10.1023/A:1021397624349.

Bandaranayake, W.M., 1998. Traditional and medicinal uses of mangroves. Mangroves salt marshes 2, 133–148. https://doi.org/10.1023/A:1009988607044.

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

Basak, U.C., Das, A.B., Das, P., 1996. Chlorophylls, carotenoids, proteins and secondary metabolites in leaves of 14 species of mangrove. Bull. Mar. Sci. 58, 654–659.

Basak, U.C., Das, A.B., Das, P., 1998. Seasonal changes in organic constituents in leaves of nine mangrove species. Mar. Freshw. Res. 49, 369–372. https://doi.org/10.1071/MF97029.

BBS, 2021. Statistical Year Book of Bangladesh, Bureau of Statistics, Planning Division, Ministry of Planning, Government of the People's Republic of Bangladesh. Dhaka, Bangladesh.

BBS, 2010. Statistical Year Book of Bangladesh, Bureau of Statistics, Planning Division, Ministry of Planning, Government of the People's Republic of Bangladesh. Dhaka, Bangladesh.

Bellinger, E.G., 1992. A key to common algae: Freshwater, estuarine and some coastal species. Institution of water and environmental management London.

Benner, R., Peele, E.R., Hodson, R.E., 1986. Microbial utilization of dissolved organic matter from the leaves of the red mangrove, *Rhizophora mangle*, in the Fresh Creek Estuary, Bahamas. Estuar. Coast. and Shelf Sci. 23, 607–619. https://doi.org/10.1016/0272-7714(86)90102-2.

Biao, X., Kaijin, Y., 2007. Shrimp farming in China: operating characteristics, environmental impact and perspectives. Ocean and Coast. Mang., 50 (7), 538-550. https://doi.org/10.1016/j.ocecoaman.2007.02.006.

Binh, C.T., Phillips, M.J., Demaine, H., 1997. Integrated shrimp– mangrove farming systems in the Mekong Delta of Vietnam. Aquacult. Res. 28, 599– 610. https://doi.org/10.1046/j.1365-2109.1997.00901.x.

Bosire, J.O., Dahdouh-Guebas, F., Kairo, J.G., Kazungu, J., Dehairs, F., Koedam, N., 2005. Litter degradation and CN dynamics in reforested mangrove plantations at Gazi Bay, Kenya. Biol. Cons. 126(2), 287–295. https://doi.org/10.1016/j.biocon.2005.06.007.

Bosma, R.H., Debrot, D., Rejeki, S., Tonneijck, F., Yuniati, A.W., Sihombing, W., 2020. Associated mangrove Aquaculture Farms: Building with Nature to restore eroding tropical muddy coasts. Ecoshape technical report, Dordrecht, The Netherlands.

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.

Bosma, R.H., Nguyen, H.T., Siahainenia, A.J., Tran, H.T.P., Tran, H.N., 2014. Shrimp-based livelihoods in mangrove silvo-aquaculture farming systems. Rev. Aquac. 6, 1-18. https://doi.org/10.1111/raq.12072.

Boyd, C.E., 2020. Water Quality : An Introduction. Book chapter, Pages, 135-162. Springer Nature. https://doi.org/10.1007/978-3-030-23335-8.

Boyd, C.E., 2018. Dissolved oxygen dynamics. Global Aquaculture Alliance, 20 August 2018.

Bozkurt, E., Kabdasli, S., 2013. Investigation of the effect of sea waves on coastal ecosystem. Fresenius Environmental Bulletin 22(5), 1482-1488.lletin.

Brett, M.T., Bunn, S.E., Chandra, S., Galloway, A.W.E., Guo, F., Kainz, M.J., Kankaala, P., Lau, D.C.P., Moulton, T.P., Power, M.E., Rasmussen, J.B., Taipale, S.J., Thorp, J.H., Wehr, J.D., 2017. How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? Freshwater Biology. 62, 833-853. https://doi.org/10.1111/fwb.12909.

Busacker, G.P., Adelman, T.R., Goolish, E.M., 1990. Methods for fish biology. American Fishery Society, Bethesda, MD, p 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. Ecology and Society. 15(2):15. https://doi.org/10.5751/ES-03331-150215.

C

Cadiz, P., Sinutok, S., Chotikam, P., 2021. Photosynthetic characteristics and leaf litter production of mangrove forests in Trang, southern Thailand. Mar. and Freshw. Res. 72 (12), 1798-1810. https://doi.org/10.1071/MF21073.

Chaffey, D.R., Miller, F.R. and Sandom, J.H. 1985. A Forest inventory of the Sundarbans, Bangladesh. Main Report. Project Report 140. Overseas Development Administration, U.K.

Chainark, S., Boyd, C.E., 2010. Water and sediment quality, phytoplankton communities and channel catfish production in sodium nitrate-treated ponds. J. Appl. Aquac. 22,171–185.

Chanda, A., Akhand, A., Manna, S., Das, S., Mukhopadhyay, A., Das, I., Hazra, S., Choudhury, S.B., Rao, K.H., Dadhwal, K., 2016. Mangrove associates versus true mangroves: a comparative analysis of leaf litter decomposition in Sundarban. Wet. Ecol. and Manag 24, 293-315. https://doi.org/10.1007/s11273-015-9456-9.

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.

Clough, B., Johnston, D., Xuan, T.T., Phillips, M.J., Pednekar, S.S., Thien, N.H., Dan, T.H., Thong, P.L., 2002. Silvofishery farming systems in Ca Mau province, Vietnam. World Bank, NACA, WWF and FAO Consortium Program on Shrimp Farming and the Environment, Bangkok. 70pp.

Crane, D.P., Ogle, D.H., Shoup, D.E., 2019. Use and misuse of a common growth metric: guide for appropriately calculating and reporting specific growth rate. Reviews in Aquaculture 1-6. https://doi.org/10.1111/raq.12396.

Crane, D.P., Einhouse, D.W., 2016. Changes in growth and diet of smallmouth bass following invasion of Lake Erie by the round goby. J. of Gr. La. Res. 42, 405–412. https://doi.org/10.1016/j.jglr.2015.12.005.

Cundell, A.M., Brown, M.S., Stafford, R., Mitchell, R., 1979. Microbial degradation of *Rhizophora mangle* leaves immersed in the sea. Estuar Coast Mar Sci 9:281–286.

Cunniff, P., 1995. AOAC international. Official methods of analysis, 16th edition. Washington, DC: Association of Official Analytical Chemist.

D

Dag, O., Dolgun, A., Konar, N.M., 2018. Onewaytests: An R Package for One-Way Tests in Independent Groups Designs. The R Journal, 10 (1), 175-199.

Dauda, A.B., Ajadi, A., Tola-Fabunmi, A.S., Akinwole, A.O., 2019. Waste production in aquaculture: Sources, components and managements in different culture systems. Aquaculture and Fisheries 4 (3), 81-88. https://doi.org/10.1016/j.aaf.2018.10.002.

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.

Debrot, A.O., Veldhuizen, A., van den Burg, S.W.K., Klapwijk, C.J., Islam, M.N., Alam, M.I., Ahsan, M.N., Ahmed, M.U., Hasan, S.R., Fadilah, R., Noor, Y.R., Pribadi, R., Rejeki, S., Damastuti, E., Koopmanschap, E., Reinhard, S., van Scheltinga, C.T., Verburg, C., Poleman, M., 2020. Non-Timber Forest Product Livelihood-Focused Intervention in Support of Mangrove Restoration: A Call to Action. Forest 11 (11), 1224. https://doi.org/10.3390/f11111224.

de Carvalho, C.C.C.R., Caramujo, M.J., 2017. Carotenoids in aquatic ecosystem and aquaculture: a colorful business with implications for human health. Front. Mar. Sci. https://doi.org/10.3389/fmars.2017.00093.

Deng, Y., Borewicz, K., van Loo, J., Olabarrieta, M.Z., Kokou, F., Sipkema, D., Verdegem, M.C.J., 2021. In-Situ Biofloc Affects the Core Prokaryotes Community Composition in Gut and Enhances Growth of Nile Tilapia (Oreochromis niloticus). Microbial Ecology. https://doi.org/10.1007/s00248-021-01880-y.

De Schryver, P., Crab, R., Deforidt, T., Boon, N., Verstraete, W., 2008. The basics of bio-flocs technology: the added value for aquaculture. Aquaculture, 277, 125-137. https://doi.org/10.1016/j.aquaculture.2008.02.019.

Dhankhar, J., Kadian, S.S., Sharma, A., 2012. Astaxanthin: a potential carotenoid. Int. J. Phar. Res. 3 (5), 1246-1259.

Dhar, A.R., Uddin, M.T., Roy, M.K., 2019. Assessment of organic shrimp farming sustainability from economic and environmental viewpoints in Bangladesh. Environmental Research 180 (8). https://doi.org/10.1016/j.envres.2019.108879.

DoF: Department of Fisheries, 2021. Year book of fisheries statistics of Bangladesh, 2019-20.

DOF: Department of Fisheries, 2016. National Fish Week, Compendium (In Bengali), Dhaka; Department of Fisheries, Ministry of Fisheries and Livestock, Government of Bangladesh.

Du, N., Li, W., Qiu, L., Zhang, Y., Wei, X., Zhang, X., 2020. Mass loss and nutrient release during the decomposition of sixteen types of plant litter with contrasting quality under three precipitation regimes. Ecol. Evolu. 10 (7), 3367-3382. https://doi.org/10.1002/ece3.6129.

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

E

Ebeling, J.M., Timmons, M.B., Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture. 257, 346-358. http://dx.doi.org/10.1016/j.aquaculture.2006.03.019.

Eding, E.H., Kamstra, A., Verreth, J.A.J., Huisman, E.A., Klapwijk, A., 2006. Design and operation of nitrifying trickling filters in recirculating aquaculture: a review. Aquacultural Engineering. 34, 234-260. https://doi.org/10.1016/j.aquaeng.2005.09.007.

Edmondson, W., 1982. Kiefer, F., and Fryer, G., 1978. Das Zooplankton der Binnengewässer. Teil 2. E. Schweizerbartsche Verlag, Stuttgart. 380 p. DM108. Limnology and Oceanography 27, 196-196.

Ellis, W. L., Bowles, J. W., Erickson, A. A., Stafford, N., Bell, S. S., & Thomas, M., 2006. Alteration of the chemical composition of mangrove (*Laguncularia racemosa*) leaf litterfall by freeze damage. Estuarine, Coastal and Shelf Science, 68(1-2), 363-371.

Enríquez, F., 2003. Variación del Bentos, con énfasis en la comunidad de poliquetos en una Laguna de Descarga de una granja camaronicola. Tesis de Maestría, Universidad de Sonora, Hermosillo, Sonora. 98 pp.

F

FAO, 2022. Fisheries and Aquaculture: National Aquaculture Sector Overview : Bangladesh.https://www.fao.org/fishery/en/countrysector/naso_bangladesh

FAO, 2020. The state of world fisheries and aquaculture 2020. In: Sustainability in Action. Fisheries and Aquaculture Technical paper (Rome).

FAO, 2007. The world's mangrove 1980-2005. Rome: Food and Agriculture Organization of the United Nations.

FAO, 2003. FAO's Global Estimate of Mangroves. http://www.fao.org/english/newsroom/news /2003/15020-en.html. Accessed on 17 November 2014.

FAO, 1986. NACA Training Manual Series No. 2. SHRIMP CULTURE: POND DESIGN, OPERATION AND MANAGEMENT. Food and Agriculture Organization (FAO) of the United Nations.

Fazi, S., Rossi, L., 2000. Effects of macro-detritivores density on deaf detritus processing rate: a macrocosm experiment. Hydrobiologia 435, 127–134. https://doi.org/10.1023/A:1004033410895.

Fiori, L.F., Cionek, V.M., Sacramento, PA., Benedito, E., 2015. caloric content of leaves of five tree species from the riparian vegetation in a forest fragment from south Brazil. Acta Limnol. Bras. 27(3), 247-253. https://doi.org/10.1590/S2179-975X6114.

Fitzgerald, W.J., 2000. Integrated mangrove forest and aquaculture systems in Indonesia. Mangrove-friendly aquaculture proceedings: SEAFDEC, pp. 21– 34.

Fitzgerald, W.J., 1999. Integrated mangrove forest and aquaculture systems in Indonesia. Mangrove-friendly aquaculture proceedings: SEAFDEC Aquaculture Department, January 11-15, 1999, Iloilo City, Philippines.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197-227. https://doi.org/10.1016/S0044-8486(01)00526-9.

Freeland, W.J., Calcott, P.H., Anderson, L.R., 1985. Tannins and saponin: interaction in herbivore diet. Biochem. Syst. Ecol. 13, 189–193. https://doi.org/10.1016/0305-1978(85)90078-X.

Friendly, M., 2017. vcdExtra: 'vcd' Extensions and Additions. R package version 0.7-1. https://CRAN.R-project.org/package=vcdExtra.

G

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 postlarvae of giant tiger shrimp *Penaeus monodon*. Aquac. Environ. Interac. 6, 1–10. https://doi.org/10.3354/aei00117.

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 postlarvae. J. Exp. Mar. Bio. Ecol. 426–427, 28–38. https://doi.org/10.1016/j.jembe.2012.05.015.

Gemede, H.F., Ratta, N., 2014. Antinutritional factors in plant foods: Potential health benefits and adverse effects. Int. J. Nutri. Food Sci. 3(4), 284-289. https://doi.org/10.11648/j.ijnfs.20140304.18.

Gladstone-Gallagher, R.V., Lundquist, C.J., Pilditch, C.A., 2014. Mangrove (*Avicennia marina* subsp. australasica) litter production and decomposition in a temperate estuary. NZ J Mar. Freshw. Res. 48(1), 24–37. https://doi.org/10.1080/00288330.2013.827124.

Gobler, C.J., Clark, H.R., Grifth, A.W., Lusty, M.W., 2017. Diurnal fluctuations in acidification and hypoxia reduce growth and survival of larval and juvenile Bay Scallops (*Argopecten irradians*) and Hard Clams (*Mercenaria mercenaria*). Frontiers in Marine Science. https://doi.org/10.3389/fmars.2016.00282.

Gonelimali, F.D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., Hatab, S., 2018. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Fron. In Microbio. https://doi.org/10.3389/fmicb.2018.01639.

Goswami, T., and Ghosal, S., 2022. From rice fields to brackish water farms: changing livelihoods in agrarian coastal Bengal, India. Asi.-pac. J. of regio. Sci. https://doi.org/10.1007/s41685-022-00229-8.

Grossart, H.P., Massana, R., McMahon, K.D., Walsh, D.A., 2020. Linking metagenomics to aquatic microbial ecology and biogeochemical cycles. Limnology and Oceanography. 65, S2-S20. https://doi.org/10.1002/lno.11382.

H

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.

Hajra, A., Mazumder, A., Verma, A., Ganguly, D.P., Mohanty, B.P., Sharma, P., 2013. Antinutritional factors in plant origin fish feed ingredients. Advances in Fish Research Vol.-V, 93-202.

R

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., Hu, X., Zhang, S., 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.

Hoque, A.K.F and Datta, D.K., 2005.The Mangroves of Bangladesh. International Journal of Ecology and Environmental Sciences 31 (3): 245-253.

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., Siddique, M.R.H., Abdullah, S.M.R., Saha, S., Ghosh, D.C., Rahman, M.S., Limon, S.H., 2014. Nutrient dynamics associated with leaching and microbial decomposition of four abundant mangrove species leaf litter of the Sundarbans, Bangladesh. Wetlands 34, 439–448. https://doi.org/10.1007/s13157-013-0510-1.

Hossain, M., Siddique, M.R.H., Rahman, M.S., Hossain, M.Z., Hasan, M.M., 2011. Nutrient dynamics associated with leaf litter decomposition of three agroforestry tree species (*Azadirachta indica*, *Dalbergia sissoo*, and *Melia azedarach*) of Bangladesh. J. For. Res. 22, 577–582. https://doi.org/10.1007/s11676-011-0175-7.

Hossain, M., Limon, S.H., Rahman, M.S., Azad, A.K., Islam, M.S., Khairuzzaman, M., 2009. Nutrients (N, P and K) dynamics associated with the leaf litter of two agroforestry tree species of Bangladesh. iForest 2, 183–186. https://doi.org/10.3832/ifor0510-002.

Hossain, M.S., Lin, C.K., Hussain, M.Z., 2001. Goodbye Chakaria Sundarbans: The oldest mangrove forest. Wet. Sci. Prac. 18 (3), 19-22. https://doi.org/10.1672/0732-9393(2001)018[0019:GCSTOM]2.0.CO;2.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical Journal 50(3), 346--363.

Hutchison, J., Spalding, M., Ermgassen, P., 2014. The Role of Mangroves in Fisheries Enhancement. Publisher: The Nature Conservancy and Wetlands International.

I

Ibrahima, A., Gillon, D., Joffre, R., 2010. Leaf litter decomposition of Mediterranean tree species in relation to temperature and initial water inhiibitions under microcosm experiment. Res J Agric Biol Sci 6:32–39.

Ibrahima, A., Biyanzi, P., Halima, M., 2008. Changes in organic compounds during leaf litter leaching: laboratory experiment on eight plant species of the Sudano-guinea of Ngaoundere, Cameroon. iForest 1, 27–33. https://doi.org/10.3832/ifor0450-0010027.

Imgraben, S., Dittmann, S., 2008. Leaf litter dynamics and litter consumption in two temperate South Australian mangrove forests. J. Sea Res. 59(1–2), 83–93. https://doi.org/10.1016/j.seares.2007.06.004.

Islam, M.N., Ratul, S.B., Sharmin, A., Rahman, K.S., Ashaduzzaman, M., & Uddin, G.M.N., 2019. Comparison of calorific values and ash content for different woody biomass components of six mangrove species of Bangladesh Sundarbans. J. Ind. Acad. Wood Sci. 16(2), 110-117. https://doi.org/10.1007/s13196-019-00246-9.

Islam, S.M.D., Bhuiyan, M.A.H., 2016. Impact scenarios of shrimp farming in coastal region of Bangladesh: an approach of an ecological model for sustainable management. Aquacult. Int. 24, 1163–1190. https://doi.org/10.1007/s10499-016-9978-z.

Islam, S., Feroz, S. M., Ahmed, Z. U., Chowdhury, A. H., Khan, R. I., Al-Mamun, A., 2016. Species richness and diversity of the floristic composition of the Sundarbans mangrove reserve forest, Bangladesh in relation to spatial habitats and salinity. *Malaysian For*, *79*, 7-38.

Islam, S.N., 2010. Threatened wetlands and ecologically sensitive ecosystems management in Bangladesh. In: Frontiers Earth Science in China, Springer-Verlag Berlin, Berlin 4(4): 438–448.

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.

IUCN., 2005. Early observations of tsunami effects on mangrove and coastal forests www.iucn.org. 7 January 2005.

J

Jackson, M.L. 1973. Soil Chemical Analysis, New Delhi, India: Prentice-Hall of India Pvt. Ltd.

Jackson, M.L., 1962. Soil Chemical Analysis. Prentice Hall Inc., Englewood cliffs, New Jersey, USA.

John Fox and Sanford Weisberg, 2019. An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage.

Johnston, D., Trong, N.V., Tien, D.V., Xuan, T.T., 2000. Shrimp yields and harvest characteristics of mixed shrimp–mangrove forestry farms in southern Vietnam:

R

factors affecting production. Aquaculture 188 (3), 263–284. https://doi.org/10.1016/S0044-8486(00)00348-3.

K

Kabir, K.A., Verdegem, M.CJ., Verreth, J.A.J., Phillips, M.J., Schrama, J.W., 2020. Dietary non-starch polysaccharides influenced natural food web and fish production in semi-intensive pond culture of Tilapia. Aquaculture 528, 735506. https://doi.rog./10.1016/j.aquaculture.2020.735506.

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.

Kamruzzaman, M., Ahmed, S., Osawa. A., 2017. Biomass and net primary producivity of mangrove communities along the Oligohaline zone of Sundarbans, Bangladesh. Forest Ecosystems. 4 (16). https://doi.org/10. 1186/s40663-017-0104-0.

Karim, A.H.M.Z., Mustari, S., 2015. Shrimp cultivation and coastal livelihood: a focus on Bangladesh coastal vulnerability. Asian Social Science 11(28). http://dx.doi.org/10.5539/ass.v11n28p109.

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.

Kamruzzaman, M., Sharma, S., Hoque, A.T.M.R., Hagihara, A., 2012. Litter fall of three subtropical mangrove species in the family Rhizophoraceae. J. Oceanogr. 68(6), 841–850. https://doi.org/10.1007/s10872-012-0134-8.

Khan, M.N.I., Suwa, R., Hagihara, A., 2007. Carbon and nitrogen pools in a mangrove stand of *Kandelia obovata* (S., L.) Yong: vertical distribution in the soil-vegetation system. Wet. Ecol. Manage. 15(2), 141–153. https://doi.org/10.1007/s11273-006-9020-8.

Kibria, G., 2013. Mangrove Forests-Its Role in Livelihoods, Carbon Sinks and Disaster Mitigation. Project: Pollution and Climate Change Impacts. https://doi.org/10. 13140/RG.2.1.1848.8800.

Kristensen, E., S. Bouillon, T. Dittmar, and C. Marchand., 2008. Organic carbon dynamics in mangrove ecosystems: A review. Aquatic Botany, 89(2), 201–219.

Krzyzowska, M., Tomaszewska, E., Soliwoda, R.K., Bien, K., Orlowski, P., Celichowski, G., Grobelny, J., 2017. Tannic acid modification of metal nanoparticles: possibility for new antiviral applications. Nanostr. Oral Medi. Pages 335-363. https://doi.org/10.1016/B978-0-323-47720-8.00013-4.

Kumar, V., Sinha, A., Makkar, H., De Boeck, G., Becker, K., 2012. Phytate and phytase in fish nutrition. J. Anim. Physiol. Anim. Nutr. 96, 335–364. https://doi.org/10.1111/j.1439-0396.2011.01169.x.

Kyralleou, M., Kallithraka, S., Theodorou, N., Teissedre, P.L., Kotseridis, Y., Koundouras, S., 2017. Changes in Tannin Composition of Syrah Grape Skins and Seeds during Fruit Ripening Under Contrasting Water Conditions. Molecules 22 (9) 1453. https://doi.org/10.3390/molecules22091453.

L

Lee, S.Y., 1989. Litter production and turnover of the mangrove *Kandelia candel* (L.) Druce in a Hong Kong tidal shrimp pond. Estuar Coast Shelf Sci 29:75–87.

Lindholm-Lehto, P., Pulkkinen, J., Kiuru, T., Koskela, J., Vielma, J., 2020. Water quality in recirculating aquaculture system using woodchip denitrification and slow sand filtration. Environmental Science and Pollution Research. 27, 17314-17328. https://doi.org/10.1007/s11356-020-08196-3.

Little, A.E.F., Robinson, C.J., Peterson, S.B., Raffa, K.F., Handelsman, J., 2008. Rules of Engagement: Interspecies Interactions that regulate Microbial Communities. Annu. Rev. Micro. 62, 375-401. https://doi.org/10.1146/annurev.micro.030608.101423.

M

Magalhaes, A., Pereira, L.C.C., da Costa, R.M., 2015. Relationship between copepod community structure, rainfall regimes, and hydrological variables in a tropical mangrove estuary (Amazon cost, Brazil). Hel. Mar. Res. 69, 123-136. https://doi.org/10.1007/s10152-014-0421-4.

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, Forests and People, 5(100094). https://doi.org/10.1016/j.tfp.2021.100094.

Makkar, H.P.S., and Singh, B., 1993. Effect of storage and urea addition on detannification and in sacco dry matter digestibility of mature oak (*Quercus incana*) leaves. Animal Feed Science and Technology. 41: 247-259.

Mangiafico, S., 2020. rcompanion: Functions to Support Extension Education Program Evaluation. R package version 2.3.26. https://CRAN.R project.org/package=rcompanion.

Marschner, B., Noble, A.D., 2000. Chemical and biological process leading to the neutralisation of acidity in soil incubated with litter materials. Soil Bio. Biochem. 32, 805-813. https://doi.org/10.1016/S0038-0717(99)00209-6.

Martinez-Cordova L.R., Campana-Torres A., M. Martinez-Porchas, 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).

Martinez-Cordova L.R., Enriquez-Ocana, L.F., 2007. J. Biol. Sci. 7, 12-17. https://doi.org/10.3844/ojbsci.2007.12.17.

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.

McKinnon, A. D., Trott, L.A., Alongi, D.M., Davidson, A., 2002. Water column production and nutrient characteristics in mangrove creeks receiving shrimp farm effluent. Aquaculture Research, 33(1), 55–73.

Mishra, G., Kumar, R., 2016. Plant litter decomposition : drivers insight to the ecological process. European Journal of Biological Research. http://dx.doi.org/10.5281/zenodo.58752.

N

Nagarajan, B., Pandiarajan, C., Krishnamoorthy, M., Sophia, P., 2008. Reproductive fitness and success in mangroves: implication on conservation. In: Sengupta M, Dalwani R, editors. Proceedings of Taal 2007: the 12th World Lake Conference; p.29–33. International Lake Environment Committee Foundation, held in Jaipur (India).

Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Little, D.C., Lubchenco, J., Shumway, S.E., 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*. Aquacult. Int. 14, 467–477. https://doi.org/10.1007/s10499-006-9049-y.

Nga, B.T., Tinh, H.Q., Tam, D.T., Scheffer, M., Roijackers, R., 2005. Young Mangrove Stands Produce a large and High quality Litter Input to Aquatic Systems. Wet. Eco. And mang. 13, 569-576. https://doi.org/10.1007/s11273-004-6073-4.

Nga, B.T., Roijackers, R., 2002. Decomposition of *Rhizophora apiculata* leaves in a mangrove-shrimp system at The Thanh Phu state farm, Ben Tre province, Vietnam. Selected papers of the workshop on integrated management of coastal resources in the Mekong delta. Saigon, Vietnam, pp 95–100.

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.

Estua. Coasta. Shelf Sci. 67(1–2), 239–250. https://doi.org/10.1016/j.ecss.2005.11.022.

O

Obadoni, B.O., and Ochuko, P.O., 2002. Phytochemical studies and comparative efficacy of crude extracts of some homeostatic plants in Edo and delta states of Nigeria. Glob. J. Pure Appl. Sci. 8(2), 203-208. https://doi.org/10.4314/gjpas.v8i2.16033.

Ogle, D.H., Wheeler, P., Dinno. A., 2020. FSA: Fisheries Stock Analysis. R package version 0.8.31. https://github.com/droglenc/FSA.

P

Pandit, N.P., Nakamura, M., 2010. Effect of high temperature on survival, growth and feed conversion ratio of Nile Tilapia, *Oreochromis niloticus*. Our Nat. 8:219–224. https://doi.org/10.3126/on.v8i1.4331.

Parisenti, J., Brito, C.C.S., Tramonte, V.L.C.G., Beirão, L.H., Moreira, C.C., Ourique, F., 2011. Total carotenoid content of shrimp commercialized in Florianopolis/SC and evaluation of color preference for consumers. Alim. Nutr., Araraquara, 22 (1), 17-20.

Paul, B.G., Vogl, C.R., 2012. Key performance characteristics of organic shrimp aquaculture in Southwest Bangladesh. Sustainability, 4 995-1012. https://doi.org/10.3390/su4050995.

Peterson, B.G., Carl, P., 2020. Performance Analytics: Econometric Tools for Performance and Risk Analysis. R package version 2.0.4. https://CRAN.R-project.org/package=PerformanceAnalytics.

Peng, Y., Chen, G., Li, S., Liu, Y., Pernetta, J.C., 2013. Use of degraded coastal wetland in an integrated mangrove–aquaculture system: a case study from the South China Sea. Ocean Coast Manage 85:209–213.

Porchas-Cornejo, M.A., Martínez-Porchas, M., Martínez-Córdova, 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 Journal of Aquaculture - Bamidgeh, IJA_64.2012.709, 7 pages.

Porchas-Cornejo, M.A., Martínez-Córdova, L.R., Ramos-Trujillo, L., Hernández-López, J., Martínez-Porchas, M., Mendoza-Cano, F., 2010. Effect of promoted natural feed on the production, nutritional and immunological parameters of *Litopenaeus vannamei* (Boone, 1931) semi-intensively farmed. Aqua. Nut. 17(2): e622-e628. https://doi-org.ezproxy.library.wur.nl/10.1111/j.1365-2095.2010.00809.x.

R

Premanathan, M., Arakaki, R., Izumi, H., Kathiresan, K., Nakano, M., Yamamoto, N., Nakashima, H., 1999. Antiviral properties of a mangrove plant, *Rhizophora apiculata* Blume, against human immunodeficiency virus. Antiviral Res. 44, 113–122. https://doi.org/10.1016/S0166-3542(99)00058-3.

Prescott, G.W., 1962. Algae of the Western Great Lakes Area Wm. C. Brown Co., Dubuque, lowa. 977 pp.

Primavera, J.H., Altamirano, J.P., Lebata, M.J.H.L., delos Reyes, A.A. Jr., Pitogo, C.L., 2007. Mangroves and shrimp pond culture effluents in Aklan, Panay Is., central Philippines. Bulletin of Marine Science 80, 795–804.

Primavera, J.H., 2006. Overcoming the impact of aquaculture on the coastal zone. Ocean & Coastal Management 49, 531-545.

Primavera, J.H., 2000. Integrated mangrove-aquaculture system in Asia. Integrated coastal zone Management Autumn Edition, 121–130.

Primavera, J.H., 1993. A critical review of shrimp pond culture in the Philippines. Rev. Fisher. Sci., CRC Press, Boca Raton, FLA, USA 1, 151–201. https://doi.org/10.1080/10641269309388539.

Primavera, J.H., Altamirano, J.P., Lebata, M.J.H.L., delos Reyes, A.A. Jr., Pitogo, C.L., 2007. Mangroves and shrimp pond culture effluents in Aklan, Panay Is., central Philippines. Bulletin of Marine Science 80, 795–804.

R

Rahman, K.S, Islam M.N., Ahmed, M.U., Bosma, R.H., Debrot, A.O., Ahsan, M. N., 2020. Selection of mangrove species for shrimp based silvo-aquaculture in the coastal areas of Bangladesh. J. Coast. Cons. 24 (59). https://doi.org/10.1007/s11852-020-00770-8.

Rahman, M.S., Hossain, G.M., Khan, S.A., Uddin, S.N., 2015. An annotated checklist of the vascular plants of Sundarbans Forests of Bangladesh. Ban. J. of Plan. Tax. 22(1). https://doi.org/10.3329/bjpt.v22i1.23862.

Rajendran, N., Kathiresan, K., 2000. Biochemial changes in decomposing leave of mangrove. Chem. Ecol. 17, 91–102. https://doi.org/10.1080/02757540008037664.

R Development Core Team R: A Language and Environment for Statistical Computing, version 4.0.2. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.r-project.org/ (accessed on December 2020).

Reef, R., Feller, I.C., Lovelock, C.E., 2010. Nutrition of mangroves. Tree Physiol. 30(9), 1148–1160. DOI: 10.1093/treephys/tpq048.

Rebello, V.T., George, M.K., Paulton, M.P., Sathianandan, T.V., 2014. Morphometric structure of the jumbo tiger prawn, *Penaeus monodon*, Fabricius, 1798 from southeast and southwest coast of India. J. Mar. Biol. Asso. Ind. 55 (2).doi: 10.6024/jmbai.2013.55.2.01784-02.

Rejeki, S., Debrot, A.O., van den Brink, A.M., Ariyati, R.W., Lakshmi Widowati, L., 2020. Increased production of green mussels (*Perna viridis*) using longline culture and an economic comparison with stake culture on the north coast of Java, Indonesia. Aquac. Res. 00, 1-8. https://doi.org/10.1111/are.14900.

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*) postlarvae. Biodiversitas 20, 2750-2757.

Revathi, P., Senthinath T.J., Thirumalaikolundusubramanian P.,Nagaranjan, P., 2014. An overview of antibiotic profile of mangrove plants. International Journal of Pharmacy and Pharmaceutical Sciences Vol 6, Issue 3, 2014.

Rodríguez, C.E.B., García, A.C., Ponce-Palafox, J.T., Hernández, M.S., Puga-López, D., Arredondo-Figueroa, J.L., Cárdenas, L.M., 2017. The Color of marine shrimps and its role in the aquaculture. Int. J. of Aqua. Fish.

Roijackers, R., Nga, B.T., 2002. Aquatic ecological studies in a mangrove-shrimp system at The Thanh Phu state farm, Ben Tre province, Vietnam. Selected papers of the workshop on integrated management of coastal resources in the Mekong delta. Saigon, Vietnam, pp 85–94.

Rojas-Tirado, P., Pedersen, P.B., Pedersen, LF., 2017. Bacterial activity dynamics in the water phase during start-up of recirculating aquaculture systems. Aquac. Eng. 78, 24-31. https://doi.org/10.1016/j.aquaeng.2016.09.004.

Romañach, S.S., DeAngelis D.L., Koh, H.L., Li, Y., Teh, S.Y., Barizan, R.S.R., and Zhai, L., 2018. Conservation and restoration of mangroves: Global status, perspectives, and prognosis. Ocean & Coastal Management 154, 72–82. https://doi.org/10.1016/j.ocecoaman.2018.01.009.

Rout, P., Kumar, N., Basak, U.C., 2015. Assessment of anti-nutritional properties in four edible fruits of mangroves of Odisha coast. Int. Journal of Current Biotechnology 3(8), 11-16.

Rowell, D.L., 1981. Oxidation and reduction. p. 401–461. In D.J. Greenland and M.H.B. Hayes (ed.) The chemistry of soil processes. John Wiley & Sons, Chichester.

S

Satpati, G.G., Barman, N., Pal, R., 2013. A study on green algal flora of Indian Sundarbans mangrove forest with special reference to morpho taxonomy. J. Algal Biomass Utln 4 (1), 26-41.

Saxena, V., Mishra, G., Saxena, A., Vishwakarma K. K., 2013. A comparative study on quantitative estimation of tannins in *Terminalia chebula, Terminalia belerica, Terminalia arjuna and Saraca indica* using spectrophotometer. Asian Journal of Pharmaceutical and Clinical Research 6(3), 148-149.

Schanderi, S.H., 1970. Methods Food Analysis. Academic press, pp 709.

Scwamborn, R., Ekau, W., Voss, M., Paul, U.S., 2002. How important are mangroves as a carbon source for decapod crustacean larvae in a tropical estuary? Mar . Ecol. Prog. Se. 229, 195-205.

Seidman, E.R., Lawrence, A.L., 1985. Growth, feed digestibility, and proximate body composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. J. World Mari. Soc. 16, 333–346. https://doi.org/10.1111/j.1749-7345.1985.tb00214.x.

Shamsuzzaman, M.M., Islam, M.M., Tania, N.J., Al-Mamun, M.A., Barman, P.P., Xu, X., 2017. Fisheries resources of bangladesh: Present status and future direction. Aquaculture and Fisheries, 2(4), 14-156. https://doi.org/10.1016/j.aaf.2017.03.006.

Shoup, D.E., Lane, W.D., 2015. Effects of turbidity on prey selection and foraging return of adult largemouth bass in reservoirs. Nort. Ame. J. of Fishe. Man. 35, 913–924. https://doi.org/10.1080/02755947.2015.1067260.

Shoup, D.E., Callahan, S.P., Wahl, D.H., Pierce, C.L., 2007. Size-specific growth of bluegill, largemouth bass and channel catfish in relation to prey availability and limnological variables. Journal of Fish Biology 70, 21–34. https://doi.10.1111/j.1095-8649.2006.01204.x.

Silva, C.A.R., Oliveira, S.R., Rego, R.D.P., Mozeto, A.A., 2007. Dynamics of phosphorus and nitrogen through litter fall and decomposition in a tropical mangrove forest. Mar. Environ. Res. 64(4),524–534. https://doi.org/10.1016/j.marenvres.2007.04.007.

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.

Sudheer, N.S., Philip, R., Singh, I.S.B., 2011. In vivo screening of mangrove plants for anti WSSV activity in *Penaeus monodon*, and evaluation of *Ceriops tagal* as a potential source of antiviral molecules. Aquaculture 311, 36–41. https://doi.org/10.1016/j.aquaculture.2010.11.016.

Sukardjo, S., 2000. Indonesia: mangrove-friendly aquaculture. In: Primavera, J.H., Garcia, L.Ma.B., Castranos, M.T., Surtida, M.B. (Eds.), Mangrove-Friendly Aquaculture. SEAFDEC, pp. 105– 139.

Sze, C.P., Ahmad, R.SH.HJ., 2000. Malaysia: mangrove-friendly aquaculture. In: Primavera, J.H., Garcia, L.Ma.B., Castranos, M.T., Surtida, M.B. (Eds.), Mangrove-Friendly Aquaculture. SEAFDEC, pp. 81– 94.

T

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.

Tal, Y., Schreier, H.J., Sowers, K.R., Stubblefield, J.D., Place, A.R., Zohar, Y., 2009. Environmentally sustainable land-based marine aquaculture. Aquaculture. 286, 28-35. https://doi.org/10.1016/j.aquaculture.2008.08.043.

Tam, N.F.Y., Wong, Y.S., Lan, C.Y., Wang, L.N., 1998. Litter production and decomposition in a subtropical mangrove swamp receiving wastewater. J. Exp. Mar. Biol. Ecol. 226, 1 –18. https://doi.org/10.1016/S0022-0981(97)00233-5.

Tanan, S., Tansutapanich, A., 2000. Thailand: Mangrove-friendly aquaculture. In: Primavera, J.H., Garcia, L.Ma.B., Castranos, M.T., Surtida, M.B. (Eds.), Mangrove-Friendly Aquaculture. SEAFDEC, pp. 57– 65.

Thong, P.Y., 2017. Phytoplankton in aquaculture ponds: friend or foe? Global Aquaculture Alliance. April 24, 2017.

Timothy, R.P., Yoshiaki, M., Carol, M.L., 1984. A Manual of Chemical and Biological Methods for Seawater Analysis, Pergamon Press, Oxford, UK.

Tinh, T.H., Koppenol, T., Hai, T.N., Verreth, A.J., Verdegem, M.C.J., 2021. Effect of carbohydrate sources on a biofloc nyrsery system for whiteleg shrimp (*Litopenaeus vannamei*). Aquaculture, 531, 735795. https://doi.org/10.1016/j.aquaculture.2020.735795.

Tomas, C., 1997. Identifying Marine Phytoplankton. 1st Edition.

Tran, N.H., 2005. Effects of mangrove leaf litters on the integrated mangrove-shrimp farming systems in Ca Mau Province, Vietnam. PhD thesis, Asian Institute of Technology, Thailand.

Tran, H.T., Bui, D.T., 2013. Cost–benefit analysis of mangrove restoration in Thi Nai Lagoon, Quy Nhon City, Vietnam. Asian Cities Climate Resilience Working Paper Series 4. IIED, London, England.

Troell, M., Naylor, R.L., Metian, M., Beveridge, M., Tyedmers, P.H., Folke, C., Arrow, K.J., Barrett, S., Crepin, A.S., Ehrlich, P.R., Gren, A., Kautsky, N., Levin, S.A., Nyborg, K., Osterblom, K., Polasky, S., Scheffer, M., Walker, B.H., Xepapadeas, T., 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.

U

UNEP, 2014. The importance of mangrove to people : A call to action. Cambridge: UNEP World Conservation Monitoring Centre.

Uribe, E.A., Archundia, M.P.F., Luna-Figueroa., 2018. The effect of live food on the coloration and growth in guppy fish, *Poecilia reticulata.* Agricultural Sciences 9, 171-179. https://doi.org/10.4236/as.2018.92013.

V

Vance, D.J., Haywood, M.D.E., Staples, D.J., 1990. Use of a mangrove estuary as a nursery area by post larval and juvenile Banana Prawns, *Penaeus merguiensis* de Man, in Northern Australia. Estua. Coast. Shelf Sci. 31, 689-701. https://doi.org/10.1016/0272-7714(90)90020-R.

Verweij, M. C., Nagelkerken, I., Hans, I., Ruseler, S.M., Mason, P.R.D., 2008. Seagrass nurseries contribute to coral reef fish populations. Limnology and Oceanography 53(4), 1540-1547.

Victor, S., Golbuu, Y., Wolanski, E., Richmond, R.H., 2004. Fine sediment trapping in two mangrove-fringed estuaries exposed to contrasting land-use intensity, Palau, Micronesia. Wetlands Ecology and Management 12, 277–283. https://doi.org/10.1007/s11273-005-8319-1.

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. Endocr. 298, 113585. https://doi.org/10.1016/j.ygcen.2020.113585.

W

Wade, N.M., Preston, N.P., Glencross, B.D., 2013. Mechanisms of shrimp coloration. Global Seafood Alliance. https://www.globalseafood.org/advocate/mechanisms-of-shrimp-coloration .

Warnes, G.R., Bolker, B., Lumley, T., Johnson, R.C., 2018. Contributions from Randall C. Johnson are Copyright SAIC-Frederick, Inc. Funded by the Intramural Research Program, of the NIH, National Cancer Institute and Center for Cancer Research under NCI Contract NO1-CO-12400. gmodels: Various R Programming Tools for Model Fitting. R package version 2.18.1. https://CRAN.R-project.org/package=gmodels.

Weatherburm, M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39, 971–974.

Wetzel, R.Z., 1995. Death, detritus, and energy flow in aquatic ecosystems. Fresh Water Biology 35(1), 83-89. https://doi.org/10.1111/j.1365-2427.1995.tb00388.x.

Wheeler, E.L., Ferrel, R.E., 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem. 49, 312.

Wichienchot, S., Thammarutwasik, P., Jongjareonark, A,, Chansuwan, W., Hmadhlu, P., Hongpattarakere, T., Itharat, A., Ooraikul, B., 2011. Extraction and analysis of prebiotics from selected plants from south Thailand. Songklanakarin J. Sci. technol.33(5):517-523.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wickins, J.F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture 9, 19–37.

Win, U.T., 2000. Myanmar: mangrove-friendly aquaculture. In: Primavera, J.H., Garcia, L.Ma.B., Castranos, M.T., Surtida, M.B. (Eds.), Mangrove-Friendly Aquaculture. SEAFDEC, pp. 145– 154.

Wickins, J.F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture 9, 19–37.

Win, U.T., 2000. Myanmar: mangrove-friendly aquaculture. In: Primavera, J.H., Garcia, L.Ma.B., Castranos, M.T., Surtida, M.B. (Eds.), Mangrove-Friendly Aquaculture. SEAFDEC, pp. 145– 154.

World Bank, 2018. **Why is Bangladesh Vulnerable to Disasters?** Bangladesh Disaster Risk and Climate Resilience Program.

Y

Yahaya, M.A., Nodu, M., Ekine, A. O., Ajuogu, P. K., 2018. The Phytochemical and Proximate Composition of White Mangrove Leaves (*Laguncularia Racemosa*). Advance Research Journal of Multidisciplinary Discoveries,32(15), 85-89.

Z

Zabbey, N., Tanee F.B.G., 2016. Assessment of asymmetric mangrove restoration trials in Ogoniland, Niger Delta, Nigeria: lessons for future intervention. Ecological Restoration 34(3), 245-257. https://doi.org/10.3368/er.34.3.245.

Zimmerman, R.J., Minello, T.J., 1984. Densities of *Penaeus aztecus*, *P. setiferus* and other natant macrofauna in a Texas salt marsh. Estuaries 7, 421-433. https://doi.org/10.2307/1351623.

Summary

Intensification of shrimp farming has been identified as one of the main causes for mangrove destruction in the coastal region of many countries. The idea to develop mangrove-shrimp co-management was developed during the last decades of the 20th century as an effort to help restore the mangrove ecosystem and make shrimp culture sustainable. The present research is part of a broader effort to introduce silvo-aquaculture, combining mangrove and shrimp, to Bangladesh. Mangrove- shrimp co-management has very high potential from an ecological and economical point of view. Mangrove leaf litter are shown to enhance shrimp production by enhancing natural food production. In addition, the nutrient use efficiency of artificial feed is found to be enhanced by providing leaf litter as extra nutrient source, a form of synergism between natural and artificial feed. Therefore the main aim of this thesis is to assess the impact of leaf litter from different mangrove species on pond performance, water quality and natural food availability in mangrove shrimp nursery tanks and ponds.

First, the nutrient and anti-nutrient content in leaf litter of selected mangrove species and their effect on shrimp performance were determined (**Chapter 2**). The observed effects on post-larval shrimp differed between mangrove species, showing a clear ranking of mangrove species in terms of their ability to enhance shrimp growth. Leaf litter from different mangrove species, not only led to different concentrations of plankton in the PL nursery tank system, but also correlated with shrimp growth performance.

Chapter 3 explored possible effects of interactions between leaf litter from the four chosen mangrove species and pelleted feed on shrimp growth and survival in shrimp PL nursery tanks. Leaf litter and feed combined, resulted in a 21 to 33% higher weight gain of shrimp PL than based on the additive contributions of only leaf litter or only feed, indicating a synergistic effect of the two food sources on shrimp growth. Among the different mangrove species tested, *S. apetala* (Sa; 23.1%) contributed the most to total weight gain followed by *A. officinalis* (Ao; 21.6%), *S. caseolaris* (Sc; 21.6%) and *H.*

S

fomes (Hf; 10%). The lower feed conversion ratio (FCR) (0.18–0.27) in the treatments combining leaf litter and supplemental feed as compared to the feed-only treatment (0.41) indicated that leaf litter (directly or indirectly by stimulating natural food production) contributed to the nutrition of the shrimp. The observed synergistic effect between supplemental feed and leaf litter is an opportunity for farmers to reduce shrimp production costs and simultaneously raise benefits.

Chapter 4 explored the effect of different combinations of leaf litter from different mangrove species on shrimp larval performance in tanks and small (mesocosm) ponds. Three 3-mangrove-species and one 4-mangrove-species combinations of mangrove leaf litter were tested. Under controlled conditions in tanks, mixed leaf litter and feed resulted in 22 to 32% higher weight gain of PL than based on the additive contributions of only leaf litter or only feed, indicating a similar synergistic effect of the two food sources on shrimp growth as observed in Chapter 3 Although the nutrient input level and PL stocking density in ponds were less than half the input or density in tanks, the shrimp grew 3.5 times larger in the mesocosm ponds. The different combinations of mangrove leaf litter employed influenced water quality and stimulated the production of phytoplankton and zooplankton food, which allowed greater shrimp weight gain.

In **Chapter 5,** the effect of leaf litter on shrimp growth, color and product appeal to farmers, exporters and local consumers was explored. The body color of shrimp not exposed to leaf litter was lighter than of shrimp grown in tanks receiving leaf litter. The majority of *Sa*-reared shrimp at harvest were significantly darker ($P < 0.05$) in body color than larvae reared with leaf litter from other mangrove species. The shrimp body color of shrimp not exposed to any leaf litter at all was lighter than of shrimp reared in presence of all types of leaf litter tested. Within each category of shrimp value-chain actors interviewed, more than 50% of respondents preferred dark colored shrimp, and none preferred pale-colored shrimp. 100% from among exporters and local consumers, respectively, 100% and 60% linked dark body color to higher price, while the opposite was the case among non-mangrove farmers. Aside from production volume, mangrove leaf litter was found to strongly enhance the color properties and hence perceived quality of black tiger shrimp to the consumer. Inclusion of mangroves in and along shrimp culture ponds is recommended as a valuable way of improving both the commercial profitability and sustainability of shrimp aquaculture in Bangladesh and elsewhere.

Finally, in the general discussion (**Chapter 6**), the effectiveness of mangrove leaf litter application in combination with pelleted supplemental feed in PL nursery systems is reviewed against existing concepts and the functioning of mangrove-shrimp rearing systems.

The main conclusion of the thesis are:

- Application of mangrove leaf litter contributes to individual growth and total production in shrimp nursery systems.
- When applied at 1 kg m^{-3} and 0.56 kg m^{-3} culture volume in tanks and mesocosm ponds, respectively, the anti-nutrient content in leaf litter does not negatively impact shrimp performance.
- Crude fiber content strongly affects the decomposition rate of mangrove leaf litter.
- Leaf litter decomposition at the described leaf litter loading rates releases nutrients that stimulate plankton production, which in turn enhance the total system performance of shrimp nursery systems.
- For the input range and culture duration, applied in this Thesis project, joint application of supplemental pelleted feed and mangrove leaf litter created synergy for a more than additive positive effect on shrimp growth.
- The traditional shrimp farmers in Bangladesh need to be introduced to and trained in mangrove-shrimp co-management rearing techniques to ensure they benefit from mangrove-shrimp aquaculture.

Overall, our results show that inclusion of mangroves in shrimp pond culture has great as yet largely untapped potential to enhance pond productivity and make shrimp

farming more environmentally sustainable. A paradigm shift is needed with respect to mangroves vis-a-vis shrimp pond aquaculture. Whereas these areas today are largely denuded of mangrove trees, these should be seen as valuable resource for the shrimp farmer of which the leaves can serves as a healthy and inexpensive source of shrimp food. Proper management of mangrove trees in and around shrimp ponds is an opportunity with which to make shrimp farming more resilient, with important benefits for the coastal communities and the coastal ecosystem.

Acknowledgement

Throughout my PhD research journey, I was accompanied and guided by many committed persons both from the Netherlands and Bangladesh. I would like to express my humble gratitude to all of them.

First, I would like to thank my promotor **Dr. Marc Verdegem**, co-promotor **Dr. Adolphe (Dolfi) O. Debrot** and daily supervisor **Prof. Dr. Nazmul Ahsan**. Without their full and wholehearted support, this hard work of 4 years would not come to fruition.

Dr. Marc Verdegem, my promoter, has been the most important person in my entire PhD research journey. I wish I could express how much grateful I am to him! I can clearly remember 29th March 2018, my very 1st day at AFI as a 1st year PhD student. We had our first meeting on my PhD proposal writing and he directed me with all his care, seasoned knowledge and profound insights. From the beginning to the submission of my WIAS final proposal, he was my co-promotor. Later on he became my promotor. I recall how sincerely and intensely he taught me statistics. There were times when I got tired of learning but he was never ever tired of teaching. I believe that in depth learning of statistics from him has been the turning point of my PhD research. His step by step, immense and skilful guidance, particularly his unique expertise in presenting the scientific results, helped me a lot write the manuscript for publication in the peer reviewed journal. His invaluable feedback has tremendously sharpened my thoughts for my PhD research. After returning to Bangladesh, I will indeed very deeply miss all the discussions that I had with him from the very beginning to the end of this PhD research. I wish him and his family a healthy and happy life.

I would like to thank **Dr. Adolphe (Dolfi) O. Debrot** a lot for providing me with such a great deal of support, particularly in reviewing the manuscript. I will never forget the days immediately before the submission of my thesis when his wife was hospitalized for being seriously ill, yet he worked day and night for reviewing my write up. My best wishes for him and his wife.

I would like to thank **Prof. Nazmul** for the unforgettable support that he provided as a research guardian in Bangladesh. He visited my research units in Satkhira and Khulna and guided me to work in several laboratories in Bangladesh.

Thanks to **Roel Bosma** for his contribution to design my PhD research and help in proposal writing. Also, thanks to **Moin** for his support in setting up my research plots in Satkhira and Khulna and working as a research associate.

I am grateful to **Prof. Geert** for his support, especially for appointing me as a guest researcher/ employee for last couple of months. He is the most cheerful and caring Chair holder I have ever seen. I like his thematic and rhythmic write up.

I am very thankful to all AFI colleagues for their support and cooperation. I would like to convey my special thanks to the AFI Secretaries **Annet** and **Marjon** for their kind and great support for my admission, visa processing, accommodation etc. I am thankful to my first office mates **Roel**, **Twan** and **Davood** and my present office (E 1216) mates **Mark**, **Kaylee**, **Corrie** and **Eleanor** for being so helpful. I would like to thank **Kabir** who supported me mentally all the way. Thanks to **Marit** and **Yale** for their support. Thanks are due to **Vivi**, **Vina** and **Fola-Sade** for their inspiration especially during the last days of my thesis writing.

I would like to thank **Sazzad Ansaree**, **Kamonasish**, **Uthpaul**, **Sujon**, **Sudip**, **Pradip**, **Faisal**, **Tanveer**, **Latiful, Mohsina** and others from Bangladeshi community for inspiring me always. My thanks are due to **Prof. Muslima** (FMRT), **Prof. Nazrul** (FWT), **Prof. Nasif** (Economics), **Prof. Moshiur**, **Shefa** (my MSc student), **Tanmoy**, **Suborna** (Bachelor student) who helped me complete some parts of research successfully.

I would like to thank the Director General (**DG**) of the Department of Fisheries for inspiring me always from Bangladesh. I would like to thank the present and past honourable **Secretary** of the Ministry of Fisheries and Livestock Bangladesh for approving my study leave and helping me to continue my research.

Thanks are due to **Selim Reza Hasan**, Country Manager, Solidaridad Network Asia, Bangladesh. I would like to thank **Sanjay** (FWT), **Uzzwal** (Lab assistant, FWT nutrient lab), **Omar Ali** (Lab assistant, FMRT water quality lab), **Reaz**, **Rana**, **Dr. Iqbal** (Pharmacy), **Nipa Adhikary** (Soil science) and **Masud** who helped me in the laboratory analyse leaf litter, soil and water quality.

Thanks to **Noman** for his support. He was the safeguard of my shrimp in Satkhira and he helped me to construct new ponds. I also thank **Habib** and **Bappy** for their help in feeding the shrimp in my absence.

I thank a lot **Tipu** and **Lipi** in Belgium. They made my trip to France and Switzerland for some relaxation after a long and tiresome write-up of my thesis.

I am grateful to Hafez Maw. **Hafizur Rahman**, all the inhabitants of **Moulavi Para** and **Jhalbari** for their best wishes always for me. Their whole hearted love always inspire me to do good deeds.

Finally, I am grateful to all of my family members for supporting me all the way during my PhD journey. I cannot but express my thanks and gratefulness to my parents (**Abba** and **Ma**) who are always my inspiration and blessings. They taught me how to lead a simple life with honesty and integrity and how to earn love from people. They never asked anything from me. They have been just praying for my healthy life.

My biggest thanks to my dear wife **Lopa** and sons **Abian** and **Ahian** who accompanied me all the way, especially at the last part of the journey here in the Netherlands. I believe that we made this big journey all together and reached a successful end.

I lost my maternal grandmother (**Nani**) and an aunt (**Boro Mami**) during this PhD journey and this is the only sad part of this journey. They loved me a lot . I pray for their eternal peace.

My thanks and gratitude to all of my friends both at home and abroad. I am truly sorry if I missed anybody to acknowledge.

A

About the author

Md. Iftakharul Alam was born in June 1978 in Khulna, Bangladesh. He completed his Bachelor of Science (Honors) in Fisheries & Marine Resources Technology (FMRT) in 2002 with distinction from Khulna University (KU), Bangladesh. He completed his 1st Master of Science in the same subject from the same university in 2005. In that year he took part in 24th Bangladesh Civil Service (BCS) examination. He secured 1st position in Fisheries cadre in that nationwide competitive examination and joined as Upazila Fisheries Officer (UFO). In 2009, he started his 2nd Master's degree in Aquaculture at Ghent University, Belgium with VLIR-uos scholarship. After successfully completing the course, he joined his previous job in the Department of Fisheries (DoF) under the Ministry of Fisheries and Livestock. In 2018, he was selected for a PhD programme at Wageningen University and Research (WUR) in the Netherlands with NWO fellowship in collaboration with Solidaridad Netwrok Asia and Khulna University. His PhD research is about introducing Mangrove-shrimp co-management in the coastal region of Bangladesh. His hobby is to play volleyball and he was the captain of the volleyball champion team in the 40th Foundation Training Course (FTC) at the Public Administration Training Center (PATC). His country has hard working and talented peoples and is full of natural resources. He dreams of a developed and prosperous Bangladesh.

Contact : iftakharku97@yahoo.com

List of publications

Alam, M.I., Ahmed, M.U., Yeasmin, S., Debrot, A.O., Ahsan, M.N., Verdegem, M.C.J., 2022. Effects of mixed leaf litter of four mangrove on shrimp postlarvae (*Penaeus monodon*, Fabricius, 1798) performance in tank and mesocosm conditions in Bangladesh. Aquaculture 551, 737968. https://doi.org/10.1016/j.aquaculture.2022.737968.

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

Alam, M.I., Ahsan, M.N., Debrot, A.O., Verdegem, M.C.J., 2021. 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) postlarvae. Aquaculture 542, 736865. https://doi.org/10.1016/j.aquaculture.2021.736865.

Debrot, A.O., Veldhuizen, A., van den Burg, S.W.K., Klapwijk, C.J., Islam, M.N., **Alam, M.I.,** Ahsan, M.N., Ahmed, M.U., Hasan, S.R., Fadilah, R., Noor, Y.R., Pribadi, R., Rejeki, S., Damastuti, E., Koopmanschap, E., Reinhard, S., van Scheltinga, C.T., Verburg, C., Poleman, M., 2020. Non-Timber Forest Product Livelihood-Focused Intervention in Support of Mangrove Restoration: A Call to Action. Forest 11 (11), 1224. https://doi.org/10.3390/f11111224.

Natrah, FMI., **Alam, M.I.,** Pawar, S., Harzevili, A.S., Nevejan, N., Boon, N., Sorgeloos, P., Bossier, P., Defoirdt, T., 2012. The impact of quorum sensing on the virulence of *Aeromonas hydrophila* and *Aeromonas salmonicida* towards burbot (*Lota lota* L.) larvae. Veterinary microbiology 159 (1-2), 77-82. https://doi.org/10.1016/j.vetmic.2012.03.014.

Alam, M.I., Yeasmin, S., Khatun, M.M., Rahman, M.M., Ahmed, M.U., Debrot, A.O., Ahsan, M.N., Verdegem, M.C.J., 2022. Effect of mangrove leaf litter on shrimp (*Penaeus monodon*, Fabricius, 1798) growth and color. Aquaculture Reports (under review).

Alam, M.I., Rahman, M.S., Ahmed, M.U., Debrot, A.O., Ahsan, M.N., Verdegem, M.C.J., 2022. Mangrove forest conservation vs shrimp production: uncovering a sustainable co-management model and policy solution in coastal Bangladesh. Forest Policy and economics (Under review).

Alam, M.I., Ahmed, M.U., Debnath, S., Debrot, A.O., Ahsan, M.N., Verdegem, M.C.J (....). A cost-benefit assessment of current mangrove use in extensive shrimp farms of coastal south-west Bangladesh. (Under preparation).

Training and supervision plan

Training and supervision		
Name PhD candidate	Md. Iftakharul Alam	
Group	Aquaculture and Fisheries (AFI)	
Co-promotor	Adolphe (Dolfi) O. Debrot	
Promotor	Dr. M.C.J. Verdegem	

Education and training	**Year**
The Basic Package (2 ECTS[1])	
WIAS Introduction Day	**2018**
WGS Scientific Integrity and Ethics in Animal Sciences	**2018**
Disciplinary Competences (10 ECTS)	
Writing the research proposal	**2018**
Statistics for the Life Sciences	**2018**
WIAS/PE&RC advanced statistics course Design of Experiments	**2018**
Summer School on agroecology and animal production	**2018**
Professional Competences (5 ECTS)	
Course on how to supervise BSc and MSc thesis students	**2018**
WIAS course Techniques for Scientific Writing and Presenting	**2018**
Writing a project proposal (ESMF: Environment and Social Management Frame Work) following world's bank guideline	**2018**
Reviewed WIAS PhD proposal of Satya Prakash	**2021**
Societal Relevance (4 ECTS)	
Ethics for Social Sciences Research	**2018**
Focused Group Discussion (FGD) in Bangladesh	**2018-2020**
Participation in the Sunday Talkies / Online	**2021**
Presentation Skills (4 ECTS)	
12th Asian Fisheries and Aquaculture Forum	**2019**
Seminar on Mangrove Polder (Organiser and Presenter) Bangladesh	**2019**
WIAS Science Day : Oral presentation	**2021**
WINNER-2021 (Oral presentation as a keynote speaker)	**2021**
Teaching competences (6 ECTS)	
Conducted Classes, Sessional and Field works for MS students	**2020**
Supervising BSc Thesis student at Khulna university, Bangladesh	**2020/2021**
Supervising MSc thesis students at Khulna University, Bangladesh	**2020/2021**
Total = 31 ECTS	

[1]One ECTS credit equals a study load of approximately 28 hours

Colophon

The work presented in this thesis was conducted at the Aquaculture and Fisheries Group, Wageningen University. The scholarship for the author was provided by NWO in collaboration with Solidaridad Network Asia and final year was supported by Wageningen university.

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover design and lay out

Md.Iftakharul Alam, Firoj Ahemd and Kajol

Printing

This thesis was printed by ProefschriftMaken | www.proefschriftmaken.nl