KORESPONDENSI PAPER

Judul : The Effect of Decomposing Mangrove Leaf Litter and Its Tannins on The Water Quality, Growth and Survival of Tiger Prawn (Penaeus monodon) Post-Larvae

Jurnal : Biodiversitas Journal of Biological Diversity

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[biodiv] Submission Acknowledgement

From: Ahmad Dwi Setyawan (smujo.id@gmail.com)

To: roel.bosma@wur.nl; resti_wisnoe@yahoo.com; rrwidowati@yahoo.com

Date: Wednesday, May 15, 2019, 12:59 PM GMT+7

Hello,

sri rejeki sulaiman has submitted the manuscript, "The Effect of Decomposing Mangrove Leaf Litter and Its Tannins on The Water Quality, Growth and Survival of Tiger Prawn (Penaeus monodon) Post-Larvae" to Biodiversitas Journal of Biological Diversity.

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Ahmad Dwi Setyawan

Biodiversitas Journal of Biological Diversity

[biodiv] Editor Decision

From: Nor Liza (smujo.id@gmail.com)

To: sri_rejeki7356@yahoo.co.uk; roel.bosma@wur.nl; resti_wisnoe@yahoo.com; rwidowati@yahoo.com

Date: Tuesday, May 21, 2019, 08:50 AM GMT+7

sri rejeki sulaiman, Roel H Bosma, Restiana Wisnu Ariyati, Lestari Lakhsmi Wiidowati, 25853737:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The Effect of Decomposing Mangrove Leaf Litter and Its Tannins on The Water Quality, Growth and Survival of Tiger Prawn (Penaeus monodon) Post-Larvae".

Our decision is: Revisions Required

Nor Liza
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Biodiversitas Journal of Biological Diversity

L-Reviewer Comments.docx
11.9kB

Reviewer Comments

The manuscript entitled "The effects of decomposing mangrove leaf litter and its tannins on the water quality, growth and survival of tiger prawn (*Penaeus monodon*) post-larvae" assessed two mangrove species leaves decomposition in containers with tiger prawn in Indonesia. Results indicated that survival of such prawn depends on the decomposing leaves concentration. Overall, leaching experiments of mangrove leaves are not that common and usually depended on species and benthonic/pelagic organisms. Hence, results from this study could be worth publishing with some minor modifications/suggestions as follows:

Add more information regarding the two mangrove species morphology/chemical nature. For instance, which mangrove species presents more pigments? Is there some sort of seasonality regarding biochemical properties?, etc.

Add more information about the tiger prawn species such as behavior or persistency against pollutants.

There is an inconsistency using the Oxford comma.

There are some missing references throughout the text.

In the abstract, authors mentioned 30 aerated tanks but in the conclusion, authors mentioned tanks without water exchange. Please clarify this in the methods section.

Some format of the references are not according to the format of this journal. Kindly check and correct accordingly

In the references, names of journals should be abbreviated. Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviation (www.issn.org/2-22661-LTWA-online.php)

[biodiv] Editor Decision

From: Nor Liza (smujo.id@gmail.com)

To: sri_rejeki7356@yahoo.co.uk; roel.bosma@wur.nl; resti_wisnoe@yahoo.com; rwidowati@yahoo.com

Date: Saturday, June 15, 2019, 09:20 PM GMT+7

sri rejeki sulaiman, Roel H Bosma, Restiana Wisnu Ariyati, Lestari Lakhsmi Wiidowati, 25853737:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The Effect of Decomposing Mangrove Leaf Litter and Its Tannins on The Water Quality, Growth and Survival of Tiger Prawn (Penaeus monodon) Post-Larvae".

Our decision is: Revisions Required

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Biodiversitas Journal of Biological Diversity

H-Reviewer comments.docx
15kB

Reviewer comments: This manuscript represents the findings of a study designed to investigate the any influence(s) of decomposing mangrove leaf litter on the growth and survival of tiger prawn (*Penaeus monodon*) post-larvae. Experimental tubs with 5 treatments utilising *A. marina* and *R. apiculate* leaf matter of varying concentration and handling procedures were employed.

The mansucript is structured appropriately and provides justified objectives and sound background for the study. The results and discussions are presented in a straight forward manner but requires addition detail (see specific comments below).

It is the reviewer's belief that this work contributes to the field of research. However, a large majority of revisions/comments require attention by the author(s). As such I recommend publication of the article after major revisions.

General Overall Comments

- Major modifications and an overall thorough improvement of the manuscript is required regarding spelling, sentence structure, text formatting, grammar, and consistency of units, terms, super/subscript text, use of italics and hyphens. These must be significantly improved. Care must be taken to avoid these issues which currently negatively impact the quality of the manuscript.

- Improvements to the manuscript must also be made with regard to cross-referencing appropriate tables and figures.

SPECIFIC COMMENTS/EXAMPLES REQUIRING ATTENTION

Abstract

- Improve spelling and use of spaces, for example Line 9 Shrimpfarmingin Demak, to be changed to Shrimp farming in Demak. Many instances of the lack of spaces between words, numbers, units and symbols require attention. Eg. Lines 12, 14, 15, 17, and 21. Look to amend text here and in all other relevant instances throughout manuscript.

Line 13: PL is explained as post larvae. Please provide distinction of PL-21

Line 14: include units after 0.125

Line 17: add rate after specific growth and survival, e.g. specific growth rate (SGR, % day^{-1}) and survival rate (SR, %)

Line 22: change tense to past, e.g. “had no toxic”

Introduction

Line 28: missing reference ()

Lines 32 & 36: *silvo-aquaculture* and silvo-aquaculture. Be consistent with use of italicized text

Line 51: amend and add space - ‘Yakupitaga 2005).Using R.’ to ‘Yakupitaga 2005). Using R.’

Line 52: is the question rather just whether tannin was released by the decomposing leaves but also at what rate. Please specify if so.

Lines 52-53: In accordance with the manuscript title, look to include water quality aspect into the objectives of the study

Materials and Methods

- Look to restructure section with emphasis on;

o 1. Text describing geographical location/context, background information of farming/pond characters

o 2. Text describing experiment design and characteristics of tanks and leaves, with a summary table of design, treatments, replicates, etc., before

o 3. Discussing treatment specific methodologies/handling of leaves and leaf concentrations

- Look to provide subsection regarding leaf litter starting conditions/characteristics (e.g. chemical and nutrient content), which will be influential in observed study results and outcomes

Lines 62-72: Please revise information/commentary is repeated

Line 84: clearly specify PL_W

Line 84: specify acclimatisation conditions and duration

Line 84: specify attributes of replacing identical individuals (e.g. weight and or length)

Line 86: specify total rate of added feed was 10% of initial total body weight as described in abstract

Line 92: include the term temperature before T, abbreviation

Line 98: include () regarding year of referenced material. E.g. Rice et al. (2012). Look to amend text here and in all other relevant instances throughout manuscript.

Line 108: confirm this is appropriate commentary for this section and provide detail regarding influence of wind? The cross-reference Figure 2A and 2B does not relate to the Figure 2 currently in the manuscript. Please emend manuscript accordingly.

Line 112: add full stop

Line 112 & 119 Equations: confirm consistant use in equations and description, e.g. W_t and W_o or BW_t and BW_o; $N_t$and N_0 or Nt and No

Line 119 Equation: Format equation correctly

Line 129: Amend spelling of ‘Sapiro-Wil test’ to ‘Shapiro-Wil test’

Results and Discussion

- Remove Discussion from heading. Section 4 of the manuscript is labelled as Discussion

Line 137: use term or acronym for dissolved oxygen as dissolved oxygen (DO) was already explained in manuscript text

Line 137: provide explanation as to the large variation of salinity fluctuations between 19 and 25 ppt, if the experimental plastic tanks are closed systems.

Line 140: Superscript -1 for mg L^{-1}

Line 142: italics for species genus

Line 144 Table 1: Table caption does not include dissolved oxygen. Amend table caption accordingly. Superscript -1 for mg L^{-1}

Line 159 Table 2: Table caption not consistent with Table 1, when discussion mean and standard deviation (mean ± standard deviation). AR and RA in table caption not consistent with treatment acronyms in table (*Am* and *Ra*). Confirm use of A and a throughout table.

Line 154 & 156: confirm use of Table 2A and Table 2B

Figure 2: not discussed in results. Add commentary/relevance – also provide correlation analysis results and R^2 value

Line 178: confirm use of Table 3A

Table 3: Table caption not consistent with Table 1, when discussion mean and standard deviation (mean ± standard deviation). Superscript -1 for day^{-1}

Line 192: confirm use of Table 3A

Line 203: amend Amarina to *A. marina*

Discussion

- Authors to provide commentary how do the selected litter concentrations for the study treatments relate to the natural leaf litter rates for the select mangrove species in the study regions. Is there any seasonal variation? and if so discuss any potential implications for farming practices/survivability of prawn/s.

Lines 240 & 241: Incomplete sentence. Amend accordingly

Line 243: amend 'did not had' to 'did not have'

Line 253: remove full stop (.)

Line 255: Add commentary regarding the use of minced leaf litter and its relevance/occurrence in situ

Lines 269: The authors should provide potential explanations as to the difference in SR trends between species. Additionally, how this may influence farming practices with some commentary of the species composition of mangroves on the banks of farm areas.

Acknowledgments

Line 294: amend 'NOW-WOTROwhich' to 'NOW-WOTRO which'

References

- Keep reference formats consistent. For example use of italics for volume number and consistent font (Line 382)

[biodiv] Editor Decision

From: Smujo Editors (smujo.id@gmail.com)

To: sri_rejeki7356@yahoo.co.uk; roel.bosma@wur.nl; resti_wisnoe@yahoo.com; rrwidowati@yahoo.com

Date: Monday, August 5, 2019, 04:00 PM GMT+7

sri rejeki sulaiman, Roel H Bosma, Restiana Wisnu Ariyati, Lestari Lakhsmi Wiidowati, 25853737:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The Effect of Decomposing Mangrove Leaf Litter and Its Tannins on The Water Quality, Growth and Survival of Tiger Prawn (Penaeus monodon) Post-Larvae".

Our decision is: Revisions Required

Smujo Editors
editors@smujo.id

Reviewer A:

Dear Editor,

Please find attached is the reviews for the manuscript "01-AUG-SGB-The effects of decomposing mangrove leaf litter and its tannins".

Overall, the research is well executed and nicely written. The results might also have management implication in shrimp farming management althtough this is not yet deeply elaborated in the manuscript. As such I suggest to add this in the Discussion as well as few changes as per suggestion in the reviews.

Thank you and best regards,

Reviewer

Recommendation: Revisions Required

Biodiversitas Journal of Biological Diversity

A-01-AUG-SGB-The effects of decomposing mangrove leaf litter and its tannins_InternalRev.docx
955.5kB

The effects of decomposing mangrove leaf litter and its tannins on water quality and the growth and survival of tiger prawn (*Penaeus monodon*) post-larvae

Abstract. Shrimp farming in Demak, Indonesia is often practiced in silvo-aquaculture systems in which mangrove trees are planted on pond bunds. As such, mangrove leaves and its substrates may have impact on penaeid shrimp production. In this area, mangrove re-growth is usually proceeded with *Avicennia marina* while planting is mostly done with *Rhizophora apiculata*. We compared the effects of decomposing fresh leaves of *A. marina* and *R. apiculata* on water quality and on the performance of *Penaeus monodon* post larvae (PL). A hundred of PL21 (post larvae aged 21 days with weight of 0.28 g) were stocked in each of 30 aerated tanks containing 800 liters of brackish water (salinity of 21 ppt) for 37 days. Five treatments with three replicates for each mangrove species were assigned by adding into the tanks of 0.125, 0.25, and 0.5 g L^{-1} of air-dried leave, 0.125 of g L^{-1} minced leave and 0.125 g L^{-1} of leachate of minced leaves. The PLs were fed 3 times daily with pellets at 10 % of initial total body weight. Water quality parameters were recorded daily. Tannin, H_2S and NH_3-N concentrations were measured every ten days. Prawn's body weight (BW) was measured and specific growth rate (SGR, % day^{-1}) and survival rate (SR, %) were calculated after the end of experiment. Results were analyzed with ANOVA and Pearson's correlation. The results showed that tannin in decomposing mangrove leaf litter up to a concentration of 0.5 mg g^{-1} did not have a significant effect on water quality and on the growth and survival of *P. monodon* PL. However increasing leaf litter concentrations showed an increase in NH_3-N concentration due to organic matter degradation. The accumulation of NH_3-N may have caused the slow growth of shrimp PL in *A. marina* treatment. Shrimp PL in leaf litter leachates treatment have a higher growth rate than those PL in regular leaf litter in relation to nutritiitional value. Survival and growth varied from 62 ±14 to 70 ± 8% and 3.1±2.1 to 5.5±1.2% day^{-1}, respectively. Although decomposing mangrove leaves of *A. marina* and *R. apiculata* had no toxic effects on *P. monodon* PL up to a concentration of 1.25 g L^{-1}, but causing severe mortality above 2.5 g L^{-1} for shrimp in tanks without water exchange. PLEASE ADD ONE OR TWO SENTENCES TO CLOSE THE ABSTRACT EXPRESSING THE IMPLICATION OF THE RESULTS ON SHRIMP FARMING MANAGEMENT.

Keywords: *Penaeus monodon, Avicennia marina, Rhizophora apiculata*, ammonia-N, tannin

INTRODUCTION

In 1980s the international demand for prawn increased, and as a result both extensive and intensive prawn culture expanded dramatically (Primavera *et al.* ,1993; Rönnbäck, 2002). In Indonesia, the decreasing world market price for rice caused by green revolution pushed the conversion of both paddy fields and mangrove forests into shrimp ponds. While extensive prawn culture caused mainly coastal land use change (i.e. mangrove loss), several problems occured due to the intensification: intrusion of saline water upland, increase of nutrients in waterbodies due to feed waste and prawn excretions, and loss of capital due to disease outbreaks (Primavera, 1997; Rivera-Ferre, 2009). In response to unsustainable systems, integrated mangrove-shrimp aquaculture systems (i.e. silvo-aquaculture) have been developed as environmentally and socioeconomically sustainable strategies for poor small-scale farmers (Primavera, 2000; Fitzgerald, 2002; Rönnbäck, 2002). In Indonesia, this technology was started in 1976 by the State Forestry Corporation, with the aim of rehabilitating and conserving mangrove forest, and resolving forestry-fisheries conflicts (Primavera, 2000).

Although silvo-aquaculture systems are more ecologically friendly with mangrove ecosystems than other types of aquaculture (Primavera, 2000), they also have problems of sustainability. Decaying mangrove leaves are known to accumulate at pond ground, causing an increase in tannin levels, which together with the shade of the mangrove trees creates an acidic and anoxic environment, which ultimately results in lower shrimp production (Johnston et al., 2000; Clough et al., 2002; Nga et al., 2006). As an example, the prawn production from a silvo-aquaculture pond with *Avicennia marina* and *Rhizophora apiculata* mangroves in Purworejo village, Demak district, Central Java, was at the low end with yield of 75 - 105 kg ha^{-1} per year (Tonneijck et al, 2015).

Fitzgerald (2000) stated that high tannin concentrations may be potentially toxic to penaeid shrimp in mangrove-shrimp aquaculture systems. Mangroves contain high levels of tannins (Robertson, 1988), which can rise as much as 20% of the dry weight of plant material (Hernes et al., 2001). Tannins, generally divided into hydrolyzed and condensed tannins, are anti-nutritional elements with zero nutritional value, affecting protein utilization and nutritional digestibility of various herbivorous and detritivorous crustaceans and fish species (Neilson et al., 1986; Conde et al., 1995 cited by Erickson et al., 2004; Becker & Makkar, 1999; Maitra & Ray, 2003; Hammann & Zimmer, 2015). The negative impact of *R. apiculata* mangrove on shrimp performance (Primavera, 2000) was confirmed in an experiment that showed that leaf concentrations higher than 0.5 g L^{-1} were very lethal where leaf effects differed between mangrove species (Hai and Yakupitaga, 2005). Using *R. apiculata* as a reference species, this study aimed to assess whether tannin was released by the decomposing leaves of *A. marina* and *R. apiculata* and which form of their leaves litter that contribute more on water quality degradation cvg, whether this affected the growth and survival rate of *P. monodon*.

MATERIALS AND METHODS

Study period and location

The study was conducted for 40 days in Demak District, Central Java, Indonesia. The coastal areas of Demak once had extensive mangrove forests (about 6000 ha), but these areas had been converted into aquaculture where some area are applied silvo-aquaculture. The mangrove species *A. marina* and *R. apiculata* co-dominate the dikes of the pond and were therefore selected as the species used in this study. The experimental station is located in Tambakbulusan village in the Sub-district of Karang Tengah, about six kilometers from the capital of Demak.

Experimental procedure

Thirty-three tanks of 1 m^3 (1x1x1 m) were used to test the effects of decomposing mangrove leaves on water quality and on the growth and survival of *P. monodon* PL. The experimental plastic tanks of 1 m^3 were filled with ± 5 cm pond bottom substrate, clay-loam soil (pH 6.5) and 800 liters of brackish water (salinity 21 ppt). The prawn PL were stocked 4 days later to let the suspended particles sediment. The water in the tanks was not exchanged but the volume was maintained by adding water from the same source regularly. Each tank was continuously aerated using Resun® LP-60 low noise air pump to maintain dissolved oxygen level above 5 mg L^{-1}, thus largely above the recommended level and the 3 mg L^{-1} generally found in silvo-aquaculture (Boyd, 1989; Binh et al., 1997; Johnston et al., 2002). The experimental tanks were covered with dark netting to reduce water temperature fluctuation and light intensity.

Tiger prawn (*P. monodon*) larvae of 21 days old, known as PL-21, at average initial body weight of 0.28 g were bought from the Centre of Brackish Water Research Institution (Balai Besar Penelitian Budidaya Air Payau = BBPBAP) in Jepara. PL-21 were randomly stocked in each tank at a density of 100 PLs m^{-2} that was acclimatized previously. During the acclimatization, dead PL were replaced by new identical individuals.

Commercial shrimp pellet produced by Central Proteinaprima Tbk. was added three times a day at 07:00, 12:00 and 18:00 to the tanks with PL at the total rate of 10% of the total stocked and adjusted after the weekly weighing. The feed was put at a 40 x 40 cm feeding tray submerged at the bottom of each tank. The pellet contained 41% protein, 5% fat, 2% fiber, 13% ash and 11% moisture.

Mature green leaves of *A. marina* and *R. apiculata* were collected from mangrove pond and transported to the experimental station. The leaves were air-dried in the
shade to a constant weight and separated species-wise in litter bags made of nylon nets of 3 mm mesh size (Figure 1A-B), and then added to the tanks nine days after collection. Stones were tied to the litter bags to make them sink.

The minced leaves were obtained by cutting the dried leaves into small pieces and mincing the cust in an electrical blender (Figure 1C). The leave leachate was obtained by soaking 100 g of blended leaf litter in 2 L filtered brackish water for 30 minutes, then sieving through a woven wire sieve (100 µm mesh size). Only the solution was added to the tanks (Figure 1D).

Experiment conducted by Hai and Yakupitaga (2005) found that the concentration of leaves higher than 0.5 g L^{-1} was very lethal. As such, three concentrations of mangrove leaves were applied in our study: 0.125, 0.25 and 0.5 g L^{-1}. Two additional leaves treatments were applied to analyze the effects of presumed faster release of tannins to allow faster decomposition and tannin dilution: (a) 0.125 g L^{-1} minced 100 g leaf litter was cut into small pieces and minced using a blender after which added species-wise in the litter bags made from pantyhose stockings (Figure 1C); (b) 0.125 g L-
1 leachate 100 g of blended leaf litter was soaked in 2 L filtered brackish water for brackish water for 30 minutes, after which sieved through a woven wire sieve (100 µm mesh size), and only
the solution was added species-wise (Figure 1D).

All 5 treatments were done in three replications.

Figure 1. The decomposing mangrove leaves were separated according to different treatment: (A) *R. apiculata* leaves; (B) *R. apiculata* leaves; (C) Minced *R. apiculata* leaves; (D) *R. apiculata* litter leachate

Data collection

Water quality parameters

Seven water quality parameters were measured including temperature (T), pH, salinity, dissolved oxygen (DO), tannin, hydrogen sulfide (H_2S), and unionized ammonia as nitrogen (NH_3-N). Water temperature was observed daily using an electronic thermometer with precision of 0.1°C, pH using HANNA ® HI98129 pH meter with precision of 0.01, and salinity using ATAGO®PAL-06s refractometer 06S refractometer with precision of 1 ppt. The DO was recorded on day 6, 12, 22, 23, 30 and 33 of the study using a YSI@Pro DO meter (read-out in 0.1 mg L^{-1}).

Tannin, hydrogen sulfide (H_2S) and unionized ammonia as nitrogen (NH_3-N) were measured according to standard methods as described in Rice et al. (2012). Therefore, every ten days three water samples were taken from each replicate treatment: 500 ml for tannin analysis and 200 ml for H_2S and NH_3-N analysis. For the control, only the two/three samples were aggregated before analysis. Samples for H_2S analysis were preserved by adding 8 drops of 2*N* zinc acetate and 10 drops of sodium hydroxide (NaOH) solution to pH > 9, while NH_3-N samples were preserved by adding sulfuric acid (H_2SO_4) to pH < 2. The 93 samples were kept in styrofoam cool boxes and being transported to Balai Besar Teknologi Pencegahan Pencemaran Industri, Semarang, for analyses. The tannin content was analysed colorimetrically by the Folin phenol method, while H_2S and NH_3-N were analyzed by the Iodometric and Phenate method, respectively.

Figure 2: (A) Initial body weight was measured in group of ten individuals; (B) final body weight was measured per individual

Growth performance and survival rate

The initial body weight was determined for the whole population (i.e. 3300 prawn) by randomly sampling and weighing 35 groups of ten individuals in order to minimize fluctuations due to wind (Figure 2A). The final body weight was determined per tank by collecting and weighing all shrimp individually (Figure 2B). Weighing was done with an A&D® HL-100 electronic weighing scale with a precision of 0.01 g.

The specific growth rate (SGR) and survival rate (SR) were calculated with the formula of Busacker et al. (1990)

$$SGR = \frac{\ln BW_t - \ln BW_o}{t} \times 100\%$$

Where :

SGR is specific growth rate (% day-1);

*BW*t is the final body weight (g);

*BW*o is the itial body weight (g); and *t* is duration of experiment (days).

$$SR = \frac{N_t}{N_o} \times 100\ \%$$

Where

SR = is the survival rate (%);

Nt = is the number of prawn collected at sampling time t; and

No = is the number of prawns initially stocked

Data analyses

Statistical analyses were done using SigmaPlot® 12. Differences were considered significant at $P < 0.05$. The water quality parameters and prawn performance were compared by one-way analyses of variance (ANOVA). Prior to the ANOVA, all data were checked for their normality using the Saphiro-Wil test. When data were not normally distributed, a non-parametric Kruskal-Wallis test by ranks was used. If the ANOVA showed the treatment significantly affected the variables, a multiple comparison Duncan and Tukey post-hoc test was done. Correlations among the water quality parameters, between the water quality parameters and growth, and between the water quality parameters and survival rate, were analyzed using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Water quality parameters

The levels of salinity, temperature, and DO did not differ significantly between the treatments throughout the experiment (Table 1). Since the experimental tanks are closed system, the salinity levels fluctuated between 19 and 25 ppt (mean 22±1 ppt) and the water temperature between 27.2 and 33.3°C (mean 30.6±1.4 °C) but the DO slightly varried from 6.3 to 6.9 mg L^{-1} (mean 6.7±0.5 mg L^{-1}). Although the DO concentration of the culture media was high and in the upper side of the recommended range (±4 mg L^{-1}) all recorded salinity, temperature, and DO concentrations were considered as optimal for *P. monodon* PL.

Table 1. The mean ± standard deviation of salinity (ppt), Dissolved Oxygen (DO), temperature (T) and pH for each treatment

Leaf Litter (gL^{-1}) Concentration	*A. marina*			*R. apiculata*		
	Salinity (ppt)	**DO (mg L^{-1})**	***T* (°C)**	**Salinity (ppt)**	**DO (mg L^{-1})**	***T* (°C)**
A (leaf concentration)						
0.125	21 ± 1	6.6 ± 0.3	30.1 ± 1.4	23 ± 1	6.9 ± 0.5	30.6 ± 1.4
0.25	22 ± 1	6.7 ± 0.6	30.2 ± 1 .4	22 ± 1	6.8 ± 0.6	30.6 ± 1. 3
0.5	23 ± 1	6.3 ± 0.3	30.4 ± 1.4	21 ± 1	6.5 ± 0.6	30.4 ± 1.4
B (leaf treatment)						
0.125 whole	21 ± 1	6.6 ± 0.3	30.1 ± 1.4	23 ± 1	6.9 ± 0.5	30.6 ± 1.4
0.125 minced	23 ± 1	6.8 ± 0.3	30.6 ± 1.3	22 ± 1	6.8 ± 0.5	30.6 ± 1.4
0.125 leachate	22 ± 1	6.9 ± 0.4	30.9 ± 1.5	22 ± 1	6.6 ± 0.4	30.6 ± 1.3

The majority of the treatments had undetectable H_2S concentrations (<0.002 mg L^{-1}). H_2S was only detected in 11 of the 30 tanks during the research period; most measured concentrations were 0.002 and 0.003 mg L^{-1}. For both *A. marina* and *R. apiculata* the highest levels were found for the treatment with 0.125 leachate: 0.005 and 0.004 mg L^{-1}, respectively. Therefore no reliable conclusion can be made about the influence of mangrove leaf litter concentrations on H_2S production.

The NH_3-N concentration found in all treatments exceeded the optimal level for penaeid PL and were considered critical and lethal. Leaf litter concentrations increased the NH3-N concentration in both *A. marina* and *R. apiculata* treatments, but this did not differ significantly (H = 6.124, df = 3, P = 0.106) (Table 2A).

The average NH_3-N concentrations in the leaf treatments for *A. marina* compared to *R. apiculata*, 0.83 ± 0.2 and 0.79 ± 0.2 mgL^{-1}, respectively, were not significantly different (Table 2B). However, species-wise, NH_3-N concentration was not also significantly affected by the leaf treatments (H = 0.230, df = 1, P = 0.631).

Table 2. Mean and standard deviation (SD) of the concentrations of NH_3-N (m.L^{-1}) and tannin for the treatments with *A. marina* and *R. apiculata*, the P-value of the ANOVA and results of the post-hoc tests (values in the same column having a different letter are significanlty different)

Treatments	NH3-N			Tannin		
	A. marina	***R. apiculata***	**Mean**	***A. marina***	***R. apiculata***	**Mean**
A (leaf concentration)						
0.125	0.82 ± 0.3^a	0.74 ± 0.2^a	0.8 ± 0.2^A	1.77 ± 1.5^a	2.21 ± 1.5^a	2.0 ± 1.5^A
0.25	0.95 ± 0.3^a	0.82 ± 0.2^a	0.9 ± 0.2^A	1.86 ± 1.5^a	1.92 ± 1.5^a	1.9 ± 1.4^A
0.5	0.99 ± 0.2^a	0.87 ± 0.2^a	0.9 ± 0.2^A	2.16 ± 1.4^a	1.97 ± 1.5^a	2.1 ± 1.4^A
B (leaf treatment)						
0.125 Whole	0.82 ± 0.3^a	0.74 ± 0.2^a	0.8 ± 0.2^A	1.77 ± 1.5^a	2.21 ± 1.5^a	2.0 ± 1.5^A
0.125 Minced	0.75 ± 0.1^a	0.76 ± 0.2^a	0.8 ± 0.2^A	1.83 ± 1.4^a	2.21 ± 1.6^a	2.0 ± 1.5^A
0.125 Leachate	0.69 ± 0.1^a	0.76 ± 0.2^a	0.7 ± 0.2^A	2.03 ± 1.46^a	2.02 ± 1.6^a	2.0 ± 1.5^A
Mean	0.83 ± 0.2^A	0.79 ± 0.2^A		1.92 ± 1.4^A	2.07 ± 1.5^A	

The concentration of mangrove leaf litter did not significantly affect tannins in water (P = 0.967) (Table 2A). However, the average tannin concentration in the tanks increased during leaf decomposition, from 0.68 ± 0.40 mg L^{-1} on day 12 to 3.72 ± 0.23 mg L^{-1} on day 33 of decomposition for the treatments of *A. marina* and similarly from 0.49 ± 0.15 mg L^{-1} on day 12 to 3.91 ± 0.19 mg L^{-1} on day 33 of decomposition for the treatments of *R. apiculata* (Figure 3).

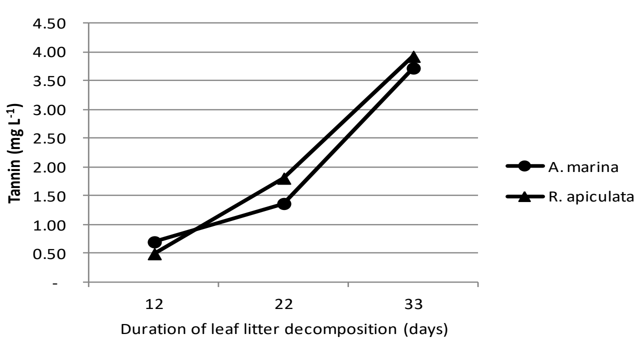

Figure 3. The mean tannin concentrations of *A. marina* and *R. apiculata* treatments increased gradually during the study period.

Although overall mean tanin concentration was recorded higher in the treatments with leaves of *R. apiculata* (2.07 ± 1.45 mg L^{-1}) compared to the treatments with *A. marina* leaves (1.92 ± 1.37 mg L^{-1}) (Table 2B), no clear trend can be detected from the treatments between the species (H = 0.257, df = 1, P = 0.612).

Shrimp growth and survival rate

Shrimp biomass relatively increased with increasing concentrations of decomposing mangrove leaves in *R. apiculate* than in *A. marina* (Table 3A). In *A. marina* tanks, the highest growth rate (SGR) was found in the 0.125 g L^{-1} concentration, while in *R. apiculata* tanks, the highest was in the 0.5 g L^{-1} concentration.

A significantly higher mean shrimp biomass was also observed in minced leaf litter (5.16 ± 0.2 % day^{-1}) and leaf litter leachate (5.24 ± 0.3 % day^{-1}) compared to leaf litter (4.03 ± 1.1% day^{-1}) in the 0.125 g L^{-1} treatments (H = 34.534, df = 2, P < 0.001) (Table 3B). In *A. marina* tanks, shrimp body weight was significantly higher in the 0.125 g L^{-1} leachate concentration than in the 0.125 g L^{-1} leaf litter concentration (Dunn's *post hoc*, Q = 4.769), while in *R. apiculata* tanks, shrimp body weight in the 0.125 g L^{-1} minced leaf litter was significantly higher than in the 0.125 g L^{-1} leaf litter concentration (Dunn's *post hoc*, Q = 4.491).

Table 3. Mean and standard deviation of the final body weight and survival rate for the treatments with *A. marina* and *R. apiculata*, and the results of the post-hoc tests (values in the same column having a different letter are significanlty different at P < 0.05)

	Shrimp growth rate (SGR) (% day^{-1})			Survival rate (SR) (%)		
Treatments	***A. marina***	***R. apiculata***	**Mean**	***A. marina***	***R. apiculata***	**Mean**
A (leaf concentration)						
0.125	4.92 ± 0.5^{bc}	4.78 ± 0.2^{bc}	4.03 ± 1.1^{B}	62 ± 16^{a}	72 ± 15^{a}	68 ± 15^{A}
0.25	4.77 ± 0.5^{bc}	5.15 ± 0.5^{b}	4.96 ±0.3^{BC}	66 ± 13^{a}	58 ± 16^{a}	62 ± 14^{A}
0.5	4.58 ± 0.1^{c}	5.51 ± 0.1^{a}	4.28 ±1.7^{AC}	86 ± 13^{a}	52 ± 2^{a}	66 ± 20^{A}
B (leaf treatment)						
0.125 Whole	4.92 ± 0.5^{b}	4.78 ± 0.2^{b}	4.03 ± 1.1^{B}	62 ± 16^{a}	72 ± 15^{a}	68 ± 15^{A}
0.125 Minced	5.02 ± 0.1^{ab}	5.29 ± 0.1^{a}	5.16 ± 0.2^{B}	75 ± 8^{a}	65 ± 4^{a}	70 ± 8^{A}
0.125 Leachate	5.41 ± 0.3^{a}	5.07 ± 0.2^{ab}	5.24 ± 0.3^{B}	58 ± 6^{a}	67 ± 4^{a}	62 ± 7^{A}
Mean	4.31 ± 1.1^{A}	5.16 ± 0.3^{B}		69 ± 13^{A}	63 ± 11^{B}	

The highest overall survival rate was recorded at 0.125 g L^{-1} minced leaf litter with a rate of 70.2 ± 7.6 % and the lowest overall was recorded at 0.25 g L^{-1} with a rate of 61.7 ± 13.9 %. However, among the concentrations, an increase in litter concentration did not lead to a significantly lower survival rate (ANOVA, F = 0.198, df = 3, P = 0.896) (Table 3A). There was also no significant difference in survival rate between litter concentrations and the control (P ≥ 0.05). SR of shrimp in the leachate treatments was also relatively lower than those in the minced treatments, but the differences were not significant (H = 2.148, df = 2, P = 0.342) (Table 3B)

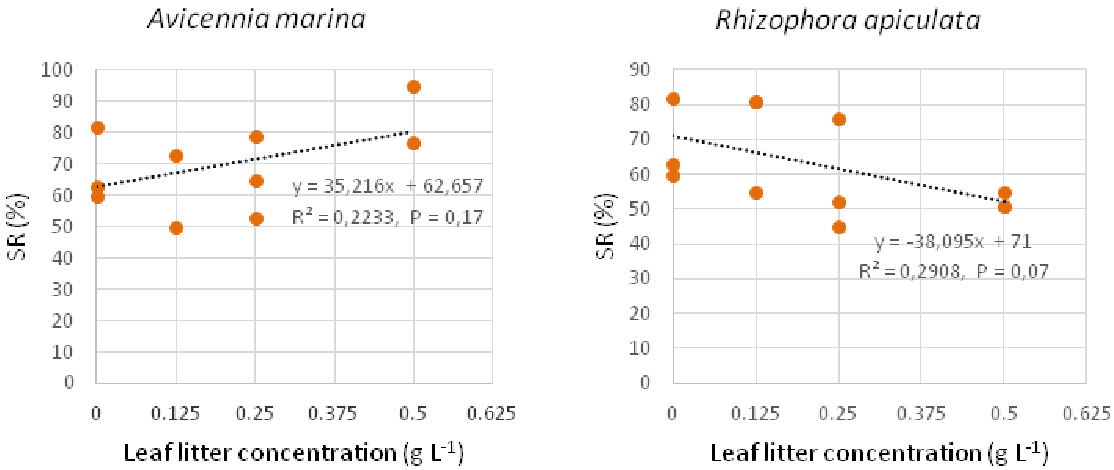

Figure 4. Multiple linear regression analysis of PL survival rate and concentrations of decomposing *A. marina and R. apiculata* leaf litter. The Pearson correlation coefficient between PL survival rate and *A. marina* leaf litter was *r* = 0.473, *P* = 0.17 and between PL survival rate and *R. apiculata* leaf litter *r* = - 0.539, *P* = 0.07.

In general, survival was significantly higher in *A. marina* treatments (mean 69 ± 13 %) than in *R. apiculata* treatments (mean 63 ± 11 %) (H = 10.464, df = 1, P = 0.001). An increase in decomposing leaf litter led to an increase in survival rate in the *A. marina* treatments (*r* = 0.473, P = 0.17), but to a decrease in survival rate in the *R. apiculata* treatments (*r* = -0.539, P = 0.07), although not significant (Figure 4).

Correlations between water quality, growth and survival

Pearson's correlation coefficient showed various water quality parameters having negative and positive effects on shrimp growth and survival between *A. marina* and *R. apiculata* treatments (Table 4)

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Table 4. Pearson's correlation coefficients (Pcc) between the water quality parameters: DO, pH, tannin and NH_3-N on *Penaeus monodon* PL growth and survival for *A. marina* and *R. apiculata* treatments.

			DO	pH	Tannin	NH_3-N
Shrimp growth	*A. marina*	Pcc	0.48	0.64	0.25	-0.71
	(n=16)	P Value	0.06	0.01	0.35	0.002
	R. apiculata	Pcc	-0.2	-0.52	-0.39	0.05
	(n=18)	P Value	0.43	0.03	0.11	0.85
Shrimp survival	*A. marina*	Pcc	-0.53	-0.49	0.07	0.53
	(n=16)	P Value	0.04	0.06	0.79	0.036
	R. apiculata	Pcc	0.07	0.36	0.55	-0.16
	(n=18)	P Value	0.79	0.14	0.02	0.54

The pH had a positive correlation with growth of shrimp in *A. marina* leaf litter (*r* = 0.64, *P* = 0.008), while this was negative in *R. apiculata* leaf litter (*r* = -0.52, *P* = 0.027). The DO levels and tannin concentration in both *A. marina* and *R. apiculata* treatments were not correlated with shrimp growth (*P* ≥ 0.05). However, the higher shrimp growth was significantly correlated to a higher shrimp mortality in both *A. marina* (*r* = -0.68, *P* = 0.004) and *R. apiculata* (*r* = -0.69, *P* = 0.002) treatments

Shrimp survival was positively correlated with tannin concentration in *R. apiculata* treatments (r = 0.55, P = 0.019). Similar results were observed for NH_3-N in *A. marina* treatments (r = 0.526, P = 0.036). Shrimp survival and NH_3-N in *A. marina* treatments were positively correlated (r = 0.53, P = 0.036), while the correlation with NH_3-N was strongly negative for growth (r = -0.71, P = 0.002). However, no significant correlation was observed between shrimp growth and NH_3-N in *R. apiculata* treatments. The pH and DO concentrations were not correlated with shrimp survival in both *A. marina* and *R. apiculata* treatments.

Discussion

Tannin concentrations

In this study, there was a relatively higher leaching of tannins in *R. apiculata* than in *A. marina* treatments within 33 days (Fig. 2). Previous studies showed that within 40 days there was a 50% reduction of the initial weight of *A. marina* and *R. apiculata* leaves, with the latter having a slower leaching of dissolved organic matter initially (e.g. tannins) (Boonruang, 1984; Robertson, 1988; Rajendran & Kathiresan, 2000). The loss through leaching may depend on various parameters such as species and environment, for example leaching is higher in the wet season compared to dry season (Robertson, 1988; Tam et al., 1990; Wafar et al., 1997). *A. marina* leaves have thinner leaf cuticle, higher initial nitrogen concentration, lower C:N ratio and contain less tannins, thus decompose relatively faster than *R. apiculata* leaves (Robertson, 1988; Camilleri, 1989; Steinke et al., 1990).

During decomposition, the nitrogen concentration in both *A. marina* and *R. apiculata* leaves initially decreases due to leaching, after which it gradually increases due to nitrogen immobilization, leading to a decrease in C:N ratio (Robertson, 1988; Benner et al., 1990; Tam et al., 1990; Dick & Osunkoya, 2000; Rajendran & Kathiresan, 2000, 2007). Microbial activity is primarily responsible for the immobilization of nitrogen (Tremblay & Benner, 2006). This may be an important mechanism of nitrogen accumulation in leaf litter.

Effect of tannin on other water quality parameters

Increasing leaf litter concentrations did not have a significant effect on DO, tannin and H_2S concentrations and means were randomly divided over the treatments (Table 1 and 3). However, the pH significantly decreased with increasing leaf litter concentrations, and the NH_3-N concentration increased to levels critical and lethal for the shrimp PL. The relatively higher NH_3-N concentrations in the *A. marina* treatments may be due to its high protein content and rapid nutrient leaching rates. The decrease in pH with increasing leaf litter concentrations could be due to the tannin content in the leaves as was observed in previous studies (Chyau et al., 2006; Nugroho et al., 2016). In contrast, Hai & Yakupitiyage (2005) observed tannin being significantly correlated with DO (r = -0.482), pH (r = 0.595) and H_2S (r = 0.738), but not with the total ammonia nitrogen (TAN). Hai & Yakupitiyage (2005) suggested that a combination of these factors could increase the toxicity of tannins to shrimp growth and survival.

Growth and survival

In this study, the highest growth rates were found in the 0.125 g L^{-1} leachate concentration with a presumed faster leaching of tannins, i.e. minced leaf litter and leaf litter leachate, compared to regular leaf litter. Minced leaf litter have been tested regarding to mimic the phase of decomposing process in nature. This is in line with findings from the study by Nga et al. (2006), who also observed significant higher growth rates when PL were grown in water containing *R. apiculata* leaf litter leachates than when grown in water containing different concentrations of leaf litter. In addition, *P. monodon* PL in *Terminalia catappa* (a mangrove associated species) leaf litter leachates had a higher growth rate than those PL in controls ($P < 0.05$) (Ikhwanuddin et al., 2014). The higher body weight of shrimp in leaf litter leachate than those in leaf litter is probably due to the leachates their higher nutritional value (i.e. leached proteins from dissolved organic carbon) (Davis et al., 2003).

Furthermore, increasing leaf litter concentrations showed an increase in NH_3-N concentration due to organic matter degradation. The accumulation of NH_3-N may have caused the slow growth in *A. marina* treatments as a strong negative correlation was observed (r = -0.709, P = 0.002). This was in line with observations from Wickins (1976) and Chin & Chen (1987), who found high NH_3-N concentrations reducing the growth of *P. monodon* and other penaeid shrimp PL, where the sensitive NH_3-N concentration was higher than 0.1 mg L^{-1}.

In the present study, the mean survival rate was significantly higher in *A. marina* treatments at 68.5 ± 13.4 % than in *R. apiculata* treatments at 62.9 ± 11.3 %. The correlation between shrimp survival might be due to the tannin concentration, which increasing tannin might be related to a higher shrimp survival. Regarding to the effect of *A. marina* in increasing the survival rate of shrimp, it is recommended that in the shrimp farm area can be covered with *A. marina* rather than *R. apiculata.* Ikhwanuddin et al. (2014) also observed a significant higher survival rate of *P. monodon* PL in treatments with a concentration of 3 g L^{-1} *T. catappa* leaf litter leachate than in controls and 1 g L^{-1}, 2 g L^{-1} and 4 g L^{-1} leaf litter leachate ($P < 0.05$). Furthermore, Harlina et al. (2015) observed *Chromolaena odorata (a mangrove associated species)* leaf extract with its active secondary metabolites such as flavonoids, tannins and alkaloids, to have no toxic effects on *P. monodon* PL up to a concentration of 1.25 g L^{-1}, but causing severe mortality above 2.5 g L^{-1}.

Shrimp mortality in treatments with high concentrations of decomposing mangrove leaf litter is probably due to the increasing NH_3 and decreasing water pH (toxic environment). However, low pH was not observed in the present study. This significant negative impact of survival rate on growth for both *A. marina* and *R. apiculata* might be due to the constant level of feeding. Feeding was not adjusted to the increased biomass. Consequently, the bigger PL might feed not only the dead PL but also the smaller PL when the latter are molting and unable to defend (Abdussamad & Thampy, 1994; Ray & Chien, 1992). Beside the cannibalism, the shrimp mortality is probably due to the high NH_3-N concentrations (toxic environment) caused by decomposing organic matter (i.e. leaf litter and dead shrimp). The NH_3-N levels in treatments with decomposing mangrove leaves of *A. marina* and *R. apiculata* were toxic for prawn in tanks without water exchange.

In conclusion, tannin in decomposing mangrove leaf litter up to a concentration of 0.5 mg g^{-1} did not have a significant effect on water quality and on the growth and survival of *P. monodon* PL. However increasing leaf litter concentrations showed an increase in NH_3-N concentration due to organic matter degradation. The accumulation of NH_3-N may have caused the slow growth of shrimp PL in *A. marina* treatments. Shrimp PL in leaf litter minced and leachates treatments have a higher growth rate than those PL in regular leaf litter. This result suggests that leaf litter leachates have a higher nutritional value as leached proteins for the shrimp.

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[biodiv] Editor Decision

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sri rejeki sulaiman, Roel H Bosma, Restiana Wisnu Ariyati, Lestari Lakhsmi Wiidowati, 25853737:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The Effect of Decomposing Mangrove Leaf Litter and Its Tannins on The Water Quality, Growth and Survival of Tiger Prawn (Penaeus monodon) Post-Larvae".

Our decision is: Revisions Required

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Reviewer A:

Dear Editor,

Please find attached is the reviews for the manuscript "01-AUG-SGB-The effects of decomposing mangrove leaf litter and its tannins".

Overall, the research is well executed and nicely written. The results might also have management implication in shrimp farming management althtough this is not yet deeply elaborated in the manuscript. As such I suggest to add this in the Discussion as well as few changes as per suggestion in the reviews.

Thank you and best regards,

Reviewer

Recommendation: Revisions Required

Biodiversitas Journal of Biological Diversity

A-01-AUG-SGB-The effects of decomposing mangrove leaf litter and its tannins_InternalRev.docx
955.5kB

The effects of decomposing mangrove leaf litter and its tannins on water quality and the growth and survival of tiger prawn (*Penaeus monodon*) post-larvae

Abstract. Shrimp farming in Demak, Indonesia is often practiced in silvo-aquaculture systems in which mangrove trees are planted on pond bunds. As such, mangrove leaves and its substrates may have impact on penaeid shrimp production. In this area, mangrove re-growth is usually proceeded with *Avicennia marina* while planting is mostly done with *Rhizophora apiculata*. We compared the effects of decomposing fresh leaves of *A. marina* and *R. apiculata* on water quality and on the performance of *Penaeus monodon* post larvae (PL). A hundred of PL21 (post larvae aged 21 days with weight of 0.28 g) were stocked in each of 30 aerated tanks containing 800 liters of brackish water (salinity of 21 ppt) for 37 days. Five treatments with three replicates for each mangrove species were assigned by adding into the tanks of 0.125, 0.25, and 0.5 g L^{-1} of air-dried leave, 0.125 of g L^{-1} minced leave and 0.125 g L^{-1} of leachate of minced leaves. The PLs were fed 3 times daily with pellets at 10 % of initial total body weight. Water quality parameters were recorded daily. Tannin, H_2S and NH_3-N concentrations were measured every ten days. Prawn's body weight (BW) was measured and specific growth rate (SGR, % day^{-1}) and survival rate (SR, %) were calculated after the end of experiment. Results were analyzed with ANOVA and Pearson's correlation. The results showed that tannin in decomposing mangrove leaf litter up to a concentration of 0.5 mg g^{-1} did not have a significant effect on water quality and on the growth and survival of *P. monodon* PL. However increasing leaf litter concentrations showed an increase in NH_3-N concentration due to organic matter degradation. The accumulation of NH_3-N may have caused the slow growth of shrimp PL in *A. marina* treatment. Shrimp PL in leaf litter leachates treatment have a higher growth rate than those PL in regular leaf litter in relation to nutritional value. Survival and growth varied from 62 ±14 to 70 ± 8% and 3.1±2.1 to 5.5±1.2% day^{-1}, respectively. Although decomposing mangrove leaves of *A. marina* and *R. apiculata* had no toxic effects on *P. monodon* PL up to a concentration of 1.25 g L^{-1}, but causing severe mortality above 2.5 g L^{-1} for shrimp in tanks without water exchange. PLEASE ADD ONE OR TWO SENTENCES TO CLOSE THE ABSTRACT EXPRESSING THE IMPLICATION OF THE RESULTS ON SHRIMP FARMING MANAGEMENT.

Keywords: *Penaeus monodon, Avicennia marina, Rhizophora apiculata*, ammonia-N, tannin

INTRODUCTION

In 1980s the international demand for prawn increased, and as a result both extensive and intensive prawn culture expanded dramatically (Primavera *et al.* ,1993; Rönnbäck, 2002). In Indonesia, the decreasing world market price for rice caused by green revolution pushed the conversion of both paddy fields and mangrove forests into shrimp ponds. While extensive prawn culture caused mainly coastal land use change (i.e. mangrove loss), several problems occured due to the intensification: intrusion of saline water upland, increase of nutrients in waterbodies due to feed waste and prawn excretions, and loss of capital due to disease outbreaks (Primavera, 1997; Rivera-Ferre, 2009). In response to unsustainable systems, integrated mangrove-shrimp aquaculture systems (i.e. silvo-aquaculture) have been developed as environmentally and socioeconomically sustainable strategies for poor small-scale farmers (Primavera, 2000; Fitzgerald, 2002; Rönnbäck, 2002). In Indonesia, this technology was started in 1976 by the State Forestry Corporation, with the aim of rehabilitating and conserving mangrove forest, and resolving forestry-fisheries conflicts (Primavera, 2000).

Although silvo-aquaculture systems are more ecologically friendly with mangrove ecosystems than other types of aquaculture (Primavera, 2000), they also have problems of sustainability. Decaying mangrove leaves are known to accumulate at pond ground, causing an increase in tannin levels, which together with the shade of the mangrove trees creates an acidic and anoxic environment, which ultimately results in lower shrimp production (Johnston et al., 2000; Clough et al., 2002; Nga et al., 2006). As an example, the prawn production from a silvo-aquaculture pond with *Avicennia marina* and *Rhizophora apiculata* mangroves in Purworejo village, Demak district, Central Java, was at the low end with yield of 75 - 105 kg ha^{-1} per year (Tonneijck et al, 2015).

Fitzgerald (2000) stated that high tannin concentrations may be potentially toxic to penaeid shrimp in mangrove-shrimp aquaculture systems. Mangroves contain high levels of tannins (Robertson, 1988), which can rise as much as 20% of the dry weight of plant material (Hernes et al., 2001). Tannins, generally divided into hydrolyzed and condensed tannins, are anti-nutritional elements with zero nutritional value, affecting protein utilization and nutritional digestibility of various herbivorous and detritivorous crustaceans and fish species (Neilson et al., 1986; Conde et al., 1995 cited by Erickson et al., 2004; Becker & Makkar, 1999; Maitra & Ray, 2003; Hammann & Zimmer, 2015). The negative impact of *R. apiculata* mangrove on shrimp performance (Primavera, 2000) was confirmed in an experiment that showed that leaf concentrations higher than 0.5 g L^{-1} were very lethal where leaf effects differed between mangrove species (Hai and Yakupitaga, 2005). Using *R. apiculata* as a reference species, this study aimed to assess whether tannin was released by the decomposing leaves of *A. marina* and *R. apiculata* and which form of their leaves litter that contribute more on water quality degradation cvg, whether this affected the growth and survival rate of *P. monodon*.

MATERIALS AND METHODS

Study period and location

The study was conducted for 40 days in Demak District, Central Java, Indonesia. The coastal areas of Demak once had extensive mangrove forests (about 6000 ha), but these areas had been converted into aquaculture where some area are applied silvo-aquaculture. The mangrove species *A. marina* and *R. apiculata* co-dominate the dikes of the pond and were therefore selected as the species used in this study. The experimental station is located in Tambakbulusan village in the Sub-district of Karang Tengah, about six kilometers from the capital of Demak.

Experimental procedure

Thirty-three tanks of 1 m^3 (1x1x1 m) were used to test the effects of decomposing mangrove leaves on water quality and on the growth and survival of *P. monodon* PL. The experimental plastic tanks of 1 m^3 were filled with ± 5 cm pond bottom substrate, clay-loam soil (pH 6.5) and 800 liters of brackish water (salinity 21 ppt). The prawn PL were stocked 4 days later to let the suspended particles sediment. The water in the tanks was not exchanged but the volume was maintained by adding water from the same source regularly. Each tank was continuously aerated using Resun® LP-60 low noise air pump to maintain dissolved oxygen level above 5 mg L^{-1}, thus largely above the recommended level and the 3 mg L^{-1} generally found in silvo-aquaculture (Boyd, 1989; Binh et al., 1997; Johnston et al., 2002). The experimental tanks were covered with dark netting to reduce water temperature fluctuation and light intensity.

Tiger prawn (*P. monodon*) larvae of 21 days old, known as PL-21, at average initial body weight of 0.28 g were bought from the Centre of Brackish Water Research Institution (Balai Besar Penelitian Budidaya Air Payau = BBPBAP) in Jepara. PL-21 were randomly stocked in each tank at a density of 100 PLs m^{-2} that was acclimatized previously. During the acclimatization, dead PL were replaced by new identical individuals.

Commercial shrimp pellet produced by Central Proteinaprima Tbk. was added three times a day at 07:00, 12:00 and 18:00 to the tanks with PL at the total rate of 10% of the total stocked and adjusted after the weekly weighing. The feed was put at a 40 x 40 cm feeding tray submerged at the bottom of each tank. The pellet contained 41% protein, 5% fat, 2% fiber, 13% ash and 11% moisture.

Mature green leaves of *A. marina* and *R. apiculata* were collected from mangrove pond and transported to the experimental station. The leaves were air-dried in the shade to a constant weight and separated species-wise in litter bags made of nylon nets of 3 mm mesh size (Figure 1A-B), and then added to the tanks nine days after collection. Stones were tied to the litter bags to make them sink.

The minced leaves were obtained by cutting the dried leaves into small pieces and mincing the cust in an electrical blender (Figure 1C). The leave leachate was obtained by soaking 100 g of blended leaf litter in 2 L filtered brackish water for 30 minutes, then sieving through a woven wire sieve (100 µm mesh size). Only the solution was added to the tanks (Figure 1D).

Experiment conducted by Hai and Yakupitaga (2005) found that the concentration of leaves higher than 0.5 g L^{-1} was very lethal. As such, three concentrations of mangrove leaves were applied in our study: 0.125, 0.25 and 0.5 g L^{-1}. Two additional leaves treatments were applied to analyze the effects of presumed faster release of tannins to allow faster decomposition and tannin dilution: (a) 0.125 g L^{-1} minced 100 g leaf litter was cut into small pieces and minced using a blender after which added species-wise in the litter bags made from pantyhose stockings (Figure 1C); (b) 0.125 g L-1 leachate 100 g of blended leaf litter was soaked in 2 L filtered brackish water for brackish water for 30 minutes, after which sieved through a woven wire sieve (100 µm mesh size), and only the solution was added species-wise (Figure 1D).

All 5 treatments were done in three replications.

Figure 1. The decomposing mangrove leaves were separated according to different treatment: (A) *R. apiculata* leaves; (B) *R. apiculata* leaves; (C) Minced *R. apiculata* leaves; (D) *R. apiculata* litter leachate

Data collection

Water quality parameters

Seven water quality parameters were measured including temperature (T), pH, salinity, dissolved oxygen (DO), tannin, hydrogen sulfide (H_2S), and unionized ammonia as nitrogen (NH_3-N). Water temperature was observed daily using an electronic thermometer with precision of 0.1°C, pH using HANNA ® HI98129 pH meter with precision of 0.01, and salinity using ATAGO®PAL-06s refractometer 06S refractometer with precision of 1 ppt. The DO was recorded on day 6, 12, 22, 23, 30 and 33 of the study using a YSI@Pro DO meter (read-out in 0.1 mg L^{-1}).

Tannin, hydrogen sulfide (H_2S) and unionized ammonia as nitrogen (NH_3-N) were measured according to standard methods as described in Rice et al. (2012). Therefore, every ten days three water samples were taken from each replicate treatment: 500 ml for tannin analysis and 200 ml for H_2S and NH_3-N analysis. For the control, only the two/three samples were aggregated before analysis. Samples for H_2S analysis were preserved by adding 8 drops of 2*N* zinc acetate and 10 drops of sodium hydroxide (NaOH) solution to pH > 9, while NH_3-N samples were preserved by adding sulfuric acid (H_2SO_4) to pH < 2. The 93 samples were kept in styrofoam cool boxes and being transported to Balai Besar Teknologi Pencegahan Pencemaran Industri, Semarang, for analyses. The tannin content was analysed colorimetrically by the Folin phenol method, while H_2S and NH_3-N were analyzed by the Iodometric and Phenate method, respectively.

Figure 2: (A) Initial body weight was measured in group of ten individuals; (B) final body weight was measured per individual

Growth performance and survival rate

The initial body weight was determined for the whole population (i.e. 3300 prawn) by randomly sampling and weighing 35 groups of ten individuals in order to minimize fluctuations due to wind (Figure 2A). The final body weight was determined per tank by collecting and weighing all shrimp individually (Figure 2B). Weighing was done with an A&D® HL-100 electronic weighing scale with a precision of 0.01 g.

The specific growth rate (SGR) and survival rate (SR) were calculated with the formula of Busacker et al. (1990)

$$SGR = \frac{\ln BW_t - \ln BW_o}{t} \times 100\%$$

Where :

SGR is specific growth rate (% day-1);

*BW*t is the final body weight (g);

*BW*o is the itial body weight (g); and *t* is duration of experiment (days).

$$SR = \frac{N_t}{N_o} \times 100\ \%$$

Where

SR = is the survival rate (%);

Nt = is the number of prawn collected at sampling time t; and

No = is the number of prawns initially stocked

Data analyses

Statistical analyses were done using SigmaPlot® 12. Differences were considered significant at $P < 0.05$. The water quality parameters and prawn performance were compared by one-way analyses of variance (ANOVA). Prior to the ANOVA, all data were checked for their normality using the Saphiro-Wil test. When data were not normally distributed, a non-parametric Kruskal-Wallis test by ranks was used. If the ANOVA showed the treatment significantly affected the variables, a multiple comparison Duncan and Tukey post-hoc test was done. Correlations among the water quality parameters, between the water quality parameters and growth, and between the water quality parameters and survival rate, were analyzed using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Water quality parameters

The levels of salinity, temperature, and DO did not differ significantly between the treatments throughout the experiment (Table 1). Since the experimental tanks are closed system, the salinity levels fluctuated between 19 and 25 ppt (mean 22±1 ppt) and the water temperature between 27.2 and 33.3°C (mean 30.6±1.4 °C) but the DO slightly varried from 6.3 to 6.9 mg L^{-1} (mean 6.7±0.5 mg L^{-1}). Although the DO concentration of the culture media was high and in the upper side of the recommended range (±4 mg L^{-1}) all recorded salinity, temperature, and DO concentrations were considered as optimal for *P. monodon* PL.

Table 1. The mean ± standard deviation of salinity (ppt), Dissolved Oxygen (DO), temperature (T) and pH for each treatment

Leaf Litter (gL^{-1}) Concentration	*A. marina*			*R. apiculata*		
	Salinity (ppt)	**DO (mg L^{-1})**	***T* (°C)**	**Salinity (ppt)**	**DO (mg L^{-1})**	***T* (°C)**
A (leaf concentration)						
0.125	21 ± 1	6.6 ± 0.3	30.1 ± 1.4	23 ± 1	6.9 ± 0.5	30.6 ± 1.4
0.25	22 ± 1	6.7 ± 0.6	30.2 ± 1 .4	22 ± 1	6.8 ± 0.6	30.6 ± 1. 3
0.5	23 ± 1	6.3 ± 0.3	30.4 ± 1.4	21 ± 1	6.5 ± 0.6	30.4 ± 1.4
B (leaf treatment)						
0.125 whole	21 ± 1	6.6 ± 0.3	30.1 ± 1.4	23 ± 1	6.9 ± 0.5	30.6 ± 1.4
0.125 minced	23 ± 1	6.8 ± 0.3	30.6 ± 1.3	22 ± 1	6.8 ± 0.5	30.6 ± 1.4
0.125 leachate	22 ± 1	6.9 ± 0.4	30.9 ± 1.5	22 ± 1	6.6 ± 0.4	30.6 ± 1.3

The majority of the treatments had undetectable H_2S concentrations (<0.002 mg L^{-1}). H_2S was only detected in 11 of the 30 tanks during the research period; most measured concentrations were 0.002 and 0.003 mg L^{-1}. For both *A. marina* and *R. apiculata* the highest levels were found for the treatment with 0.125 leachate: 0.005 and 0.004 mg L^{-1}, respectively. Therefore no reliable conclusion can be made about the influence of mangrove leaf litter concentrations on H_2S production.

The NH_3-N concentration found in all treatments exceeded the optimal level for penaeid PL and were considered critical and lethal. Leaf litter concentrations increased the NH3-N concentration in both *A. marina* and *R. apiculata* treatments, but this did not differ significantly (H = 6.124, df = 3, P = 0.106) (Table 2A).

The average NH_3-N concentrations in the leaf treatments for *A. marina* compared to *R. apiculata*, 0.83 ± 0.2 and 0.79 ± 0.2 mgL^{-1}, respectively, were not significantly different (Table 2B). However, species-wise, NH_3-N concentration was not also significantly affected by the leaf treatments (H = 0.230, df = 1, P = 0.631).

Table 2. Mean and standard deviation (SD) of the concentrations of NH_3-N (m.L^{-1}) and tannin for the treatments with *A. marina* and *R. apiculata*, the P-value of the ANOVA and results of the post-hoc tests (values in the same column having a different letter are significanlty different)

Treatments	**NH3-N**			**Tannin**		
	A. marina	***R. apiculata***	**Mean**	***A. marina***	***R. apiculata***	**Mean**
A (leaf concentration)						
0.125	0.82 ± 0.3^a	0.74 ± 0.2^a	0.8 ± 0.2^A	1.77 ± 1.5^a	2.21 ± 1.5^a	2.0 ± 1.5^A
0.25	0.95 ± 0.3^a	0.82 ± 0.2^a	0.9 ± 0.2^A	1.86 ± 1.5^a	1.92 ± 1.5^a	1.9 ± 1.4^A
0.5	0.99 ± 0.2^a	0.87 ± 0.2^a	0.9 ± 0.2^A	2.16 ± 1.4^a	1.97 ± 1.5^a	2.1 ± 1.4^A
B (leaf treatment)						
0.125 Whole	0.82 ± 0.3^a	0.74 ± 0.2^a	0.8 ± 0.2^A	1.77 ± 1.5^a	2.21 ± 1.5^a	2.0 ± 1.5^A
0.125 Minced	0.75 ± 0.1^a	0.76 ± 0.2^a	0.8 ± 0.2^A	1.83 ± 1.4^a	2.21 ± 1.6^a	2.0 ± 1.5^A
0.125 Leachate	0.69 ± 0.1^a	0.76 ± 0.2^a	0.7 ± 0.2^A	2.03 ± 1.46^a	2.02 ± 1.6^a	2.0 ± 1.5^A
Mean	0.83 ± 0.2^A	0.79 ± 0.2^A		1.92 ± 1.4^A	2.07 ± 1.5^A	

The concentration of mangrove leaf litter did not significantly affect tannins in water (P = 0.967) (Table 2A). However, the average tannin concentration in the tanks increased during leaf decomposition, from 0.68 ± 0.40 mg L^{-1} on day 12 to 3.72 ± 0.23 mg L^{-1} on day 33 of decomposition for the treatments of *A. marina* and similarly from 0.49 ± 0.15 mg L^{-1} on day 12 to 3.91 ± 0.19 mg L^{-1} on day 33 of decomposition for the treatments of *R. apiculata* (Figure 3).

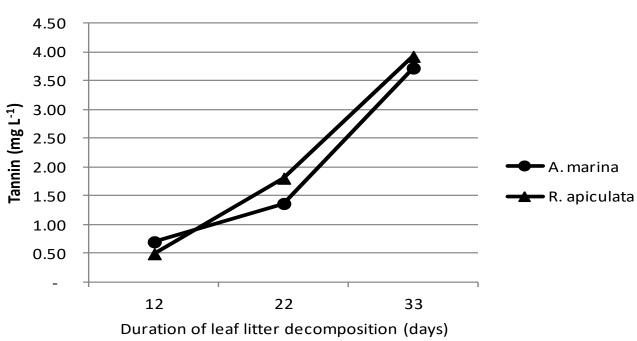

Figure 3. The mean tannin concentrations of *A. marina* and *R. apiculata* treatments increased gradually during the study period.

Although overall mean tanin concentration was recorded higher in the treatments with leaves of *R. apiculata* (2.07 ± 1.45 mg L^{-1}) compared to the treatments with *A. marina* leaves (1.92 ± 1.37 mg L^{-1}) (Table 2B), no clear trend can be detected from the treatments between the species (H = 0.257, df = 1, P = 0.612).

Shrimp growth and survival rate

Shrimp biomass relatively increased with increasing concentrations of decomposing mangrove leaves in *R. apiculate* than in *A. marina* (Table 3A). In *A. marina* tanks, the highest growth rate (SGR) was found in the 0.125 g L^{-1} concentration, while in *R. apiculata* tanks, the highest was in the 0.5 g L^{-1} concentration.

A significantly higher mean shrimp biomass was also observed in minced leaf litter (5.16 ± 0.2 % day^{-1}) and leaf litter leachate (5.24 ± 0.3 % day^{-1}) compared to leaf litter (4.03 ± 1.1% day^{-1}) in the 0.125 g L^{-1} treatments (H = 34.534, df = 2, P < 0.001) (Table 3B). In *A. marina* tanks, shrimp body weight was significantly higher in the 0.125 g L^{-1} leachate concentration than in the 0.125 g L^{-1} leaf litter concentration (Dunn's *post hoc*, Q = 4.769), while in *R. apiculata* tanks, shrimp body weight in the 0.125 g L^{-1} minced leaf litter was significantly higher than in the 0.125 g L^{-1} leaf litter concentration (Dunn's *post hoc*, Q = 4.491).

Table 3. Mean and standard deviation of the final body weight and survival rate for the treatments with *A. marina* and *R. apiculata*, and the results of the post-hoc tests (values in the same column having a different letter are significanlty different at P < 0.05)

	Shrimp growth rate (SGR) (% day^{-1})			**Survival rate (SR) (%)**		
Treatments	***A. marina***	***R. apiculata***	**Mean**	***A. marina***	***R. apiculata***	**Mean**
A (leaf concentration)						
0.125	4.92 ± 0.5^{bc}	4.78 ± 0.2^{bc}	4.03 ± 1.1^{B}	62 ± 16^{a}	72 ± 15^{a}	68 ± 15^{A}
0.25	4.77 ± 0.5^{bc}	5.15 ± 0.5^{b}	4.96 ± 0.3^{BC}	66 ± 13^{a}	58 ± 16^{a}	62 ± 14^{A}
0.5	4.58 ± 0.1^{c}	5.51 ± 0.1^{a}	4.28 ± 1.7^{AC}	86 ± 13^{a}	52 ± 2^{a}	66 ± 20^{A}
B (leaf treatment)						
0.125 Whole	4.92 ± 0.5^{b}	4.78 ± 0.2^{b}	4.03 ± 1.1^{B}	62 ± 16^{a}	72 ± 15^{a}	68 ± 15^{A}
0.125 Minced	5.02 ± 0.1^{ab}	5.29 ± 0.1^{a}	5.16 ± 0.2^{B}	75 ± 8^{a}	65 ± 4^{a}	70 ± 8^{A}
0.125 Leachate	5.41 ± 0.3^{a}	5.07 ± 0.2^{ab}	5.24 ± 0.3^{B}	58 ± 6^{a}	67 ± 4^{a}	62 ± 7^{A}
Mean	4.31 ± 1.1^{A}	5.16 ± 0.3^{B}		69 ± 13^{A}	63 ± 11^{B}	

The highest overall survival rate was recorded at 0.125 g L^{-1} minced leaf litter with a rate of 70.2 ± 7.6 % and the lowest overall was recorded at 0.25 g L^{-1} with a rate of 61.7 ± 13.9 %. However, among the concentrations, an increase in litter concentration did not lead to a significantly lower survival rate (ANOVA, F = 0.198, df = 3, P = 0.896) (Table 3A). There was also no significant difference in survival rate between litter concentrations and the control (P ≥ 0.05). SR of shrimp in the leachate treatments was also relatively lower than those in the minced treatments, but the differences were not significant (H = 2.148, df = 2, P = 0.342) (Table 3B)

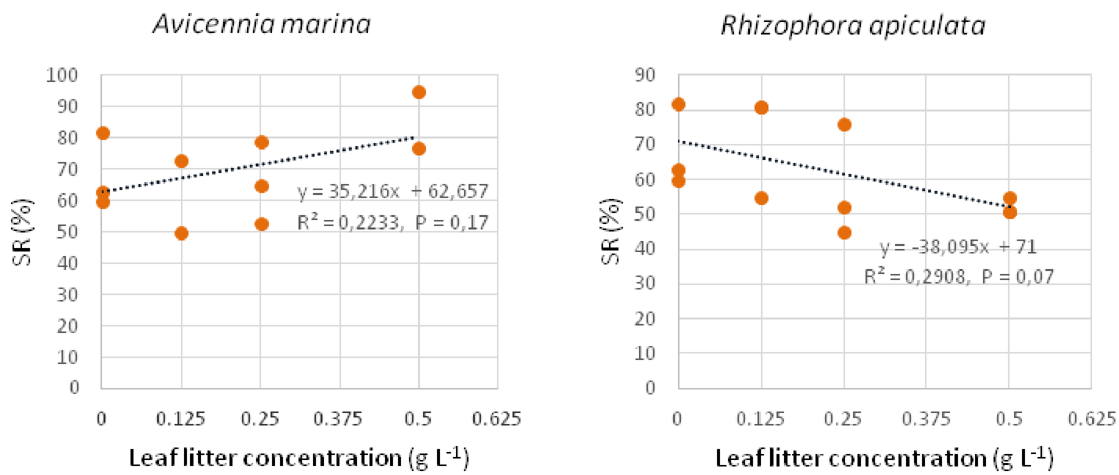

Figure 4. Multiple linear regression analysis of PL survival rate and concentrations of decomposing *A. marina and R. apiculata* leaf litter. The Pearson correlation coefficient between PL survival rate and *A. marina* leaf litter was r = 0.473, P = 0.17 and between PL survival rate and *R. apiculata* leaf litter r = - 0.539, P = 0.07.

In general, survival was significantly higher in *A. marina* treatments (mean 69 ± 13 %) than in *R. apiculata* treatments (mean 63 ± 11 %) (H = 10.464, df = 1, P = 0.001). An increase in decomposing leaf litter led to an increase in survival rate in the *A. marina* treatments (r = 0.473, P = 0.17), but to a decrease in survival rate in the *R. apiculata* treatments (r = -0.539, P = 0.07), although not significant (Figure 4).

Correlations between water quality, growth and survival

Pearson's correlation coefficient showed various water quality parameters having negative and positive effects on shrimp growth and survival between *A. marina* and *R. apiculata* treatments (Table 4)

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Table 4. Pearson's correlation coefficients (Pcc) between the water quality parameters: DO, pH, tannin and NH_3-N on *Penaeus monodon* PL growth and survival for *A. marina* and *R. apiculata* treatments.

			DO	pH	Tannin	NH_3-N
Shrimp growth	*A. marina*	Pcc	0.48	0.64	0.25	-0.71
	(n=16)	P Value	0.06	0.01	0.35	0.002
	R. apiculata	Pcc	-0.2	-0.52	-0.39	0.05
	(n=18)	P Value	0.43	0.03	0.11	0.85
Shrimp survival	*A. marina*	Pcc	-0.53	-0.49	0.07	0.53
	(n=16)	P Value	0.04	0.06	0.79	0.036
	R. apiculata	Pcc	0.07	0.36	0.55	-0.16
	(n=18)	P Value	0.79	0.14	0.02	0.54

The pH had a positive correlation with growth of shrimp in *A. marina* leaf litter (r = 0.64, P = 0.008), while this was negative in *R. apiculata* leaf litter (r = -0.52, P = 0.027). The DO levels and tannin concentration in both *A. marina* and *R. apiculata* treatments were not correlated with shrimp growth (P ≥ 0.05). However, the higher shrimp growth was significantly correlated to a higher shrimp mortality in both *A. marina* (r = -0.68, P = 0.004) and *R. apiculata* (r = -0.69, P = 0.002) treatments

Shrimp survival was positively correlated with tannin concentration in *R. apiculata* treatments (r = 0.55, P = 0.019). Similar results were observed for NH_3-N in *A. marina* treatments (r = 0.526, P = 0.036). Shrimp survival and NH_3-N in *A. marina* treatments were positively correlated (r = 0.53, P = 0.036), while the correlation with NH_3-N was strongly negative for growth (r = -0.71, P = 0.002). However, no significant correlation was observed between shrimp growth and NH_3-N in *R. apiculata* treatments. The pH and DO concentrations were not correlated with shrimp survival in both *A. marina* and *R. apiculata* treatments.

Discussion

Tannin concentrations

In this study, there was a relatively higher leaching of tannins in *R. apiculata* than in *A. marina* treatments within 33 days (Fig. 2). Previous studies showed that within 40 days there was a 50% reduction of the initial weight of *A. marina* and *R. apiculata* leaves, with the latter having a slower leaching of dissolved organic matter initially (e.g. tannins) (Boonruang, 1984; Robertson, 1988; Rajendran & Kathiresan, 2000). The loss through leaching may depend on various parameters such as species and environment, for example leaching is higher in the wet season compared to dry season (Robertson, 1988; Tam et al., 1990; Wafar et al., 1997). *A. marina* leaves have thinner leaf cuticle, higher initial nitrogen concentration, lower C:N ratio and contain less tannins, thus decompose relatively faster than *R. apiculata* leaves (Robertson, 1988; Camilleri, 1989; Steinke et al., 1990).

During decomposition, the nitrogen concentration in both *A. marina* and *R. apiculata* leaves initially decreases due to leaching, after which it gradually increases due to nitrogen immobilization, leading to a decrease in C:N ratio (Robertson, 1988; Benner et al., 1990; Tam et al., 1990; Dick & Osunkoya, 2000; Rajendran & Kathiresan, 2000, 2007). Microbial activity is primarily responsible for the immobilization of nitrogen (Tremblay & Benner, 2006). This may be an important mechanism of nitrogen accumulation in leaf litter.

Effect of tannin on other water quality parameters

Increasing leaf litter concentrations did not have a significant effect on DO, tannin and H_2S concentrations and means were randomly divided over the treatments (Table 1 and 3). However, the pH significantly decreased with increasing leaf litter concentrations, and the NH_3-N concentration increased to levels critical and lethal for the shrimp PL. The relatively higher NH_3-N concentrations in the *A. marina* treatments may be due to its high protein content and rapid nutrient leaching rates. The decrease in pH with increasing leaf litter concentrations could be due to the tannin content in the leaves as was observed in previous studies (Chyau et al., 2006; Nugroho et al., 2016). In contrast, Hai & Yakupitiyage (2005) observed tannin being significantly correlated with DO (r = -0.482), pH (r = 0.595) and H_2S (r = 0.738), but not with the total ammonia nitrogen (TAN). Hai & Yakupitiyage (2005) suggested that a combination of these factors could increase the toxicity of tannins to shrimp growth and survival.

Growth and survival

In this study, the highest growth rates were found in the 0.125 g L^{-1} leachate concentration with a presumed faster leaching of tannins, i.e. minced leaf litter and leaf litter leachate, compared to regular leaf litter. Minced leaf litter have been tested regarding to mimic the phase of decomposing process in nature. This is in line with findings from the study by Nga et al. (2006), who also observed significant higher growth rates when PL were grown in water containing *R. apiculata* leaf litter leachates than when grown in water containing different concentrations of leaf litter. In addition, *P. monodon* PL in *Terminalia catappa* (a mangrove associated species) leaf litter leachates had a higher growth rate than those PL in controls ($P < 0.05$) (Ikhwanuddin et al., 2014). The higher body weight of shrimp in leaf litter leachate than those in leaf litter is probably due to the leachates their higher nutritional value (i.e. leached proteins from dissolved organic carbon) (Davis et al., 2003).

Furthermore, increasing leaf litter concentrations showed an increase in NH_3-N concentration due to organic matter degradation. The accumulation of NH_3-N may have caused the slow growth in *A. marina* treatments as a strong negative correlation was observed ($r = -0.709$, $P = 0.002$). This was in line with observations from Wickins (1976) and Chin & Chen (1987), who found high NH_3-N concentrations reducing the growth of *P. monodon* and other penaeid shrimp PL, where the sensitive NH_3-N concentration was higher than 0.1 mg L^{-1}.

In the present study, the mean survival rate was significantly higher in *A. marina* treatments at 68.5 ± 13.4 % than in *R. apiculata* treatments at 62.9 ± 11.3 %. The correlation between shrimp survival might be due to the tannin concentration, which increasing tannin might be related to a higher shrimp survival. Regarding to the effect of *A. marina* in increasing the survival rate of shrimp, it is recommended that in the shrimp farm area can be covered with *A. marina* rather than *R. apiculata.* Ikhwanuddin et al. (2014) also observed a significant higher survival rate of *P. monodon* PL in treatments with a concentration of 3 g L^{-1} *T. catappa* leaf litter leachate than in controls and 1 g L^{-1}, 2 g L^{-1} and 4 g L^{-1} leaf litter leachate ($P < 0.05$). Furthermore, Harlina et al. (2015) observed *Chromolaena odorata (a mangrove associated species)* leaf extract with its active secondary metabolites such as flavonoids, tannins and alkaloids, to have no toxic effects on *P. monodon* PL up to a concentration of 1.25 g L^{-1}, but causing severe mortality above 2.5 g L^{-1}.

Shrimp mortality in treatments with high concentrations of decomposing mangrove leaf litter is probably due to the increasing NH_3 and decreasing water pH (toxic environment). However, low pH was not observed in the present study. This significant negative impact of survival rate on growth for both *A. marina* and *R. apiculata* might be due to the constant level of feeding. Feeding was not adjusted to the increased biomass. Consequently, the bigger PL might feed not only the dead PL but also the smaller PL when the latter are molting and unable to defend (Abdussamad & Thampy, 1994; Ray & Chien, 1992). Beside the cannibalism, the shrimp mortality is probably due to the high NH_3-N concentrations (toxic environment) caused by decomposing organic matter (i.e. leaf litter and dead shrimp). The NH_3-N levels in treatments with decomposing mangrove leaves of *A. marina* and *R. apiculata* were toxic for prawn in tanks without water exchange.

In conclusion, tannin in decomposing mangrove leaf litter up to a concentration of 0.5 mg g^{-1} did not have a significant effect on water quality and on the growth and survival of *P. monodon* PL. However increasing leaf litter concentrations showed an increase in NH_3-N concentration due to organic matter degradation. The accumulation of NH_3-N may have caused the slow growth of shrimp PL in *A. marina* treatments. Shrimp PL in leaf litter minced and leachates treatments have a higher growth rate than those PL in regular leaf litter. This result suggests that leaf litter leachates have a higher nutritional value as leached proteins for the shrimp.

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