Manuscript Details

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Title	SEDIMENTATION RATES OF NUTRIENTS AND PARTICULATE MATERIAL IN POND MARICULTURE OF SHRIMP (Litopenaeus vannamei) CARRIED OUT WITH DIFFERENT MANAGEMENT STRATEGIES
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Abstract

Marine shrimp farming is an important economic activity in tropical and subtropical regions, but its expansion has contributed to the increase of nutrients and organic matter in coastal ecosystems. Thus, this work evaluated the sedimentation rates of nutrients and particulate matter in marine shrimp (Litopenaeus vannamei) grow-out in earthen ponds. Three different stocking densities of the shrimp were evaluated over a period of approximately 79 days: M1: 92 shrimps.m-2; M2: 14 shrimps.m-2 and M3: 8 shrimps.m-2. Transparency, temperature, pH and dissolved oxygen remained within the ideal ranges for the pond production of L. vannamei, whereas the salinity was outside the recommended range. With the exception of total inorganic and organic carbon, the sedimentation rates of nutrients were significantly higher in M3 for the first sample period. This was perhaps due to the management of the first phase (greenhouse) requiring high inputs for a high initial population and the consequent accumulation of suspended solids and organic matter. The M1 showed decreases throughout the experimental period for the sedimentation rates of nutrients, which may have been subjected to bacterial decomposition. Decreasing sedimentation rates in the M2 were only observed for ammonia, nitrate and total-N. This trend may be associated with the primary production in the earthen pond system as suggested by the increasing of chlorophyll-α throughout the cultivation. In conclusion, the sedimentation rates of nutrients in marine shrimp aquaculture are influenced by a high stocking density and the quantity of feed offered per unit of production area.

Keywords	Greenhouse, Earthen ponds, Nutrients, Sedimentation rate, Shrimp
Taxonomy	Estuarine Ecosystem, Aquatic Trophic Dynamics, Crustacea, Aquatic Ecosystem Models
Manuscript category	Sustainability and Society

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Submission Files Included in this PDF

File Name [File Type]

Response letter - Aquaculture.docx [Response to Reviewers]

Highlights ABJ.docx [Highlights]

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COI ABJ.docx [Conflict of Interest]

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Data will be made available on request

All other comments and remarks can be found directly in the attached file, highlighted in yellow.

Comment – Reviewer 2	**Answer - Authors**
Introduction	
1. Page 2, first paragraph: move lines 39-40 to line 37 before mentioning the farmed shrimp species. Also should state that shrimp are also farmed in inland areas (L. vannamei) and L vannamei is the most farmed species.	The referred phrase was moved from the first paragraph to the second paragraph (lines 66-68 of the new draft). This phrase comes before mentioning farmed species. In lines 72-73 (new draft) it is mentioned that the L vannamei is the most farmed shrimp species and farmed in inland and coastal areas.
2. Lines 55-58, I think no more wild seed are used for L. vannamei (100% domesticated) and P. monodon. To avoid being too dramatic with "high environment impact" and any issues with consumers I would remove this as the shrimp industry has been improving on these matters and there is still room for that (reason of this work; prefer to keep from lines 63). Remove also disease sentence (line 59). I would suggest maintaining lines 60-62 and highlighting the waste problems, current practices to address the issue and then the willing to improve which has already been addressed from lines 63. So, better start new paragraph from line 60.	The phrase "The expansion of shrimp mariculture in Brazil near coastal areas has also led to environmental Problems..." begins the final paragraph of the new draft (lines 83-86). We have attempted to address the request of the reviewer in this paragraph.
3. Line 68, not "current research" as you are citing other guys.	We agree and have made the revision. This is line 96 in the new draft.
4. Line 73, Can you clarify other management strategies apart from different densities?	We have attempted to clarify in the final line of this paragraph by providing a brief description as to how the management strategies differed (probiotics, fertilizers, monophasic vs. biphasic stocking). This is seen in lines 102-103 of the new draft.
Methods	
5. Page 4, Line 79, any salinity value?	Salinity is now provided in line 110 of the new draft.
6. Lines 86-87, the second sentence should come after line 115.	We agree and have made this revision (lines 150-151 of the new draft)
7. Line 115, authors use the term "prawn". It is better to be consistent as from beginning the author use "shrimp".	This was a typing error. Nonetheless, this was revised (phrase in line 149 of the new draft).
Results	
8. Table 2: "Final individual biomass? Or weight?	We changed to "Mean individual mass", as has been used in Dantas et al. 2020 (published in Aquaculture) and in other recent publications, referring to the individual animal. This change is observed in Table 2.
9. If survival between M1 and M3 was similar, why there is difference in final biomass?	Different stocking densities, but also the term "final biomass" was changed to "final yield" for clarification. This was written in lines 299-301 of the new draft.

10. I believe it is better to use feed conversion rate (FCR) than apparent feed conversion (AFC) as we would like to know how the "really" amount of feed contributed to different sedimentation rates.	We agree and have made this revision in lines 223-224 of the new draft, and in the first paragraph of the discussion.
Discussion	
11. Page 8, lines 235-236, due to WSSV? If that is the case you should have detected and reported in your methodology. If the disease affected then your overall results are as well. How would you justify the impact of density and other management you tested?	Detection of WSSV now included in methodology of new draft (lines 205-209). WSSV is also discussed in the first paragraph of the discussion. In lines 386-413, we explained how certain intrinsic/biotic factors are able to stabilize pond conditions despite variations in inputs. Hence, overall results were not likely due to WSSV.
12. Why survival on M2 is significantly lower than other M1 and M3?	This was addressed in the first paragraph of the discussion.
13. Lines 243-244, following "mean salinity" I expected to see mean value in the brackets. L vannamei can withstand salinity to 60 g/L.	This was revised according to recommendation of the reviewer and is observed in line 309 of the new draft.
14. Lines 238-239, if higher AFC in M1 is related to low survival, how do you explain lower AFC in M2 which had significantly lower survival than M1? And lower AFC in M3 if it had similar survival to M1?	This is due to differences in stocking density and growth, and is noted in lines 299-304.
15. Line 322-326, If the difference was not observed due to high feed input then how do you relate this to different AFC you reported? Different AFC would mean that each treatment had different feed input and it would theoretical have differential impact on sedimentation rate	We have attempted to address the flow/accumulation of carbon/sedimentation in aquatic systems in lines 386-413. Basically, certain intrinsic factors regulate the transformation and flow of nutrients, independent of the nutrient input loads.

Highlights:

- This was the first study to analyze the sedimentation rates of nutrients and particulate material during the cultivation of the marine shrimp *Litopenaeus vannamei*.
- This study used three treatments that differed according to management strategies and stocking densities, of which were based on the use of fertilizers and whether the shrimps were stocked directly or indirectly.
- The water used in the present study was recycled in recirculating systems, being provided to the ponds only to compensate for losses to evaporation.

Sedimentation rates of nutrients and particulate material in pond mariculture of shrimp (*Litopenaeus vannamei*) carried out with different management strategies

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Running Title: Sedimentation rates in shrimp pond mariculture

Abstract

Marine shrimp farming is an important economic activity in tropical and subtropical regions, but its expansion has contributed to the increase of nutrients and organic matter in coastal ecosystems. Thus, this work evaluated the sedimentation rates of nutrients and particulate matter in marine shrimp (*Litopenaeus vannamei*) grow-out in earthen ponds. Three different stocking densities of the shrimp were evaluated over a period of approximately 79 days: M1: 92 shrimps.m^{-2}; M2: 14 shrimps.m^{-2} and M3: 8 shrimps.m^{-2}. Transparency, temperature, pH and dissolved oxygen remained within the ideal ranges for *L. vannamei* pond mariculture, whereas the salinity was outside the recommended range. With the exception of total inorganic and organic carbon, the sedimentation rates of nutrients were significantly higher in M3 for the first sample period. This was perhaps due to the management of the first phase (greenhouse) requiring high inputs for a high initial population and the consequent accumulation of suspended solids and organic matter. The M1 showed decreases throughout the experimental period for the sedimentation rates of nutrients, which may have been subjected to bacterial decomposition. Decreasing sedimentation rates in the M2 were only observed for ammonia, nitrate and total-N. This trend may be associated with the primary production in the earthen pond system as suggested by the increasing of chlorophyll-α throughout the cultivation. In conclusion, the sedimentation rates of nutrients in marine shrimp aquaculture are influenced by a high stocking density and the quantity of feed offered per unit of production area.

Keywords: Greenhouse, Earthen ponds, Nutrients, Sedimentation rate, Shrimp.

1. Introduction

Aquaculture and other agricultural activities provide food security and poverty alleviation but are generally associated with negative environmental impacts (Bartley et al., 2007; Béné et al., 2016). Commercial aquaculture is one of the fastest growing agriculture industries worldwide that has led to a robust and diverse human food supply, consisted of quality products with high added value. The expansion of aquatic farming must coincide with the optimization of production while minimizing water exchange, greenhouse gas emissions, and improve the treatment and recycling of effluents to improve the economic, social and environmental sustainability of aquaculture (Pereira and Rocha, 2015; Moura, et al., 2016; Araújo and Valenti, 2017; Soares and Henry-Silva, 2019). More specifically, the aquaculture industry needs new management strategies to overcome negative externalities (Bostock et al., 2010; Troell et al., 2014; Hatje et al., 2016; Ribeiro et al., 2016).

Among aquatic production systems, shrimp mariculture has become one of the most productive aquaculture activities worldwide, representing the second highest group of exported species in terms of value (FAO, 2018). Marine shrimp are cultivated in coastal and estuarine regions and are an important source of income for several Asian and Latin American countries, including Brazil (Wurmann et al., 2004; Liao and Chien, 2011; Castillo-Juárez et al., 2015). The two major marine shrimp species used for commercial production are the American species of *Litopenaeus vannamei* and the Asian species *Penaeus monodon*. The *L. vannamei* represents 53% of global crustacean aquaculture, and global output increased from ~2.7 million tonnes in 2010 to ~4.2 million tonnes in 2016 (FAO, 2018). The *L. vannamei* is a popular aquaculture species due to its capacity to be produced in coastal regions and inland in freshwater or saline-alkaline environments.

Post-larval shrimps are epibenthic and hence, remain above the bottom sediments in earthen grow-out ponds where most of the organic matter and nutrients accumulate. The

sedimented material is attributed to the use of fertilizers, high feed inputs, and the growth and turnover of the plankton community that eventually settles on the bottom sediments in earthen ponds as organic matter. The high accumulation of organic matter in bottom sediments leads to the depletion of dissolved oxygen due to increased microbial decomposition and aquatic respiration, which compromise the production. In general, a high portion of the nutrients from shrimp production is drained with the effluents to natural estuarine and mangrove environments (Pereira et al., 2013; Ottinger et al., 2016; Hatje et al., 2016; Ribeiro et al., 2016).

The expansion of shrimp mariculture near coastal areas has led to environmental problems such as the increased use of natural fisheries resources as a source for high quality protein and the consequent rise in organic material and nutrient loads into littoral aquatic environments (Boyd and Tucker, 2014). Commercial feed in shrimp mariculture accounts for the majority of nutrient inputs in ponds, and has higher potential impacts on soil quality when compared to other aquaculture activities since the feed settles directly on pond bottoms (Páez-Osuna and Ruiz-Fernández, 2005; Boyd and Tucker, 2014). In 2015, global shrimp mariculture was responsible for the highest consumption of fishmeal when compared to all other major aquaculture activities (Tacon et al., 2011; Hasan, 2017). Therefore, the development of modern shrimp farming must focus on the use of highly digestible feeds with adequate levels of protein and alternate protein sources as a strategy to improve environmental conditions, which may lead to less water exchange, decreased costs for pumping, and a reduction in the proliferation of pathogens (Azevedo-Santos et al., 2011, Castillo-Soriano et al., 2013; Silva et al., 2013; Brito et al., 2014; David et al., 2015; Henry-Silva et al., 2015). Only a few recent studies have focused on alternative feed sources and the conversion of commercial feed into shrimp biomass as a strategy to reduce organic matter and nutrient loads that contribute to the eutrophication of aquatic environments (Chen et al., 2012; Brito et al., 2016; Oestreich et al., 2016). Thus, the present study evaluated the sedimentation rates of nutrients and particulate material from the

aquaculture of marine shrimp (*L. vannamei*) in an estuarine region of the Brazilian semi-arid northeast, carried out with different stocking densities and management strategies (use of fertilizers and probiotics and with single-phase and biphasic stocking).

2. Materials and Methods

2.1. Area of study

The present study was carried out at a commercial marine shrimp farm located in the rural coastal region near the city of Mossoró, Rio Grande do Norte - Brazil (05°05′56″S, 37°17′12″W). The water used for cultivation was taken from hypersaline wells with salinities that varied from 41 to 61 $g.L^{-1}$. The enterprise has 80 ponds with areas varying from 2,600 to 26,000 m^2, used for the grow-out of *L. vannamei* in densities of 8 to 100 $shrimp.m^{-2}$. The region has a tropical and semi-arid climate of BSwh' according to the Köppen classification system, with annual averages of temperature of 27.4ºC, rainfall of 685.3 mm and relative humidity of 68.9%.

2.2. Experimental design

The experimental period was 79 days. The ponds were drained, sterilized and maintained sanitary and empty for thirty days before being stocked with the *L. vannamei* post-larvae. Ponds with areas of 26,000 m^2 were used for treatments with low stocking densities (8-14 $shrimp.m^{-2}$) and ponds with areas of 2,600 m^2 for treatments with high stocking densities (92 $shrimps.m^{-2}$). The shrimps were stocked with a mean individual weight of 0.004 g (post-larva stage 12). The experimental design was completely randomized with three treatments and four replicates, for a total of 12 experimental units as earthen ponds.

The type of management strategy was the factor tested with three levels: Management strategy 1 (M1): four grow-out ponds were initially stocked with a density of 92 $shrimps.m^{-2}$. The production system was managed as a single grow-out phase in which the post-larvae were

stocked directly in earthen ponds immediately after the larviculture. The ponds were initially fertilized with a mixture of 100 kg.ha^{-1} of wheat bran, 30 kg.ha^{-1} of calcium nitrate, 20 kg.ha^{-1} of silicate and 20 kg.ha^{-1} of molasses, and were maintained with biweekly applications of 30 kg.ha^{-1} of calcium nitrate and weekly of 10 kg.ha^{-1} of molasses. Management strategy 2 (M2): four grow-out ponds with an area of approximately 26,000 m^2 each were initially stocked with 14 shrimps.m^{-2}. The production system was managed as a single-phase grow-out with an initial application of fertilizers similar to the M1 treatment, but with no subsequent use of fertilizers. Management strategy 3 (M3): This treatment consisted of two distinct growth phases. The first phase was an intermediate growth phase of 30 days in a 20 x 100 meter raceway stocked with 1,000 shrimps.m^{-2}. The raceway was initially fertilized using a mixture of 250 kg.ha^{-1} of wheat bran, 45 kg.ha^{-1} of calcium nitrate and 40 kg.ha^{-1} of molasses to maintain a C/N ratio above 10 (Avnimelech, 1999). A probiotic mixture comprised of *Bacillus* sp. and *Lactobacillus* sp. were added at 0.2 kg.ha^{-1} to the production system as well. In the second phase, juveniles of *L. vannamei* were transferred from the raceway at a mean individual biomass of 0.98 ± 0.05 g to four grow-out ponds at a stocking density of 8 shrimps.m^{-2}. Each of the grow-out ponds was initially fertilized with 30 kg.ha^{-1} of calcium nitrate and 100 kg.ha^{-1} of dolomitic limestone. The ponds were fertilized weekly using 10 kg.ha^{-1} of calcium nitrate until the harvest. Feed from all procedures was manually distributed and three feeders were used to verify and control feed intake.

Three types of commercial shrimp feed with different compositions were used during the experiment (Table 1). Phase 1: feed used from stocking until 10 days of culture. Phase 2: feed used after phase 1 until the shrimp attained a mass of 3g. Phase 3 (grow-out feed): used from the moment that the shrimps attained 3g until the harvest. During the experiment, the shrimps were fed three times daily, with two offerings in the morning and one in the afternoon.

Shrimps, sediment and water samples were collected every 15 days to monitor shrimp growth for the feed management and to assess the physical and chemical parameters of the cultivation.

Table 1. Characterization of the commercial feed used in the cultivation.

(at the end of the manuscript)

2.3. Particulate matter collection:

Four sample collections were carried out in the M1 and M2 treatments, and three sample collections for the M3 because of the shorter cultivation time in grow-out ponds. Samples were collected at 30 days of cultivation and every 15 days thereafter throughout the experimental period, from September to November of 2016. The particulate matter that settled at the pond bottoms was sampled by placing tripton collectors in the ponds at a depth of approximately 1.5 meters for 24 hours. The tripton collectors were filled with distilled water before being submersed to avoid the deposition of solid material before the start of the sampling period. Samples of sedimentation were taken from the interior of the tripton collectors. To determine sedimentation rates, 150 mL water samples were obtained from the sedimentation chambers immediately after removing from the ponds and were filtered using filters (quantitative filter – nominal retention: 20-25 microns) that were previously dried and weighed (P_1). The filters with particulate material were then dried in an incubator at 60ºC for 24 hours, cooled and weighed (P_2). The differences in the masses between P_1 and P_2 provided the mass (in grams) of the total suspended materials. The concentration of the total suspended solids (TSS) was expressed in $mg.L^{-1}$ and was determined by the formula:

$$TSS = \frac{P2-P1}{V} x1000$$

Where:

P1 = initial weight of the filter with the sample (g);

P2 = weight of the filter with sample material after drying in incubator (g);

V = volume of water used for filtration (L);

1,000 = conversion to milligrams.

The sedimentation rate (SR = mg/cm^2/day) corresponds to the concentration of the material in the filtered sample, corrected for the average volume of the tripton collectors and is estimated by the equation:

$$SR = \frac{\text{Vc x C}}{\text{Ac x T}}$$

Where:

Vc = volume of the sedimentation chambers (2.36 liters);

C = concentration of the material in suspension inside the chambers (mg.L^{-1});

Ac = surface area of the sedimentation chamber opening (78.54 cm²);

T = time in days.

The settleable solids were determined using Imhoff cones. Contents of the tripton collectors were homogenized before obtaining samples to fill the Imhoff cones. The volume of the homogenized sample was recorded in the cones and the volume of the settled material (mL.L^{-1}) was measured after a period of 45 min.

2.4. Limnological variables

Water samples were taken from the surface of the ponds to determine the concentrations of ammonia, nitrate and nitrite (Mackereth et al., 1978), total nitrogen (Koroleff, 1976) and total phosphorus (Golterman et al., 1978). Total carbon (TC), total organic carbon (TOC) and total inorganic carbon (TIC) (Bloesch et al., 1977; Cobelas, 1991) were analyzed from samples collected from the water surface with test tubes (50 ml). Water samples were subjected to oxidation catalytic combustion using a VARIO-TOC analyzer to determine the different carbon concentrations. Chlorophyll-α was analyzed from 100 ml samples of the pond surface water. The samples were filtered using cellulose membrane filter – 47 mm diameter – 0.45 microns

porosity. Transparency (cm), temperature (ºC), pH, salinity ($g.L^{-1}$) and dissolved oxygen ($mg.L^{-1}$) were measured during the collection of the tripton samplers, using a Secchi disk and a water quality parameter multi-probe sensor (HORIBA U-50). All sample collections were carried out near the drain gates of the ponds for the day and night periods at 7 AM and 6 PM, respectively.

2.5. White Spot Virus Syndrome Analysis

At the end of the experiment, pleopods of 50 shrimp from each treatment were removed and stored in 95% ethanol for qPCR to detect the presence of the white spot syndrome virus (WSSV). WSSV was detected and quantified using qPCR primers and TaqMan probes (Life technologies®), and an ABI 7300 Real-time PCR system (Applied Biosystem®) using methods described in Feijó, et al. (2013).

2.6. Data analysis

The following data for the limnological variables were tested for normality (D'Agostino test) and homoscedasticity (Bartlett test): ammonia, nitrite, nitrate, chlorophyll, orthophosphate, total carbon (TC), total inorganic carbon (TIC), total organic carbon (TOC), total phosphorus, total nitrogen, and particulate material. When normality and homoscedasticity were met, means of the variables were compared using a one-way analysis of variance (ANOVA) and significant differences were determined using the Tukey test ($p<0.05$). All statistical analyses were performed using the software STATISTICA version 10.0.

3. Results

The cultivations of treatments M1, M2 and M3 lasted 63, 79 and 51 days, respectively. Mean survival for treatments M1, M2 and M3 were 42.9%, 12.2% and 39.3%, respectively (Table 2). Survival was similar between the M1 and M3 treatments, both of which were significantly higher than the survival in the M2. Significant differences in the feed conversion ratio (FCR) were shown between treatments, with the value in M1 (2.95/1) being significantly

higher than the values observed in treatments M2 (1.44/1) and M3 (0.22/1) (Table 2). At the end of the cultivations, the average values of final yield were 651.9 kg.ha^{-1} in M1, 332.2 kg.ha^{-1} in M2 and 219.0 kg.ha^{-1} in M3. The final shrimp yield in M1 was significantly higher when compared to the M2 and M3 treatments, of which the latter two treatments showed no difference between each other (Table 2). The qPCR of the shrimp tissues showed that WSSV was present in 100% of the samples from all treatments.

(Suggested location of Table 2)

Transparency, salinity and dissolved oxygen showed rising trends throughout the production cycle in the M3 treatment. Salinity of the M3 treatment was significantly higher than in the M1 and M2 treatments for the second and third sampling periods (Table 3). Temperature of the M3 presented a downward trend throughout the production cycle. The water transparency varied from 26 to 40 cm; 40 to 69 g.L^{-1} for salinity; 26 to 32.0 ºC for water temperature; 7.4 to 8.6 for pH and 4.1 to 9.7 mg.L^{-1} for dissolved oxygen throughout the cultivation period for all treatments. No significant differences were shown for the variables between the M1 and M2 treatments.

(Suggested location of Table 3)

No significant differences were shown between treatments for the different sampling periods of chlorophyll-α (Table 3). The chlorophyll-α showed a downward trend in the M1 treatment, with higher values recorded in the second sampling period (177.56 ± 160.0 µg.L^{-1}). A growing trend was shown for the chlorophyll-α in the M2 treatment from the second period thereafter, with higher values recorded in the fourth sampling period (106.86 ± 30.9 µg.L^{-1}).

No chlorophyll-α was identified in the first sampling period for the M3 treatment and showed a growing trend in the subsequent samples (Table 4).

(Suggested location of Table 4)

The sedimentation rate of the particulate matter was significantly higher in the M3 than in the M1 and M2 treatments at 15 days of cultivation, whereas the M1 treatment was significantly higher than the M2 and M3 for the remaining sampling periods (Fig. 1). The sedimentation rate of the particulate matter for the M1 was 26.03 mg/cm^2/day at 30 days and decreased to 11.59 mg/cm^2/day for the rest of the culture. The sedimentation rate for the M2 increased through the experimental period from 5.5 to 7.3 mg/cm^2/day.

The sedimentation rates of ammonia and nitrite were significantly higher in the M3 treatment (12.05 µg/cm²/day; 11.12 µg NO_2/cm²/day) when compared to those of the M1 (1.50 µg/cm²/day; 0.037 µg NO_2/cm²/day) and M2 (3.9 µg/cm²/day; 0.054 µg NO_2/cm²/day) treatments at 15 days of cultivation (Fig. 1). The sedimentation rate of nitrite in M3 was significantly lower (0.025 µg/cm²/day) than in the M1 (0.30 µg/cm²/day) at day 45. The sedimentation rate of nitrate in the M3 (13.8 µg/cm²/day) was significantly lower than in the M1 (20.5 µg/cm²/day) and M2 (22.0 µg/cm²/day) treatments at 30 days of culture. All treatments showed a general decrease in the sedimentation of nitrate toward the end of the culture.

The sedimentation rates of total phosphorus in the M3 treatment were significantly higher than in the M1 and M2 at day 15 (Fig. 1). The sedimentation rates of total phosphorus in the M1 was significantly higher than in the M2 at day 30 and 45, of which the M1 decreased and the M2 showed a slight increase at 30 days and thereafter. The average sedimentation rate of total nitrogen in the M3 treatment (0.055 mg/cm²/day) was higher than in the M1 and M2 at

15 days of culture and then decreased over the following periods. No significant differences were shown between the M1 and M2 treatments for sedimentation rates of total nitrogen throughout the experimental period. Sedimentation of total nitrogen decreased in all treatments throughout the cultivation, with average values varying from 0.02 to 0.021 mg/cm²/day for the M1, 0.019 to 0.02 mg/cm²/day for the M2 and 0.016 to 0.055 mg/cm²/day for the M3.

No significant differences were shown between treatments for the sedimentation rate of the total inorganic carbon (TIC) at any of the sampling periods (Fig. 1). Means were 0.8 ± 0.12, 0.706 ± 0.14 and 0.8 ± 0.05 mg/cm²/day for the M1, M2 and M3 treatments, respectively. The sedimentation rate of the total organic carbon (TOC) in the M3 was significantly lower than in the M1 and M2 treatments at 15 days, but then increased and was significantly higher than the M2 at 30 days and the M1 at 45 days. In general, the sedimentation of TOC in all treatments increased throughout the experimental period.

(Suggested location of Figure 1)

4. Discussion

Low survival of the shrimp in all treatments was perhaps due to the high salinity and the presence of White Spot Virus Syndrome in all three cultivation systems, of which the WSSV was present in all samples according to results of the qPCR. Maia et al. (2016) reported higher survival rates (~84%) with a density of 98 shrimps.m^{-2} and with a salinity of 22 $g.L^{-1}$, and no WSSV was observed. The significantly lower shrimp survival in the M2 system may be associated with a longer cultivation time, which is ultimately a longer exposure of the shrimp to WSSV. Costa et al. (2010) reported that survival rates of the *L. vannamei* exposed to WSSV were 65% and 5% after 29 and 51 days of cultivation, respectively, suggesting that a longer exposure time of the shrimp to WSSV decreases survival. FCR and final yield of the M1 system

were higher than those of the other two management strategies, perhaps due to the higher stocking density that required an increased feed input throughout the cultivation. The high FCR of the M2 system was perhaps due to low shrimp survival, whereas the low FCR of the M3 may have been due to the compensatory growth when using a biphasic system for stocking the shrimp (Marques and Lombardi, 2011; Marques et al., 2012). Brito et al. (2016) used different feed management strategies in the cultivation of *L. vannamei* with a stocking density and obtained a lower AFC of 1.31 with 94% survival.

The transparency, temperature, pH and dissolved oxygen of the cultivation water were within the ranges recommended for shrimp mariculture (Valenti, 1985; Trejo-Flores et al., 2016). On the other hand, mean salinity (mean = 49.6; range = 42 to 61 $g.L^{-1}$) was high in all treatments and above the values recommended in Boyd (1989), which are between 15 and 25 $g.L^{-1}$. Sedimentation rates of the nutrients and particulate matter in the M3 treatment were higher than those in the M1 and M2 for the first sampling period probably due to the high inputs of feed and fertilizer for the initial stocking density in the greenhouse raceways. Ma et al. (2013) reported a greater contribution of particulate material when cultivating marine shrimp in greenhouse ponds, but with a faster decomposition of the organic material due to the increased temperature. A reduction in particulate matter was observed in the M3 treatment after 30 days perhaps due to the reduced stocking density, which required less feed per unit of cultivation area.

Chlorophyll-α was reduced in the second sampling period for the M1 treatment, which may be associated to the decrease in nitrogenous compounds (Silva et al., 2017). In the M2 treatment, the chlorophyll-α increased at the third sampling period and other nutrients increased at the second and third periods as well. According to Costa et al. (2016), the high concentrations of nitrogen and phosphorus are related to the growth of phytoplankton communities. In turn, the phytoplankton supplements shrimp nutrition and recycles nutrients from the water column

(Ananda et al., 2019). Chlorophyll-α was undetected in the M3 treatment for the first sampling period due to the first production phase being carried out in a greenhouse, which reduces the penetration of solar energy and ultimately photosynthetic activity.

The M1 treatment showed the highest sedimentation rate of particulate material at 30 days of culture and thereafter, but decreased until the end of the cultivation. Ribeiro et al. (2016) suggested that an increase of nutrients in shrimp ponds can stimulate benthic production, which improves the decomposition of organic matter and ultimately reduces the accumulation of nutrients. On the other hand, it has been shown that up to 30% of feed in shrimp grow-out ponds are drained to the aquatic environment as excrement and unconsumed feed, which also contribute to intrinsic problems related to water quality and the productive performance of shrimp (Pillay, 2004; Gaona et al., 2017). Mean values of the particulate matter for all treatments of the present study were similar to those reported for *O. niloticus* reared in net-tanks (6.13 to 9.23 mg/cm/day) in the semi-arid northeastern region of Brazil (Moura et al., 2014). Most of the particulate matter produced in shrimp grow-out ponds is formed by a combination of chemical products, fertilizers, excrements, undigested feed, undesired organisms and detritus (Flaherty et al., 2000; Hall, 2004; Paul and Vogl, 2011). Thus, the increased sedimentation of particulate material may be related to the proportionately high feed inputs required to sustain a high shrimp biomass.

The sedimentation rates of ammonia, nitrite and nitrate were higher at 15 days of culture in the M3 treatment when compared to the other treatments, perhaps due to the use of molasses, wheat bran and calcium nitrate in the first phase of this management strategy. The sedimentation rates of nitrate were higher than those of the nitrite and ammonia in the M3 treatment, suggesting that dissolved oxygen and the bacterial community was adequate to convert nitrogenous wastes into the most stable form of nitrogen. The accumulation of nitrate

is a positive externality for shrimp aquaculture ponds since high concentrations of ammonia lead to ecdysis, oxygen depletion, and mortality (Chen and Lin, 1992).

The sedimentation rates of phosphorus and particulate material showed a similar increasing pattern in the M2, indicating an accumulation of wastes from high feed input. In general, feed is the major source of phosphorus in fed aquaculture systems (David et al., 2017b; Flickinger et al., 2020b). Aquaculture activities release high quantities of phosphorus in particulate matter to the environment because it is highly insoluble in water and poorly absorbed by the target species (Flickinger et al., 2020b). Bottom sediments have been shown to absorb approximately 66 to 84% of the phosphorus from feed in aquaculture, of which shrimp have been shown to absorb 25% of the phosphorus accumulated in the sediments (Yiyong et al., 2001; Na and Kim, 2003; Guo and Li, 2003; Sugiura, et al., 2006; Avnimelech, 2009; Moura, et al., 2014). The decrease in sedimentation of phosphorus in the M1 and M3 treatments is probably associated with the reduction in feed offered before harvesting the shrimp and the compensatory growth in the second production phase of the M3. The average sedimentation rates of the phosphorus observed in the present study (113 µg/cm²/day) were approximately 12% to 33% lower than those observed in Moura et al. (2014), which recorded sedimentation rates of 315 µg/cm²/day for the aquaculture of Nile tilapia in net-tanks.

The sedimentation of total nitrogen is proportional to the quantity of feed offered and the protein content of the feed. Funge-Smith and Briggs (1998) suggested that 24% of the total nitrogen content of the artificial feed used in marine shrimp grow-out is converted into shrimp biomass, while 35% is drained with the effluents and 31% is retained in the sediments. Haque et al. (2016) reported that 33.6 g of nitrogen per 1,000 g of feed offered to the target species accumulate at the pond bottoms. The highest sedimentation rate of total nitrogen in the M3 treatment in the present study was observed in the beginning of the culture and occurred because of the high feed input to sustain the high initial density of shrimp in the greenhouse phase of

the culture. Total nitrogen in the M3 was reduced at the second sampling period and thereafter as the second phase of the culture was carried out with a low stocking density, which required a reduction in the use of nitrogenous fertilizers per unit of production as well.

The sedimentation rate of total nitrogen in the M2 showed stability starting at the second sampling period, which may be associated to an increase in primary production when considering the increasing concentration of chlorophyll-α throughout the culture (Faria et al., 2001; Casé et al., 2008; Silva, et al.; 2017). The M1 treatment showed a trend of stabilization for the total nitrogen during the first three sampling periods and a downward trend until the end of the culture, perhaps due to the management strategy of this treatment that used molasses as a carbon source, showing a higher C/N ratio (3.1/1) than those of the M3 (1.44/1) and M2 (1.08/1). An increased C/N ratio may facilitate the maintenance of nitrogenous compounds by heterotrophic and autotrophic organisms at acceptable levels for shrimp grow-out (Avnimelech, 2009; Ballester et al., 2010; Brito et al., 2016; Xu et al., 2016). The sedimentation rates of carbon in the present study increased throughout the experimental period as well, likely due to the high production of wastes from feed, feces, and other material. TOC is expected to increase in aquatic production systems, since CO_2 is removed from the atmosphere by photosynthetic phytoplankton and converted into organic material (Boyd et al., 2010). Flickinger et al. (2020a) showed that CO_2 enters monoculture and Integrated Multi-Trophic Aquaculture (IMTA) earthen pond systems at a near constant rate, and in Amazon river prawn (*Macrobrachium amazonicum*) monoculture this atmospheric gas represented nearly 20% of all carbon inputs. Nevertheless, TOC in the water column was similar between these culture systems despite the high variation in feed carbon input, suggesting that much of the carbon remained immobilized in settled solid organic material and that the flow of carbon in the earthen ponds was limited by aerobic decomposition on the pond bottom and reuptake of CO_2 by photosynthetic organisms

in the water column, thus maintaining constant TOC and TIC water concentrations (Flickinger et al., 2020a).

Other studies on nutrient budgets in Amazon river prawn (*Macrobrachium amazonicum*) monoculture and its integration in IMTA have shown that chlorophyll-α and organic matter increase in the water column over time with no negative effects on water quality (David et al., 2017a; 2017b; Flickinger et al., 2019; Flickinger et al., 2020b). Moura et al. (2014) also observed that water quality remained adequate for the grow-out of Nile tilapia in net-cages despite the accumulation of TIC and TOC. In the present study, no negative impacts on water quality were observed with the accumulation of TOC, suggesting that inputs were insufficient to provoke high aerobic decomposition and deplete dissolved oxygen. Therefore, when considering the increase of chlorophyll-α over time and that no differences were shown between treatments, results of the TOC and TIC indicate that pelagic photosynthetic biota was absorbing atmospheric CO_2 at a similar rate between pond systems. In addition, proliferation of photosynthetic organisms may have been limited by aerobic decomposition of feed and fertilizer that accumulated on the pond bottom, and the concentration of dissolved oxygen that regulates aerobic decomposition (Flickinger et al., 2020a).

In conclusion, the present study shows elevated sedimentation rates of nutrients and particulate material during the first 60 days of *L. vannamei* grow-out when carried out in high stocking densities. No changes were shown between the tested management strategies due to the high feed input necessary to meet the nutritional requirements of the animals. The increase of primary productivity throughout the experimental period may have facilitated the maintenance of nitrogenous compounds and other nutrients at acceptable concentrations.

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Declaration

The authors of the present study declare no conflict of interest and have no affiliation with the commercial shrimp farm where the research was carried out. All the animals used in the research were commercialized for human consumption, respecting all the procedures in the capture and processing thereof.

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Tables and Figures

Table 1
Characterization of the commercial feed used in the cultivation.

Phases	CP (%)	P (%)	E.E. (%)	Granulometry (mm)
Phase 1	40	1.3	0.9	0.54 - 1.0
Phase 2	40	1.3	0.9	1.0 - 1.8
Phase 3	35	0.9	0.8	2.5

CP – Crude Protein, P – Phosphorus and E.E. – Ether Extract

Table 2
Means (± SD) of the productive performance of the *L. vannamei* grow-out carried out with different management strategies. Different letters indicate significant differences according to the Tukey test (P<0.05).

Parameters	Treatments		
	M1	M2	M3
Survival (%)	42.9 ± 5.5a	12.2 ± 3.5b	39.3 ± 0.1a
Final Individual Mass (g)	6.3 ± 0.38b	9.4 ± 1.9 a	6.9 ± 0.51b
Feed Conversion Ratio	2.95 ± 0.47a	1.44 ± 0.41b	0.22 ±0.08c
Final Yield ($kg.ha^{-1}$)	651.9 ± 99.4a	332.0 ± 149.3b	219.0 ± 56.6b

Table 3
Means (± SD) of the limnological variables in the grow-out of *L. vannamei* carried out with different managements strategies. Different letters indicate significant differences according to the Tukey test (P<0.05).

	Treatment		
Variable	M1	M2	M3
Transparency (cm)	31.2 ± 8.6a	33.7 ± 3.1a	33.2 ± 1.1a
Salinity ($g.L^{-1}$)	41.8 ± 1.4a	46.0 ± 1.6a	61.1 ± 0.9b
Temperature (°C)	28.9 ± 0.2a	29.0 ± 0.6a	28.5 ± 0.1a
pH	8.4 ± 0.1a	8.4 ± 0.1a	7.8 ± 0.2a
Dissolved Oxygen ($mg.L^{-1}$)	7.2 ± 0.7a	5.6 ± 1.2a	7.0 ± 0.8a

Table 4
Means (± SD) of the chlorophyll-α ($\mu g.L^{-1}$) of the cultivation water in the grow-out of *L. vannamei* carried out with different management strategies. Different letters indicate significant differences according to the Tukey test (P<0.05).

	Days			
Treatment	15	30	45	60
M1	145.74±97.6a	177.56±160.0a	76.56±23.0a	76.18±13.04a
M2	34.1±27.7a	10.64±4.9a	64.53±54.8a	106.86±30.9a
M3	ND	10.14±8.3a	10.98±8.98a	

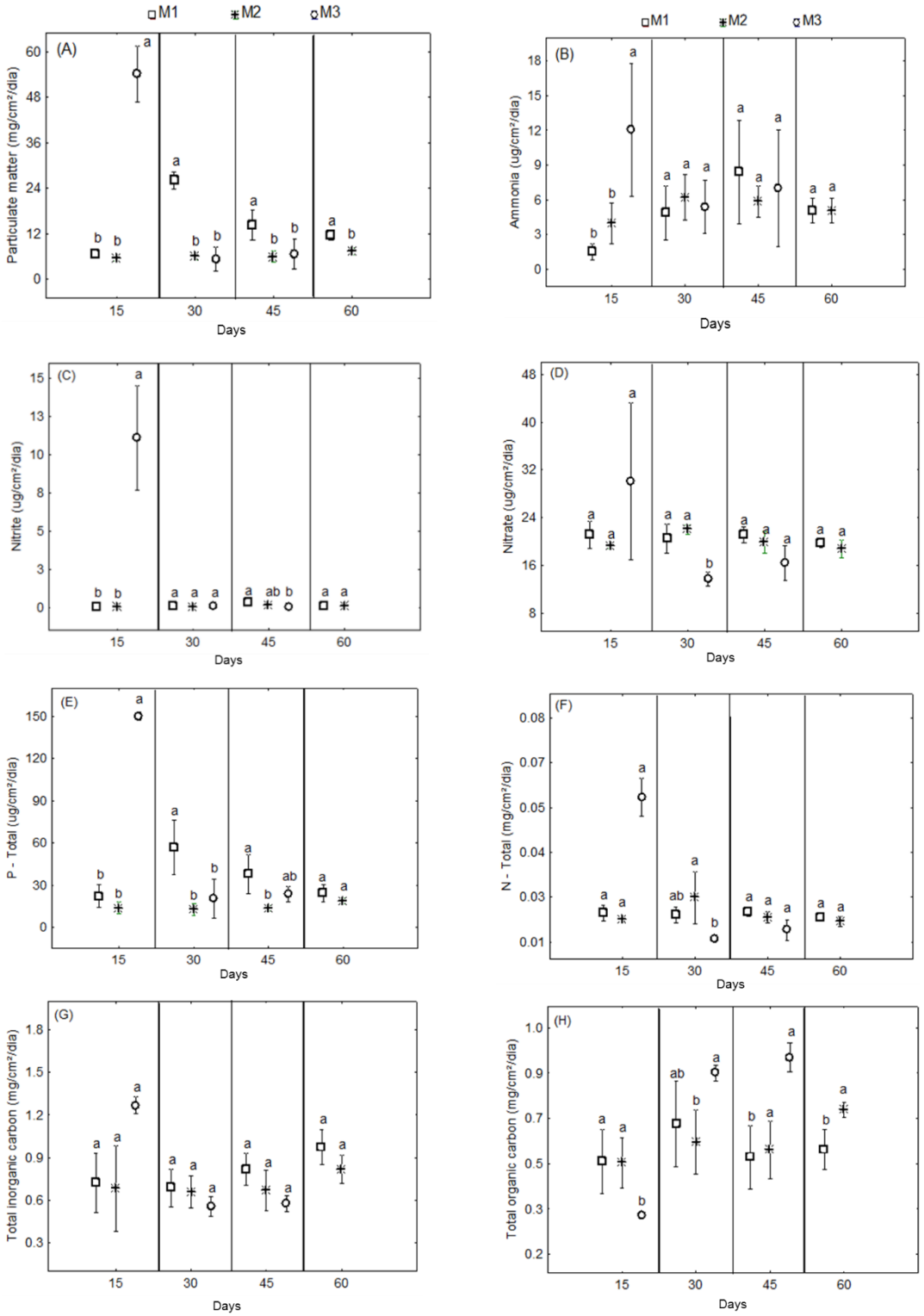

Fig. 1. Means (±SD) of the sedimentation rates of: A) particulate matter; B) ammonia; C) nitrite; D) nitrate; E) total phosphorus; F) total nitrogen; G) total inorganic carbon; and H) total organic carbon for the different treatments*. Distinct letters indicate significant differences according to the Tukey test *($p<0.05$)*.

*The initial stocking density of the shrimp in the M3 treatment was 1,000 shrimps.m^{-2}, then decreased to 8 shrimps.m^{-2} starting at 30 days of culture.

DECLARATION

The authors of the present study declare no conflict of interest and have no affiliation with the commercial shrimp farm where the research was carried out. All the animals used in the research were commercialized for human consumption, respecting all the procedures in the capture and processing thereof.

AUTHOR STATEMENT

Ambrosio Paula Bessa Junior: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Visualization; Roles/Writing – original draft; Writing – review & editing. **Dallas Lee Flickinger**: Roles/Writing – original draft; Writing – review & editing. **Gustavo Gonzaga Henry-Silva**: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing – original draft; Writing – review & editing