



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

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### ARTICLE INFO

#### Keywords:

Greenhouse  
Earthen ponds  
Nutrients  
Sedimentation rate  
Shrimp

### 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<sup>-2</sup>; M2: 14 shrimps.m<sup>-2</sup> and M3: 8 shrimps.m<sup>-2</sup>. 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- $\alpha$  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.

### 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; Cacho and Henry-Silva, 2020). 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; 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. 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). This species is targeted for aquaculture

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<https://doi.org/10.1016/j.aquaculture.2020.736307>

Received 16 September 2019; Received in revised form 17 December 2020; Accepted 20 December 2020

Available online 23 December 2020

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because it adapts well to artificial rearing conditions and can be produced in coastal regions as well as inland with fresh or saline-alkaline water.

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; 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 and the 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 high potential to alter 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). 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 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<sup>-1</sup>. The enterprise has 80 ponds with areas varying from 2600 to 26,000 m<sup>2</sup>, used for the grow-out of *L. vannamei* in densities of 8 to 100 shrimp.m<sup>-2</sup>. The region has a tropical and semi-arid climate of BSwh' according to the Köppen classification system, with annual averages of temperature of 27.4 °C, 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<sup>2</sup> were used for treatments with low stocking densities (8–14 shrimp.m<sup>-2</sup>) and ponds with areas of 2600 m<sup>2</sup> for treatments with high stocking densities (92 shrimps.m<sup>-2</sup>). 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<sup>-2</sup>. 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<sup>-1</sup> of wheat bran, 30 kg.ha<sup>-1</sup> of calcium nitrate, 20 kg.ha<sup>-1</sup> of silicate and 20 kg.

ha<sup>-1</sup> of molasses, and were maintained with biweekly applications of 30 kg.ha<sup>-1</sup> of calcium nitrate and weekly of 10 kg.ha<sup>-1</sup> of molasses. Management strategy 2 (M2): four grow-out ponds with an area of approximately 26,000 m<sup>2</sup> each were initially stocked with 14 shrimps.m<sup>-2</sup>. 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 × 100 m raceway stocked with 1000 shrimps.m<sup>-2</sup>. The raceway was initially fertilized using a mixture of 250 kg.ha<sup>-1</sup> of wheat bran, 45 kg.ha<sup>-1</sup> of calcium nitrate and 40 kg.ha<sup>-1</sup> 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<sup>-1</sup> to the production system as well. In the second phase, juveniles of *L. vannamei* were transferred from the raceway at a mean individual weight of 0.98 ± 0.05 g to four grow-out ponds at a stocking density of 8 shrimps.m<sup>-2</sup>. Each of the grow-out ponds was initially fertilized with 30 kg.ha<sup>-1</sup> of calcium nitrate and 100 kg.ha<sup>-1</sup> of dolomitic limestone. The ponds were fertilized weekly using 10 kg.ha<sup>-1</sup> 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 weight of 3 g. Phase 3 (grow-out feed): used from the moment that the shrimps attained 3 g 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.

### 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 m for 24 h. The tripton collectors were filled with distilled water before being submerged 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 µm) that were previously dried and weighed (P<sub>1</sub>). The filters with particulate material were then dried in an incubator at 60 °C for 24 h, cooled and weighed (P<sub>2</sub>). The differences in the weight between P<sub>1</sub> and P<sub>2</sub> provided the weight (in grams) of the total suspended materials. The concentration of the total suspended solids (TSS) was expressed in mg.L<sup>-1</sup> and was determined by the formula:

**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.

$$TSS = \frac{P2 - P1}{V} \times 1000$$

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);

1000 = conversion to milligrams.

The sedimentation rate (SR = mg/cm<sup>2</sup>/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:

$$SR = \frac{V_c \times C}{A_c \times T}$$

where:

V<sub>c</sub> = volume of the sedimentation chambers (2.36 L);

C = concentration of the material in suspension inside the chambers (mg.L<sup>-1</sup>);

A<sub>c</sub> = surface area of the sedimentation chamber opening (78.54 cm<sup>2</sup>);

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<sup>-1</sup>) 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 and 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- $\alpha$  was analyzed from 100 ml samples of the pond surface water. The samples were filtered using cellulose membrane filter – 47 mm diameter – 0.45  $\mu$ m porosity. Transparency (cm), temperature (°C), pH, salinity (g.L<sup>-1</sup>) and dissolved oxygen (mg.L<sup>-1</sup>) 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<sup>-1</sup> in M1, 332.2 kg.ha<sup>-1</sup> in M2 and 219.0 kg.ha<sup>-1</sup> 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.

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<sup>-1</sup> for salinity; 26 to 32.0 °C for water temperature; 7.4 to 8.6 for pH and 4.1 to 9.7 mg.L<sup>-1</sup> for dissolved oxygen throughout the cultivation period for all treatments. No significant differences were shown for the variables between the M1 and M2 treatments.

No significant differences were shown between treatments for the different sampling periods of chlorophyll- $\alpha$  (Table 3). The chlorophyll- $\alpha$  showed a downward trend in the M1 treatment, with higher values recorded in the second sampling period (177.56  $\pm$  160.0  $\mu$ g.L<sup>-1</sup>). A growing trend was shown for the chlorophyll- $\alpha$  in the M2 treatment from the second period thereafter, with higher values recorded in the fourth sampling period (106.86  $\pm$  30.9  $\mu$ g.L<sup>-1</sup>). No chlorophyll- $\alpha$  was identified in the first sampling period for the M3 treatment and showed a growing trend in the subsequent samples (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<sup>2</sup>/day at 30 days and decreased to 11.59 mg/cm<sup>2</sup>/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<sup>2</sup>/day.

The sedimentation rates of ammonia and nitrite were significantly higher in the M3 treatment (12.05  $\mu$ g/cm<sup>2</sup>/day; 11.12  $\mu$ g NO<sub>2</sub>/cm<sup>2</sup>/day) when compared to those of the M1 (1.50  $\mu$ g/cm<sup>2</sup>/day, 0.037  $\mu$ g NO<sub>2</sub>/cm<sup>2</sup>/day) and M2 (3.9  $\mu$ g/cm<sup>2</sup>/day, 0.054  $\mu$ g NO<sub>2</sub>/cm<sup>2</sup>/day) treatments at 15 days of cultivation (Fig. 1). The sedimentation rate of nitrite in M3 was significantly lower (0.025  $\mu$ g/cm<sup>2</sup>/day) than in the M1 (0.30  $\mu$ g/cm<sup>2</sup>/day).

**Table 2**

Means ( $\pm$  SD) of the productive performance of the *L. vannamei* grow-out carried out with different management strategies.

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

Different letters indicate significant differences according to the Tukey test ( $p < 0.05$ ).

**Table 3**

Means ( $\pm$  SD) of the limnological variables in the grow-out of *L. vannamei* carried out with different managements strategies.

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

Different letters indicate significant differences according to the Tukey test ( $p < 0.05$ ).

**Table 4**

Means ( $\pm$  SD) of the chlorophyll- $\alpha$  ( $\mu\text{g.L}^{-1}$ ) of the cultivation water in the grow-out of *L. vannamei* carried out with different management strategies.

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

Different letters indicate significant differences according to the Tukey test ( $p < 0.05$ ).

cm<sup>2</sup>/day) at day 45. The sedimentation rate of nitrate in the M3 (13.8  $\mu\text{g}/\text{cm}^2/\text{day}$ ) was significantly lower than in the M1 (20.5  $\mu\text{g}/\text{cm}^2/\text{day}$ ) and M2 (22.0  $\mu\text{g}/\text{cm}^2/\text{day}$ ) 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/cm<sup>2</sup>/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/cm<sup>2</sup>/day for the M1, 0.019 to 0.02 mg/cm<sup>2</sup>/day for the M2 and 0.016 to 0.055 mg/cm<sup>2</sup>/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  $\pm$  0.12, 0.706  $\pm$  0.14 and 0.8  $\pm$  0.05 mg/cm<sup>2</sup>/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.

#### 4. Discussion

The presence of WSSV during the cultivation of *L. vannamei* may have contributed negatively to the productive performance results of the shrimp. 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<sup>-2</sup> and with a salinity of 22 g.L<sup>-1</sup>, 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. The high FCR in the M1 system may be due the amount of feed given at the beginning of cultivation as a function of the high initial shrimp density. The FCR of the M2 system was lower than that of the M1, perhaps due to a lower feed input. 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 of 500 shrimps.m<sup>-3</sup> and obtained AFC values near 1.3 with survival of around 90%.

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, the salinity of the cultivations in the present study ranged from approximately 42 to 61 g.L<sup>-1</sup>, which is higher than the range of 15 to 25 g.L<sup>-1</sup> recommended in Boyd, (1989). 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. (2016) 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- $\alpha$  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- $\alpha$  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 (Shyne Ananda et al., 2019). Chlorophyll- $\alpha$  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 Volg, 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

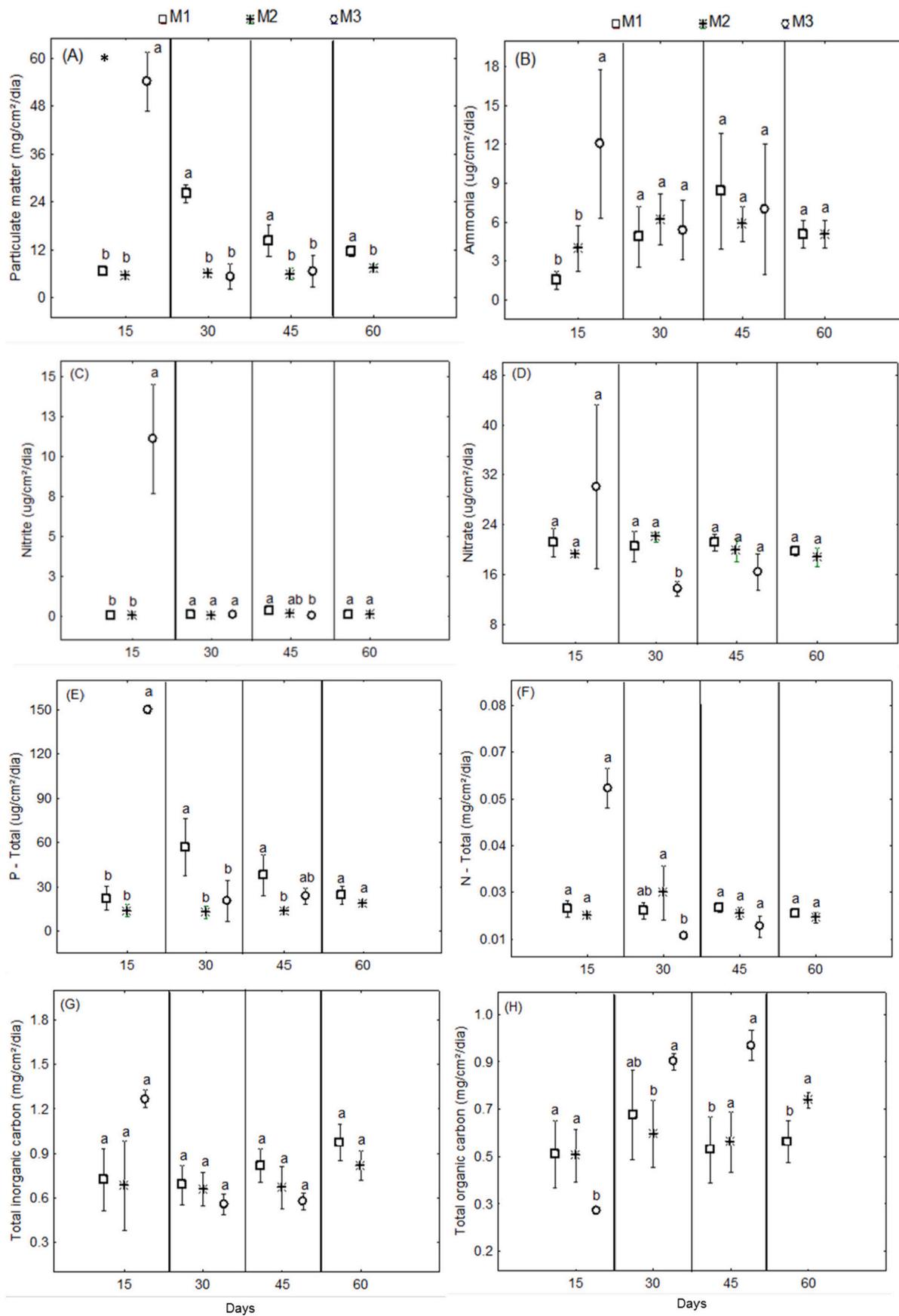


Fig. 1. – Means (±SD) of the sedimentation rates of: A) particulate matter; B) ammonia; C) nitrite; D) nitrate; E) total phosphorus; F) total nitrogen; G) total inorganic carbon; and H) total organic carbon for the different treatments. Distinct letters indicate significant differences according to the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

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, 1999; 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  $\mu\text{g}/\text{cm}^2/\text{day}$ ) were approximately 12% to 33% lower than those observed in Moura et al. (2014), which recorded sedimentation rates of 315  $\mu\text{g}/\text{cm}^2/\text{day}$  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- $\alpha$  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, 1999; 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  $\text{CO}_2$  is removed from the atmosphere by photosynthetic phytoplankton and converted into organic material (Boyd et al., 2010). Flickinger et al. (2020a) showed that  $\text{CO}_2$  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  $\text{CO}_2$  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- $\alpha$  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- $\alpha$  over time and that no differences were shown between treatments, results of the TOC and TIC indicate that pelagic photosynthetic biota was absorbing atmospheric  $\text{CO}_2$  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.

Fig. 1 (A)\* - The initial stocking density of the shrimp in the M3 treatment was 1000 shrimps. $\text{m}^{-2}$ , then decreased to 8 shrimps. $\text{m}^{-2}$  starting at 30 days of culture.

## Declaration of Competing Interest

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.

## Acknowledgements

The authors thank The National Council for Scientific and Technological Development - CNPq and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) for the financial support provided. We would also like to thank Cário Sheves Paivandre Maia for providing the facilities to carry out the experiment and the Center for the Diagnosis of Diseases of Aquatic Organisms at the Federal University of Ceará - Brazil for the molecular analyses to detect and quantify white spot syndrome virus.

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