

## Article

# Polyculture of Pacific White Shrimp *Litopenaeus vannamei* (Boone) and Red Seaweed *Gracilaria birdiae* (Greville) under Different Densities

Gustavo Gonzaga Henry-Silva <sup>1,\*</sup>, Joseanna Alves <sup>1</sup>, Dallas Flickinger <sup>2</sup>, Renata Gomes-Rebouças <sup>1</sup> and Ambrosio Bessa-Junior <sup>1</sup> 

<sup>1</sup> Departamento de Biociências, Universidade Federal Rural do Semi-Árido (UFERSA), Av. Francisco Mota 572, Costa e Silva, Mossoró 59625-900, RN, Brazil

<sup>2</sup> Department of Agriculture and Environmental Sciences, Lincoln University of Missouri, Jefferson City, MO 65101, USA

\* Correspondence: gustavo@ufersa.edu.br

**Abstract:** The present study evaluated a polyculture system with Pacific white shrimp (*Litopenaeus vannamei*) and the macroalga *Gracilaria birdiae* and its potential to remove nutrients. The experimental design consisted of a shrimp monoculture of 100 animals/m<sup>2</sup> (T0) and three multitrophic cultures with *L. vannamei* (100 animals/m<sup>2</sup>) and with *G. birdiae* at densities of 500 (T500), 1000 (T1000), and 2000 (T2000) g/m<sup>2</sup>. Nitrogen and phosphorus concentrations decreased at the beginning of the experiment in the treatments with macroalgae, but this reduction was not maintained throughout the cultivation period. The stable values of *G. birdiae* biomass were perhaps related to the high turbidity of the water. There was an increase in shrimp biomass during cultivation, which reached the final individual averages of 7.5 g (T0), 7.6 g (T500), 5.9 g (T1000), and 7.5 g (T2000), with no significant differences between treatments. Nevertheless, the absence of macroalgae growth provides no added profit for the producer. Thus, there is no economic advantage in using *G. birdiae* in an integrated multitrophic system with *L. vannamei* at a high density and conditions of high water turbidity.



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**Keywords:** *Litopenaeus vannamei*; macroalga; aquaculture; nutrients

## 1. Introduction

The environmental impacts of aquaculture are a global concern due to the discharge of particulate organic matter from wastes and unconsumed feed [1,2]. Organic wastes settle at the bottom and enrich sediments, which increase the risk of the eutrophication of the environment and ultimately the creation of harmful algal blooms that inhibit aquaculture activities [3,4]. The priority of aquaculture is to be sustainable with minimal negative impacts on the environment [5,6]. Hence, integrated multi-trophic aquaculture (IMTA) is an alternative to traditional monoculture that increases the sustainability of producing aquatic organisms by optimizing the space and time of a culture unit, and it could be an alternative that could alleviate environmental problems [7–10].

IMTA systems usually consist of a primary species of economic interest and a secondary extractive species that takes advantage of the unused nutrients of the culture system for growth [11–13]. The removal of nutrients occurs through biological processes and may be conducted using macroalgae species, which contribute to the reduction in negative impacts on the environment [14–18]. IMTA must also consider profitability to the producer [19]. Integrated aquaculture tends to be more productive due to the commercialization of both the fed species and the secondary extractive species [20,21]. One of the main markets for macroalgae is fresh or dry biomass. It is also used for extracts, which have bioactive properties when used as ingredients in food supplements, animal feed, and cosmetics [22].

The main criteria for selecting a macroalga species for production in IMTA are high growth rate, nutrient absorption capacity, ease of cultivation, and resistance to epiphytes. Macroalgae have advantages when used in IMTA systems, and some species are efficient biofiltrators [23,24]. The species of algae that grow without fixing to substrate are considered more suitable for cultivation; an example is the red algae *Gracilaria* [24,25]. The cultivation of the macroalgae of the genus *Gracilaria* in a shrimp pond has a high capacity for the absorption of nitrate and phosphate and can be a major contributor to the removal of nitrogen and phosphorus from the growing environment [26,27]. Thus, the present study evaluated the performance of Pacific white shrimp (*Litopenaeus vannamei*) when reared in a polyculture system with different densities of *Gracilaria birdiae* macroalgae. The macroalga was observed for growth and its potential to remove nutrients from the shrimp culture.

## 2. Materials and Methods

*G. birdiae* was obtained at Flecheiras beach (3°13'06.1" S and 39°15'51.4" W), which is located on the eastern coast of Ceará state in northeastern Brazil. Individuals of good physiological condition and without signs of depigmentation were collected during low tide. The macroalga was transported to the Aquaculture Sector of the Federal Rural University of the Semi-Arid in isothermal boxes. Post-larvae of Pacific white shrimp were transported in plastic bags with oxygen. The individuals of *G. birdiae* and *L. vannamei* were acclimated to experimental conditions for two weeks prior to starting the experiment.

The experimental design was completely randomized with four treatments and four replicates for a total of 16 experimental units (1000 L fiberglass tanks with an area of 1 m<sup>2</sup>); the units were supplied with seawater with a salinity of 35 and continuous aeration. The control treatment was a shrimp monoculture (100 shrimp/m<sup>2</sup>—T0). In the three multi-trophic systems, the shrimp densities were the same as those of the monoculture (100 shrimp/m<sup>2</sup>), with the macroalga at densities of 500, 1000, and 2000 g/m<sup>2</sup> of wet mass for the T500, T1000, and T2000 treatments, respectively. The experimental units were outdoors for exposure to a natural photoperiod, and they had no shading.

The shrimp post-larvae (0.005 g) were stocked in the experimental units ten days before the introduction of *G. birdiae*. Initially, a commercial feed was supplied four times daily to the shrimp (40% crude protein). When the shrimps reached 1 g, a commercial pelleted feed was offered twice daily in trays (35% crude protein). The daily amount of pelleted feed supplied to the shrimps was 6 g per tank and increased gradually when no feed was detected in the trays. The experiment was run for 90 days.

The *G. birdiae* wet biomass was sampled 30 days after the beginning of the experiment to optimize the acclimatization of the macroalgae to the experimental units. The macroalgae were tied to polypropylene ropes at a depth of 20 cm from the water surface. Then, the wet biomass of the macroalga was sampled every 15 days and weighed using an analytical balance. The mean shrimp biomass was estimated every two weeks using a sample of 10% of the shrimp population in each experimental unit. *G. birdiae* was suspended on a mesh stretched on the water surface. Survival (S) was calculated from the final number of animals divided by the number of stocked individuals and multiplied by one hundred. The apparent feed conversion (AFC) factor was calculated according to the total food offered divided by the final biomass of the shrimp. The daily growth rate was calculated using the following Equation (1):

$$\text{Daily growth rate (\%/day)} = (\text{final wet weight} - \text{initial wet weight}) / (\text{initial wet weight}) \times 100 / \text{days of culture} \quad (1)$$

The tanks were siphoned daily and replenished to compensate for evaporation and syphoning at about 10% of the water in each tank every day. The temperature (°C), pH, dissolved oxygen (mg/L), and turbidity (NTU) of the water in the cultivation systems were monitored weekly with a Horiba U-50 multiparameter probe. Water transparency was determined using a Secchi disk, and salinity was determined with a portable refractometer. Water samples were collected every two weeks to analyze the concentrations of total phos-

phorus [28] and total nitrogen using standard methods [29]. The values of the productive performance parameters of the shrimp, the biomass of the macroalgae, and the limnological variables were analyzed using the Shapiro–Wilk and Bartlett tests to evaluate normality and homoscedasticity, respectively. For the variables that showed normal distribution and homogeneous variance, analysis of variance (one-way ANOVA) was applied and, a posteriori, the Tukey test, which identified the significant differences ( $p < 0.05$ ) between treatments.

### 3. Results and Discussion

No significant differences were found in the limnological variables between treatments except for turbidity. The mean turbidity values at T0 and T500 were significantly higher than the mean values of T1000 and T2000 (Table 1). The high turbidity values may be related to the absence of *G. birdiae* in T0 and to the reduced biomass of the macroalgae in T500. The water quality in shrimp mariculture generally shows high loads of suspended solid particles, which contribute to the increase in suspended particulate matter [30,31] (Table 1).

**Table 1.** Mean ( $\pm$ SD) of temperature, pH, dissolved oxygen (OD), turbidity, transparency, and salinity for the different treatments throughout the experimental period of 90 days. T0: *L. vannamei* monoculture (100 shrimp/m<sup>2</sup>); T500: (100 shrimp/m<sup>2</sup> and 500 g/m<sup>2</sup> macroalgae *G. birdiae*); T1000: (100 shrimp/m<sup>2</sup> and 1000 g/m<sup>2</sup> of the macroalgae *G. birdiae*); T2000: (100 shrimp/m<sup>2</sup> and 2000 g/m<sup>2</sup> of the macroalgae *G. birdiae*).

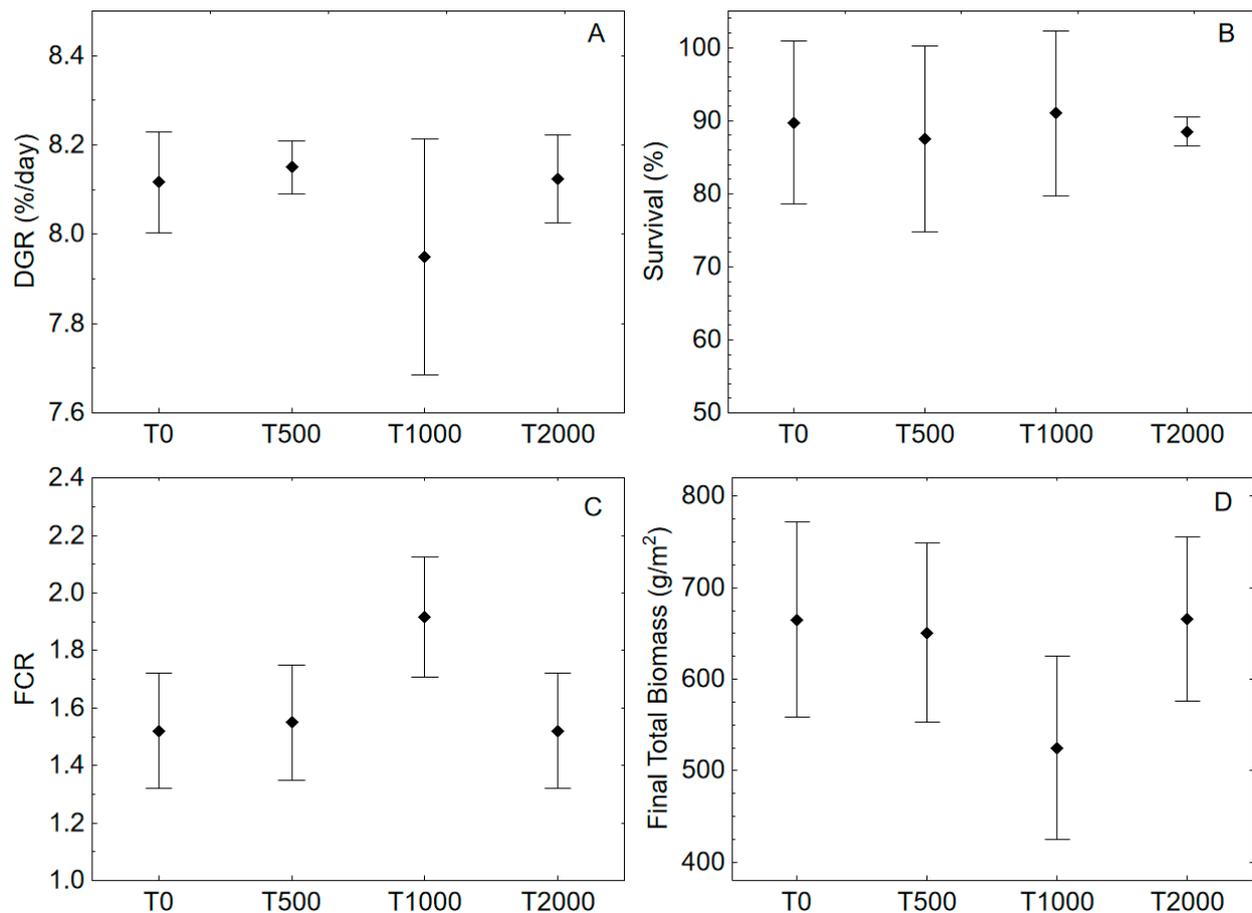
| Treatments | Temperature (°C) | pH             | DO (mg/L)     | Turbidity (NTU)               | Transparency (cm) | Salinity       |
|------------|------------------|----------------|---------------|-------------------------------|-------------------|----------------|
| T0         | 29.7 $\pm$ 0.5   | 8.35 $\pm$ 0.1 | 4.4 $\pm$ 0.2 | 160.1 <sup>a</sup> $\pm$ 69.9 | 25.0 $\pm$ 2.2    | 35.0 $\pm$ 0.9 |
| T500       | 29.6 $\pm$ 0.4   | 8.33 $\pm$ 0.1 | 4.4 $\pm$ 0.1 | 152.6 <sup>a</sup> $\pm$ 63.8 | 25.5 $\pm$ 2.4    | 35.6 $\pm$ 0.8 |
| T1000      | 29.6 $\pm$ 0.6   | 8.27 $\pm$ 0.2 | 4.5 $\pm$ 0.3 | 116.8 <sup>b</sup> $\pm$ 45.9 | 28.5 $\pm$ 3.1    | 35.2 $\pm$ 1.3 |
| T2000      | 29.4 $\pm$ 0.3   | 8.28 $\pm$ 0.1 | 4.3 $\pm$ 0.2 | 108.9 <sup>b</sup> $\pm$ 59.1 | 30.7 $\pm$ 3.5    | 35.8 $\pm$ 0.9 |

Different letters indicate a significant difference between treatments according to the Tukey test ( $p < 0.05$ ).

The shrimp biomass increased in all treatments, and the final mean individual biomass for each treatment was 7.5  $\pm$  0.6 g (T0), 7.6  $\pm$  0.3 g (T500), 5.9  $\pm$  1.5 g (T1000), and 7.5  $\pm$  0.5 g (T2000), with the daily growth rates of 8.1, 8.2, 8.0, and 8.1% per day, respectively (Figure 1A). No significant differences were observed in the survival of *L. vannamei* between treatments. These results show that the macroalgae did not compromise the growth of the shrimp. The mean survival in the monoculture (89%) was similar to that observed in the culture systems with macroalgae (86% in T500, 93% in T1000, and 89% in T2000 (Figure 1B). The mean values of the FCR also showed no difference between the treatments T0 (1.5  $\pm$  0.2), T500 (1.5  $\pm$  0.2), T1000 (1.9  $\pm$  0.3), and T2000 (1.5  $\pm$  0.2) (Figure 1C). No significant differences in the final total biomass of the shrimp were found between the mean values, which were: 665  $\pm$  82, 651  $\pm$  70, 524  $\pm$  91, and 666  $\pm$  73 g/m<sup>2</sup> in T0, T500, T1000, and T2000, respectively (Figure 1D). The results show that the integration with the macroalgae did not affect the final productivity of *L. vannamei*.

Raposo et al. [32] evaluated the performance of *L. vannamei* in a multitrophic culture system integrated with macroalgae and observed no significant differences in the biomass gained by the shrimp in the monoculture and in the polyculture with *G. birdiae*, but they observed a significant gain in the biomass of *L. vannamei* when in polyculture with the macroalgae *Ulva fasciata*. The shrimp in the previous study showed no remnants of *G. birdiae* in the digestive tract and the feces. The absence of macroalgae in the digestive tract is perhaps due to the cylindrical and hard stalks of the *G. birdiae*, which inhibits consumption by the shrimp. Hence, *Ulva fasciata* has more potential than *G. birdiae* to contribute to the growth of *L. vannamei*. Fouroughifard et al. [33] evaluated the integrated culture of *L. vannamei* with the macroalgae *Gracilaria corticata* for 45 days and found that shrimp growth and survival were significantly higher in the polyculture with *G. corticata* when compared to the shrimp monoculture. The positive results for the biomass gains and the

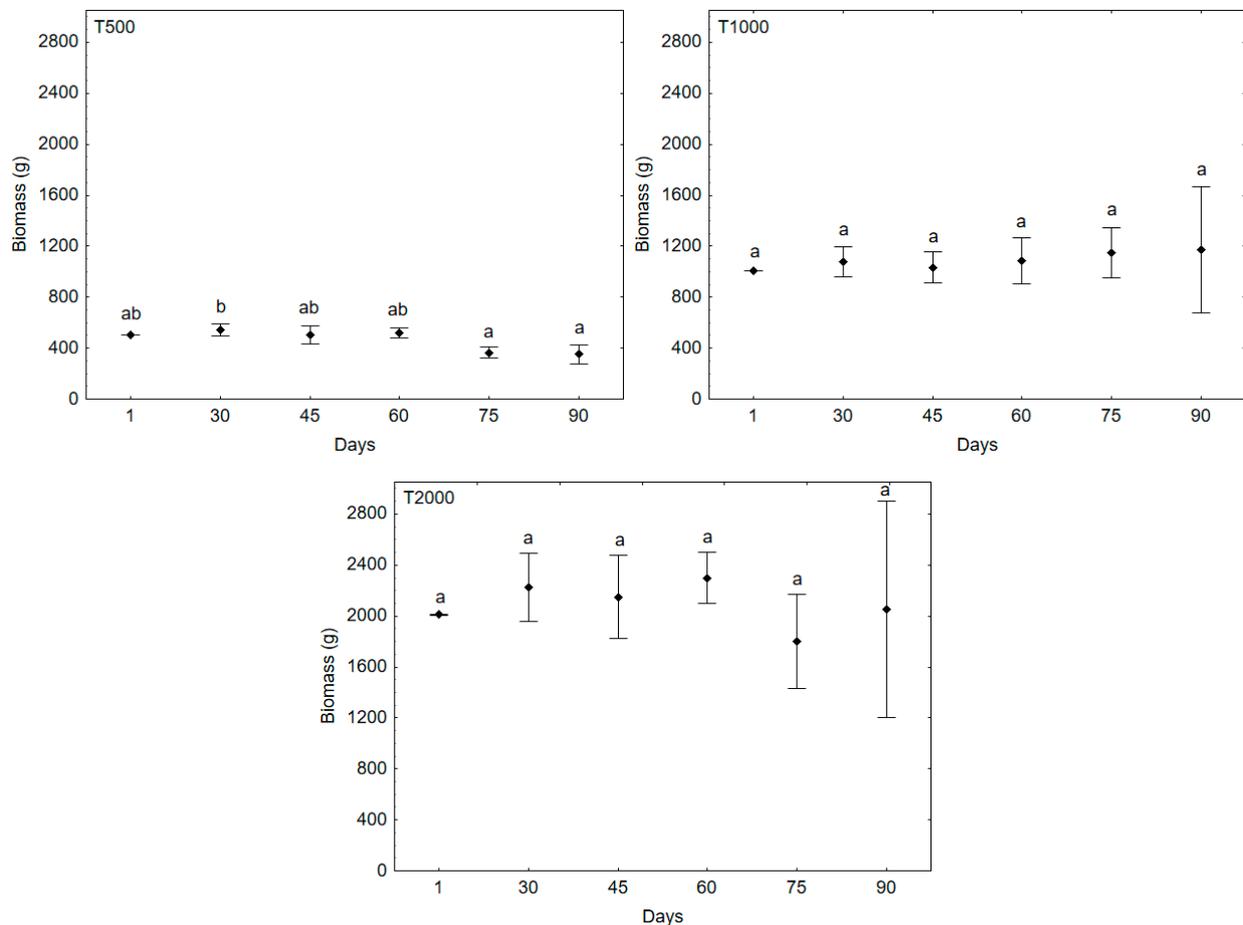
survival of *L. vannamei* in the polyculture observed by Fourrooghifard et al. [33] may be related to the density of the shrimp (25 and 50 shrimp/m<sup>2</sup>) and the cultivation time; the present study used 100 shrimp/m<sup>2</sup> and a cultivation time of 90 days. The cultivation of shrimp at higher densities provides more nutrients for the macroalgae and increases the amount of particulate matter in the suspension, which ultimately increases the turbidity. The high turbidity reduces the solar radiation necessary for photosynthesis, and the excess of particulate material deposits on the plant structures of the macroalgae compromises photosynthetic activity and, consequently, the growth of *G. birdiae*.



**Figure 1.** Mean ( $\pm$ SD) of daily growth rate—DGR (A), survival (B), feed conversion ratio—FCR (C), and final total biomass (D) of *L. vannamei*. T0: shrimp monoculture (100 shrimp/m<sup>2</sup>); T500: 100 shrimp/m<sup>2</sup> and 500 g/m<sup>2</sup> of *G. birdiae*; T1000: 100 shrimp/m<sup>2</sup> and 1000 g/m<sup>2</sup> of *G. birdiae*; T2000: 100 shrimp/m<sup>2</sup> and 2000 g/m<sup>2</sup> of *G. birdiae*. No significant differences were found according to ANOVA followed by the Tukey test ( $p > 0.05$ ).

The mean values of the *G. birdiae* initial biomass in T500, T1000, and T2000 were 500, 1000, and 2000 g, respectively, while at the end of the cultivation, the mean values were 397, 1177, and 2057 g, respectively (Figure 2). A significant difference in the *G. birdiae* biomass was only observed between days 30 and 90 in T500. The values of the macroalgae biomass stability from the beginning to the end of the experiment were probably related to the high turbidity values of the culture systems. High values of turbidity in the culture environment affect primary producers by limiting light absorption and, consequently, hindering the growth of marine macroalgae [33]. Marinho-Soriano et al. [34] concluded that the high concentration of fine particulate matter suspended in the effluent of a shrimp culture system contributed to the deposition of a thin layer of particulate material on the macroalgae, blocking the incident light and affecting its growth. Oliveira et al. [25] evaluated *G. birdiae* growth at different depths and concluded that turbidity and light

penetration influenced the growth of this species. Nelson et al. [35] also reported that macroalgae grown in effluent channels with high turbidity showed low growth rates. These studies corroborate the results obtained in the present work, which show that turbidity is a relevant factor in the productivity of marine macroalgae.

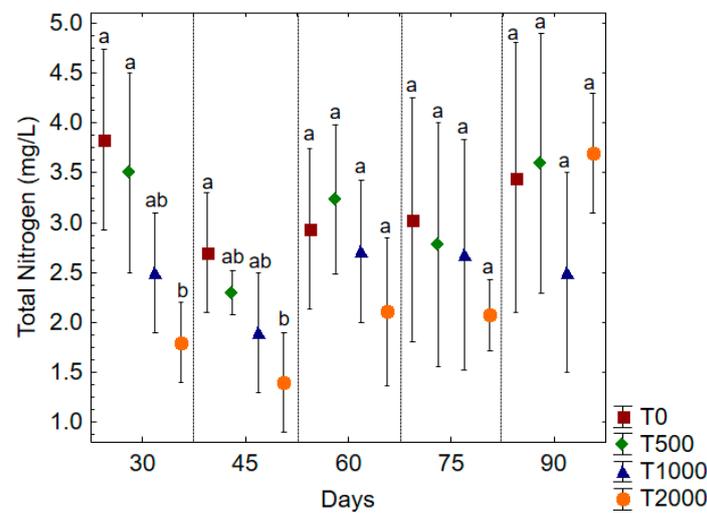


**Figure 2.** Mean ( $\pm$ SD) of *G. birdiae* wet biomass throughout the experimental period of 90 days. T500: 100 shrimp/m<sup>2</sup> and 500 g/m<sup>2</sup> of *G. birdiae*; T1000: 100 shrimp/m<sup>2</sup> and 1000 g/m<sup>2</sup> of *G. birdiae*; T2000: 100 shrimp/m<sup>2</sup> and 2000 g/m<sup>2</sup> of *G. birdiae*. Different letters indicate a significant difference between sampling periods according to the Tukey test ( $p < 0.05$ ).

No significant increase in the *G. birdiae* biomass was shown during the cultivation period. Therefore, the macroalgae provide no economic gains for the producer. Thus, there would be no economic advantages in using *G. birdiae* in an integrated multitrophic system with *L. vannamei* at a high density and conditions of high water turbidity.

At 30 days, water from the monoculture (T0) and the treatment with the lower density of macroalgae (T500) showed significantly higher TN concentrations than those observed in the treatment with a higher density of *G. birdiae* (T2000), while T1000 did not differ significantly from the other treatments. The mean TN values on day 30 were 3.8, 3.5, 2.5, and 1.7 mg/L in T0, T500, T1000, and T2000, respectively. The same occurred on day 45, with T0 showing TN values that were significantly higher (2.7 mg/L) than those found in T2000 (1.4 mg/L). However, after day 60, no significant differences were observed between treatments (Figure 3). These results show that during the first 45 days of the experiment, there was a reduction in nitrogen in the treatments with macroalgae, probably due to the absorption of nutrients by *G. birdiae*. After this period, there was no significant difference in the nitrogen consumption or the production between treatments, perhaps

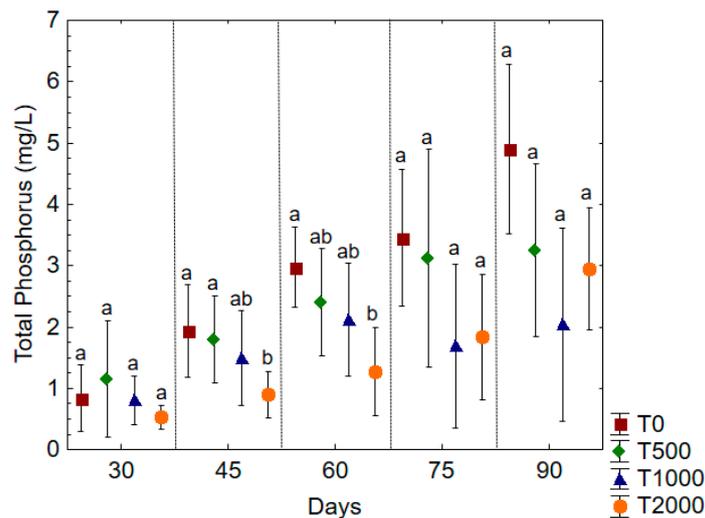
because of the increase in the shrimp biomass and the stabilization of the macroalgae biomass, compromising the absorption of the excess available nutrients.



**Figure 3.** Mean ( $\pm$ SD) of total nitrogen throughout the experimental period of 90 days. T0: shrimp monoculture (100 shrimp/m<sup>2</sup>); T500: 100 shrimp/m<sup>2</sup> and 500 g/m<sup>2</sup> of *G. birdiae*; T1000: 100 shrimp/m<sup>2</sup> and 1000 g/m<sup>2</sup> of *G. birdiae*; T2000: 100 shrimp/m<sup>2</sup> and 2000 g/m<sup>2</sup> of *G. birdiae*. Different letters indicate a significant difference between treatments according to the Tukey test ( $p < 0.05$ ).

On day 45 the monoculture treatment (T0) and the treatment with lower macroalgae density (T500) showed significantly higher TP concentrations when compared to the treatment with the highest density of *G. birdiae* (T2000). The mean TP concentration values in this period were 1.9, 1.8, 1.5, and 0.9 mg/L for T0, T500, T1000, and T2000, respectively. On day 60 of the experiment, T0 showed significantly higher concentrations of TP (2.9 mg/L) when compared to the TP concentration in the treatment with higher macroalgae density (T2000) (1.3 mg/L). In the same period, no difference was shown between T500 and T1000, with means of 2.5 and 2.2 mg/L, respectively. No significant differences were observed between the treatments after day 75 (Figure 4). The turbidity of the culture systems was probably from biological debris as well as the microalgae and other bacteria.

The absorption efficiency of the macroalgae was limited until day 60 of the cultivation for TN and from day 75 for TP, probably due to the increase in feed intake for the shrimp, which was rich in nitrogen and phosphorus, and the accumulation of these nutrients in plant tissue. Macroalgae have a high nutrient absorption rate in the first hours of exposure; the rate decreases as the nutrients accumulate in their tissues [27]. *G. birdiae* appeared to reduce TN and TP in the culture system, and more so at the end of the experiment, but no significant difference in these concentrations was shown between the treatment with no macroalgae and the polyculture treatments. This is perhaps due to the small increase in macroalgae biomass being insufficient to assimilate nutrients from the higher feed input that were proportional to the shrimp biomass.



**Figure 4.** Mean ( $\pm$ SD) of total phosphorus throughout the experimental period of 90 days. T0: shrimp monoculture (100 shrimp/m<sup>2</sup>); T500: 100 shrimp/m<sup>2</sup> and 500 g/m<sup>2</sup> of *G. birdiae*; T1000: 100 shrimp/m<sup>2</sup> and 1000 g/m<sup>2</sup> of *G. birdiae*; T2000: 100 shrimp/m<sup>2</sup> and 2000 g/m<sup>2</sup> of *G. birdiae*. Different letters indicate a significant difference between treatments according to the Tukey test ( $p < 0.05$ ).

#### 4. Conclusions

The growth and survival of *L. vannamei* marine shrimp are not affected in the integrated system with *G. birdiae*. However, a high density of *L. vannamei* during a complete shrimp production cycle can provide a large amount of particulate matter in the water column, increasing turbidity and consequently limiting the increase in *G. birdiae* biomass in the culture system and the ability to remove nutrients.

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