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Estimating nitrogen and phosphorus saturation point for *Eichhornia crassipes* (Mart.) Solms and *Salvinia molesta* Mitchell in mesocosms used to treating aquaculture effluent

Estimativa do ponto de saturação de nitrogênio e fósforo para *Eichhornia crassipes* (Mart.) Solms e *Salvinia molesta* Mitchell em mesocosmos utilizados para o tratamento de efluente de aquicultura

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Abstract: Aim: To evaluate the growth of *Eichhornia crassipes* (Mart.) Solms and *Salvinia molesta* Mitchell in tanks used for treating aquaculture effluent and compare the results with literature data in order to estimate the nutrients saturation point. **Methods**: An experiment with six rectangular fiberglass tanks were separated in two treatments, inflow and outflow (higher and lower nutrient concentration), and the two macrophytes above cited was carried out during 50 days. A floating quadrat with 0.25 m^2 of *E. crassipes* and *S. molesta* at inflow and outflow of the tanks was collected weekly for fresh mass measurement. At the beginning and end of the experiment samples of macrophytes were oven-dried at 60 °C until constant weight to determine the dry mass. Dry mass of plants was estimated by a simple linear regression analysis between fresh mass and dry mass (DM). **Results**: The N and P concentrations were significantly higher ($P<0.05$) in the inflow (mean of 0.66 mg L^{-1} and 233.6 µg L^{-1}, respectively) than in the outflow of the tanks (mean of 0.38 mg L^{-1} and 174.7 µg L^{-1}, respectively). However, no significantly different plant growth was observed for either higher or lower concentration. For both higher and lower nutrient concentrations, the biomass gain for *E. crassipes* was, respectively, 428.5 and 402.7 g DM.m^2. For *S. molesta*, biomass gain was 135.2 and 143.1 g DM.m^2, in the higher and lower concentrations, respectively. Others studies reported high growth of *E. crassipes* and *S. molesta* in concentrations of nitrogen (0.14 – 0.18 mg L^{-1}) and phosphorus (14.2 – 77.0 µg L^{-1}) lower than this study. **Conclusion**: The comparison of *E. crassipes* and *S. molesta* growth in this study with others allow us to assume that the saturation point of *E. crassipes* should be 0.26 mg L^{-1} of nitrogen and 77 µg L^{-1} of phosphorus and for *S. molesta* below 0.19 mg L^{-1} of nitrogen and 15.1 µg L^{-1} of phosphorus.

Keywords: aquatic macrophytes, nutrients, growth, freshwater prawn, effluents.

Resumo: Objetivo: Avaliar o crescimento de *Eichhornia crassipes* (Mart.) Solms e *Salvinia molesta* Mitchell em tanques utilizados para o tratamento de efluente de aquicultura e comparar os resultados com dados da literatura a fim de estimar o ponto de saturação de nutrientes. **Métodos**: Um experimento com seis tanques retangulares divididos em dois tratamentos, entrada e saída (maior e menor concentração de nutrientes, respectivamente), e as duas macrófitas aquáticas citadas acima foi realizado durante 50 dias. Um quadrante de 0,25 m^2 com amostras de *E. crassipes* e *S. molesta* da entrada e da saída dos tanques foi coletado semanalmente para estimar a massa fresca das macrófitas. No início e no final do experimento amostras das macrófitas foram secas em estufa a 60 °C até peso constante para determinar a massa seca. A massa seca das macrófitas foi estimada por regressão linear simples entre a massa fresca e a massa seca (MS). **Resultados**: As concentrações de N e P foram significativamente ($P<0.05$)

maiores na entrada (em média 0,66 mg L^{-1} e 233,6 μg L^{-1}, respectivamente) do que na saída dos tanques (em média 0,38 mg L^{-1} e 174,7 μg L^{-1}, respectivamente). Entretanto, não foi observada diferença significativa no crescimento das macrófitas cultivadas na maior e menor concentração de nutrientes. Na maior e menor concentração, o ganho de biomassa de *E. crassipes* foi, respectivamente, de 428,5 e 402,7 g MS.m^2. Para *S. molesta*, o ganho de biomassa foi de 135,2 e 143,1 g de MS.m^2, na entrada e saída respectivamente. Estudos já demostraram elevado crescimento de *E. crassipes* e *S. molesta* em concentrações de nitrogênio (0.14 – 0.18 mg L^{-1}) e fósforo (14.2 – 77.0 μg L^{-1}) menores que às deste estudo. **Conclusão**: A comparação do crescimento de *E. crassipes* e *S. molesta* observado neste estudo com os de outros nos permite assumir que o ponto saturação de nutrientes de *E. crassipes* deve estar abaixo de 0.26 mg L^{-1} de nitrogênio e 77 μg L^{-1} de fósforo; e de *S. molesta* pode estar abaixo de 0.19 mg L^{-1} de nitrogênio e 15.1 μg L^{-1} de fósforo.

Palavras chave: macrófitas aquáticas, nutrientes, crescimento, camarão de água doce, efluente.

1. Introduction

Several studies have demonstrated the important role of macrophytes in the structure and function of aquatic ecosystems (Wetzel, 2001; Chambers et al., 2008; Mormul et al., 2013). In fact, macrophytes play a vital role in primary production as an important food resource for aquatic and terrestrial organisms. These plants provide both living (grazing food webs) and dead organic (detritivorous food webs) matter, and affect other aquatic assemblages such as species diversity of attached organisms (Thomaz and Cunha, 2010), invertebrates (Mormul et al., 2010), macroinvertebrates (Batista-Silva et al., 2012), and fish diversity (Súarez et al., 2013). The aquatic macrophytes affect nutrient cycle through active and passive transfers of chemical elements from sediment to water (Camargo et al., 2003). The relatively slow decomposition of fibrous material of macrophytes (e.g., cellulose, lignin and hemicellulose) also contributes to the return of carbon to sediment (Bianchini Junior et al., 2008; Bianchini Junior et al., 2010; Thomaz and Esteves, 2011).

The primary production of the community of aquatic macrophytes in many environments can be greater than other aquatic primary producers (Wetzel, 2001; Kalff, 2002), especially in aquatic ecosystems with high availability of nutrients, large littoral regions and flood plains. Nutrients are heterogeneously distributed in natural habitats (Jackson and Caldwell, 1993; Gross et al., 1995); however, human activities have increased availability of nutrients in some aquatic ecosystems creating conditions to the macrophytes cover the surface of many lakes, reservoirs, rivers and ponds (Thomaz et al., 2003; Wilson et al., 2005). There are several examples of proliferation of some macrophytes species in aquatic environments impacted and non-impacted by organic pollution (Pieterse and Murphy, 1990; Thomaz et al., 2005; Camargo et al., 2006; Pitelli et al., 2008). A recent study showed that the growth of *Salvinia molesta* Mitchell was approximately 12 times higher in a reservoir impacted by aquaculture effluent (high concentration of nitrogen and phosphorus) than in a non-impacted reservoir (Pistori et al., 2010).

The growth rate of *Eichhornia crassipes* (Mart.) Solms is among the highest of any plant known (Gopal, 1987). *Eichhornia crassipes* outside its native range can grow quickly and reach very high densities (Julien et al., 1996). Although the macrophytes favor habitat complexity and heterogeneity in aquatic ecosystems (Cunha et al., 2012), their high growth rates associated with excess of nutrients from human activities impact negatively the environment and economic development by depleting oxygen in the water, interfering in the generation of electrical power, limiting the discharge capacity of lowland rivers, and obstructing the movement of boats (Vereecken et al., 2006; UNEP and GEAS, 2013).

Several authors have evaluated the growth of aquatic macrophytes under different nutrient concentrations and demonstrated that higher concentrations provide faster growth and higher final biomass (Bini et al., 1999; Camargo et al., 2003; Thomaz et al., 2007; Bianchini Junior et al., 2010). However, few studies have sought to assess the nutrient concentrations in which an aquatic macrophyte reaches maximum growth. The nutrient concentrations for maximum growth of an aquatic macrophyte may be estimated by the Michaelis-Menten function that determines the half-saturation constant that corresponds to the nutrient concentration where productivity is one-half of maximum productivity (Toerien et al., 1983; Davis and McDonnell, 1997; Carr et al., 1997). Recently, Thomaz et al. (2007) observed that the growth of the submerged macrophyte *Egeria najas*

Planch increased with increasing sediment nutrient up to certain concentrations of N and P. Above these concentrations, the authors reported no further plant growth. Nitrogen and phosphorus saturation points of aquatic macrophyte species, even those considered important aquatic weeds, are little known. Based on this information, we hypothesized that there is a nutrient saturation point, above which there is no growth of macrophytes. Therefore, the objective of this study was evaluated the growth of two free-floating species considered important aquatic weeds (*Eichhornia crassipes* and *Salvinia molesta*) in two concentrations (higher and lower nutrient concentrations) of nitrogen and phosphorus, and compare the results with literature data in order to estimate the saturation point.

2. Material and Methods

The experiment consisted of evaluating *Eichhornia crassipes* and *Salvinia molesta* growth by comparing two treatments with higher and lower nutrient concentrations with three replicates each. The experimental design scheme is shown in Figure 1. The experimental unit consisted of rectangular fiberglass tanks with capacity of 1.6 m^3 placed outdoors (Jaboticabal SP/Brazil 21° 15' 22" S and 48° 18' 48" W).

The experiment was conducted with continuous effluent input from a pond with a surface area of 193 m^2 and an average depth of 1.1 m (212.3 m^3). This pond was used to maintain the freshwater prawn *Macrobrachium rosenbergii* De Man, 1879 broodstock. The tanks used to grow the free-floating aquatic macrophytes were separated into two sections by wooden boards to prevent wind from moving the plants from one section to another. The section close to the inflow from the effluent pond (inflow water) presented higher nutrient concentration while the section close to the outflow of effluent (outflow water) had lower nutrient concentrations. The pond water effluent was transported by gravity until the tanks. The water flow rate in each tank was regulated to 2.0 – 2.2 L min^{-1}, controlled and adjusted by flow rate meters installed at the inlet of the tanks. Residence time was approximately 12 hours.

The macrophytes were collected from stream-preserved ecosystems of southern São Paulo State, Brazil (23° 50' - 24° 15' S and 46° 35'- 47° 00' W) and selected according to similar size and appearance. The macrophyte biomass was homogeneously

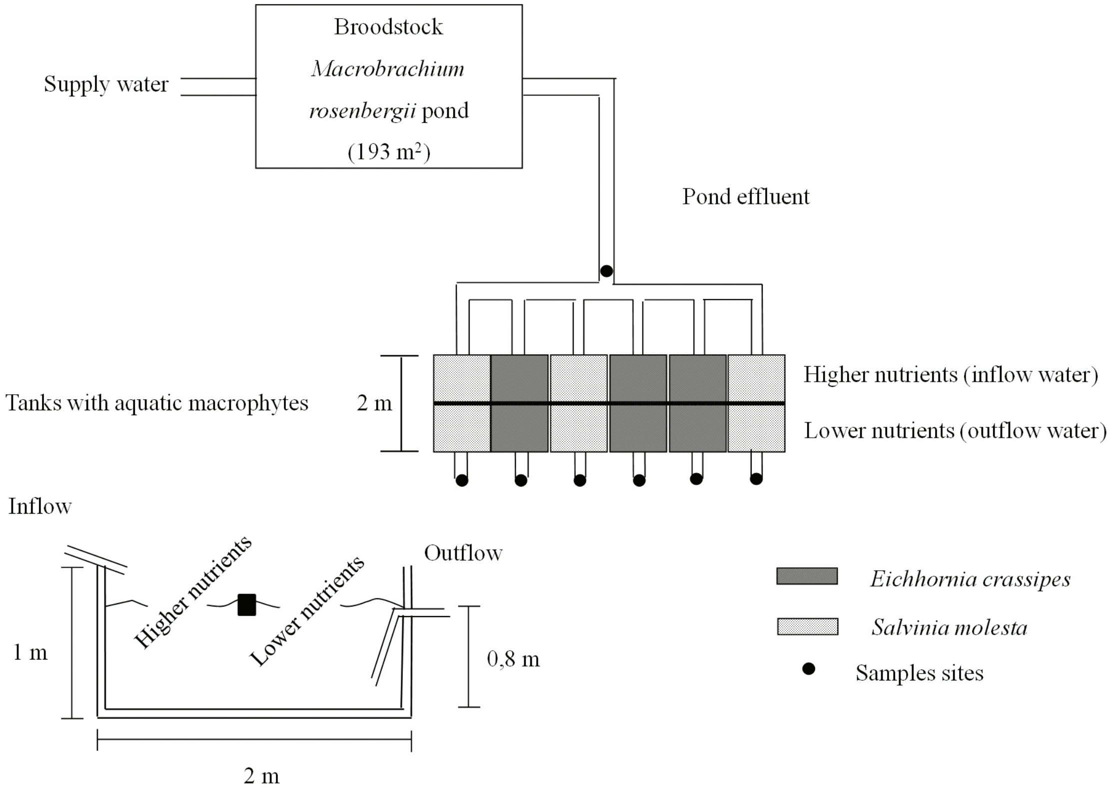

Figure 1. Schematic diagram of the experiment with free-floating aquatic macrophytes grown under different nitrogen and phosphorus concentrations.

distributed to cover approximately 80% of the tanks' surfaces to prevent phytoplankton growth. *Eichhornia crassipes* initial density was 13.5 kg fresh mass m^{-2} (0.669 kg dry mass m^{-2}) while for *S. molesta*, initial density was 3.7 kg fresh mass m^{-2} (0.180 kg dry mass m^{-2}).

The experiment lasted 50 days (from March to April, 2007). Inflow and outflow water samples from the tanks populated with *E. crassipes* (outflow-Ec) and *S. molesta* (outflow-Sm) were analyzed at 8, 29 and 50 days of experiment. In the laboratory, approximately 0.5 L of water was filtered to determine concentrations of ammonia nitrogen (NH_3-N) (Koroleff, 1976), nitrite (NO_2-N), nitrate (NO_3-N) (Mackereth et al., 1978) and orthophosphate (PO_4-P) (Golterman et al., 1978). The concentration of total inorganic nitrogen (TIN) was given by the sum of the inorganic forms of nitrogen (NH_3-N, NO_2–N and NO_3-N). The non-filtered samples were used to determine total Kjeldahl nitrogen (TKN) (Mackereth et al., 1978) and total phosphorus (TP) (Golterman et al., 1978).

A floating quadrat with 0.25 m^2 of *E. crassipes* and *S. molesta* at the tank inflow and outflow was collected at seven day intervals for measuring the fresh mass (FM, $g.m^{-2}$) and returned to the respective tanks. At the experiment beginning and end, a quadrat (0.25 m^2) of *E. crassipes* and *S. molesta* was also collected from each tank inlet and outlet to determine N and P concentrations. These samples were oven-dried at 60 °C until constant weight to determine the dry mass (g $DM.m^{-2}$). Subsequently, the plants were ground to determine total nitrogen (TN % DM) by the Kjeldahl method and total phosphorus (TP % DM) (Allen et al., 1974).

Plants dry mass ($g.m^{-2}$) was estimated by a simple linear regression analysis between fresh mass (FM) and dry mass (DM) masses.

Plant growth was determined by the biomass gain according to equation 1. For this, we used the highest value of biomass for both species of macrophytes, *E. crasssipes* and *S. molesta*.

$$G = DM_{(Hb)} - DM_{(i)} \tag{1}$$

where G = Growth; $DM_{(Hb)}$ = dry mass (*highest biomass* – g m^{-2}) and $DM_{(i)}$ = initial dry mass (g m^{-2}).

The stock of nitrogen and phosphorus (g of N or P m^2) in the macrophyte biomass was calculated according to equation 2:

$$S = \frac{DM.\%P}{100} \tag{2}$$

where S = stock; DM = dry mass (g) and %P = %P DM.

Nitrogen and phosphorus accumulated in the macrophyte biomass were determined by the difference between the amount of N and P accumulated in the highest biomass and the initial amount.

To evaluate the difference of nitrogen and phosphorus concentrations between the inflow and outflow of tanks, the data of total Kjeldahl nitrogen (TKN), total phosphorus (TP), total inorganic nitrogen (TIN) and P-orthophosphate (PO_4-P) were submitted to T-test for dependent samples. The same test was also applied to compare the growth (*G*) of *E. crassipes* and *S. molesta* at tank inlet and outlet; and to verify differences between N and P stocks is the macrophytes' biomass in the higher and lower nutrient concentrations.

3. Results

The T-test applied to nitrogen and phosphorus concentration data proved them significantly different between tank inflow and outflow. The TKN, TP, TIN and PO_4-P concentrations were significantly higher ($P<0.05$) in the inflow than in the outflow for the two aquatic macrophytes (Table 1 and 2). TKN and TIN concentrations were 1.9 times higher in the inflow than outflow of tanks with *E. crassipes*, whereas TP and PO_4-P concentrations were 1.5 and 2.8 times higher, respectively (Table 1). In the tanks with *S. molesta*, TKN and TIN concentrations were on average 1.6 and 1.3 times higher in the inflow compared

Table 1. Mean nitrogen and phosphorus concentrations in the inflow and outflow of tanks with *Eichhornia crassipes* resulting from the T-test.

Limnological variables	***Eichhornia crassipes***								
	Days of experiment								
	8			**29**			**50**		
	Inflow	**Outflow**	***P* value**	**Inflow**	**Outflow**	***P* value**	**Inflow**	**Outflow**	***P* value**
TKN (mg L^{-1})	0.69	0.37	0.008	0.61	0.42	0.005	0.68	0.26	0.006
TP (μg L^{-1})	235.7	222.0	0.044	225.0	127.3	<0.001	240.3	103.5	<0.001
TIN (μg L^{-1})	209.3	95.6	0.001	101.2	80.6	<0.001	198.7	80.2	0.001
PO_4-P (μg L^{-1})	60.9	20.0	0.009	64.0	15.7	<0.001	68.0	32.5	0.016

TKN = Total Kjeldahl nitrogen; TP = total phosphorus; TIN = Total inorganic nitrogen; PO_4-P = P-orthophosphate.

to outflow. TP and PO_4-P concentrations were 1.7 times higher in the inflow than outflow of the tanks populated with *S. molesta* (Table 2). Regarding to growth of macrophytes, no significant difference was observed ($P<0.05$) between higher and lower nutrient concentrations (Table 3).

Eichhornia crassipes growth curve showed a period of adaptation to the effluent condition at the beginning of the experiment. *Eichhornia crassipes* biomass peaked on day 43 (an average 1,076.7 g $DM.m^{-2}$) for both nutrients concentrations (Figure 2). *Salvinia molesta* biomass peaked on day 29 (303.2 ± 17.7 g $DM.m^{-2}$) in the higher and 36th days (320.8 ± 10.1 g $DM.m^{-2}$) in the lower nutrient concentrations. After these days *S. molesta* biomass decreased in both concentrations (Figure 2).

Nitrogen contents in *E. crassipes* biomass increased from 1.80% DM (first day) to 2.35% DM on the 43th day of experiment. Phosphorus contents increased from 0.56% DM (first day) to 0.60% DM. In the *S. molesta* biomass, the contents of nitrogen increased from 1.93% (first day) to 2.76% DM (at 29th day) in the inflow and to 2.54% DM in the outflow (at 36th day). The contents of phosphorus increased from 0.30% (first day) to 0.54% DM on the 29th day in the inflow and to 0.56% DM on the 36th day of experiment in the outflow.

At the beginning of the experiment, the nitrogen stock in *E. crassipes* and *S. molesta* biomasses was 11.3 ± 0.9 and 3.5 ± 0.04 g m^2, respectively. The initial phosphorus stock was 2.0 ± 0.1 and 0.56 ± 0.04 g m^2 in the *E. crassipes* and *S. molesta* biomasses, respectively. No significant difference ($P<0.05$) was observed between inflow and outflow for the N and P stock in the *E. crassipes* and *S. molesta* biomasses (Table 4).

Table 2. Mean nitrogen and phosphorus concentrations in the inflow and outflow of tanks with *Salvinia molesta* resulting from the T-test.

Limnological variables	*Salvinia molesta*								
	Days of experiment								
	8			29			50		
	Inflow	Outflow	*P* value	Inflow	Outflow	*P* value	Inflow	Outflow	*P* value
TKN (mg L^{-1})	0.69	0.41	0.003	0.61	0.45	0.049	0.68	0.36	0.049
TP (μg L^{-1})	235.7	213.5	0.017	225.0	182.2	0.037	240.3	199.6	0.011
TIN (μg L^{-1})	209.3	179.1	0.006	101.2	83.5	0.006	198.7	129.1	<0.001
PO_4-P (μg L^{-1})	60.9	24.8	0.011	64.0	42.4	0.012	68.0	44.4	0.045

TKN = Total Kjeldahl nitrogen; TP = total phosphorus; TIN = Total inorganic nitrogen; PO_4-P = P-orthophosphate.

Table 3. Mean biomass gain of plants in the inflow (higher nutrient concentrations) and outflow (lower nutrients concentrations) and T-test values. (n = 3; ± standard deviation).

Aquatic macrophyte	Biomass gain (g $DM.m^2$)		T-test *P* value
	Inflow	Outflow	
Eichhornia crassipes	428.5 ± 200.6	402.7 ± 65.6	0.83
Salvinia molesta	135.2 ± 13.7	143.1 ± 12.2	0.12

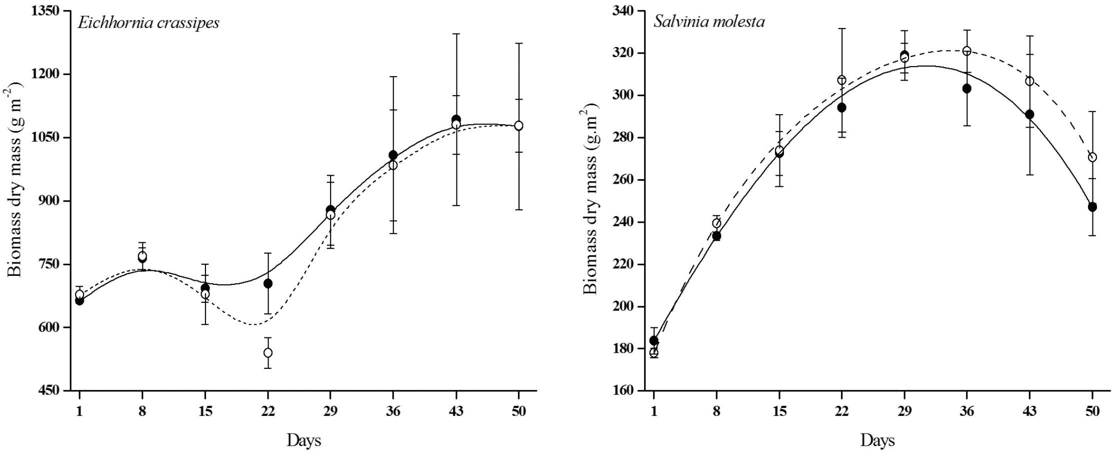

Figure 2. Growth curves of *Eichhornia crassipes* and *Salvinia molesta* in the higher (●) and lower (○) nutrient concentrations.

4. Discussion

The results show that *E. crassipes* and *S. molesta* growth was similar in inflow and outflow, since no significant difference was observed in the biomass gains for the different nutrient concentrations for either species. Even considering the small number of replicates (n = 3) and the large standard deviations we observe that the growth curves of the two species in higher and lower nutrient concentrations were similar. However, high nutrient concentrations allow the plants to grow faster and reach high biomass (Thomaz et al., 2007; Pistori et al., 2010). In a reservoir impacted by aquaculture effluent, Pistori et al. (2010) reported that *S. molesta* reached carrying capacity of 36.4 g DM.m^{-2} after 100 days of experiment, whereas in a non-impacted reservoir the value was 3.0 g DM.m^{-2} after 200 days. Other authors demonstrated that the growth rates of many species of aquatic macrophytes increase as nutrients availability also increases, but the increase of nutrients concentrations favor the growth of plants up to a certain concentration. For example, in a polluted river in Canada, Carr and Chambers (1998) observed a direct relationship between P concentrations in the sediment and the biomass of submerged macrophytes up to about 200 mg P.g^{-1}. Above this concentration there was no increase of plant biomass. *Egeria najas* relative growth rate increased with increasing sediment nutrients up to about of 100 mg P g.DM and 60 mg N g.DM, but above these concentrations Thomaz et al. (2007) reported no growth for this species. These findings support the hypothesis that there is a saturation point, above which there is no growth of macrophytes.

In our study the concentrations of nutrients in the tank outflow were higher than those observed in others studies, but this does not imply greater biomass gain of the two species. Henry-Silva et al. (2008) observed high biomass gain of *E. crassipes* (2,649.2 g DM.m^{-2}) and *S. molesta* (321.0 g DM.m^{-2}) in water with average TKN and TP concentrations 2.0 and 3.0 times lower than our study, respectively. On the other hand, in water with similar NKT (0.5 mg L^{-1}) and PT (287 µg L^{-1}) concentrations, Henry-Silva and Camargo (2005) reported *E. crassipes* biomass (736.7 g DM.m^{-2}) 1.5 times lower than our study. Therefore, the nutrient saturation point may be below the concentrations observed in this study, because high *E. crassipes* growth was also observed in environment with lower NKT (0.35 mg L^{-1}) and TP (77.0 µg L^{-1}) concentrations (Henry-Silva, 2001). Rubim and Camargo (2001) also observed high relative growth rate (0.11-0.20 g day^{-1}) of *S. molesta* in water with nutrient concentration of 14.2 µg L^{-1} TP and 0.14 mg L^{-1} TKN. Great biomass gain (173 g) was observed by Benassi and Camargo (2000) for *S. molesta* in an experiment that analyzed the competition process between this species and *Pistia stratiotes* L. in low nutrient concentrations (30.8 µg L^{-1} TP and 0.31 mg L^{-1} TKN). These results allow us to assume that nutrient concentrations that determine the maximum growth of *S. molesta* are lower than the concentrations that produce maximum growth of *E. crassipes*. When we compare the results (biomass gain and maximum biomass) of our study with others (Henry-Silva and Camargo, 2005; Henry-Silva et al, 2008; Benassi and Camargo, 2000), it is possible to verify greater differences. These differences can be due to the influence of variables other than nitrogen and phosphorus availability, like temperature and light.

Although, the existence of a saturation point can be possible, for *E. crassipes* this point for TP is under 103.5 µg L^{-1} and for TKN 0.26 mg L^{-1} (this study) and 77.0 µg L^{-1} for TP and 0.35 mg L^{-1} for TKN (Henry-Silva, 2001). For *S. molesta* the saturation point is under 199.6 for TP and 0.36 for NKT (this study) and 15.1 µg L^{-1} for TP and 0.19 mg L^{-1} for NKT (Henry-Silva et al., 2008). The N and P contents in the biomass of the two plants were not significantly (P>0.05) different between tanks' inflow and outflow. This data reinforces the existence of saturation point for nitrogen and phosphorus for aquatic macrophytes, because it suggests that plants absorb enough amounts of N and P to maximum growth.

In conclusion, probably there is a nutrient saturation point for *E. crassipes* and *S. molesta*, because these macrophytes grew similarly in the higher and the lower concentrations of nutrients.

Table 4. Mean of nitrogen (N) and phosphorus (P) stocks in the biomasses of aquatic macrophytes observed in the inflow and outflow of the tanks and *P* values (n = 3; ± standard deviation).

	Eichhornia crassipes		T-test *P* value	*Salvinia molesta*		T-test *P* value
	Inflow	Outflow		Inflow	Outflow	
N (g m^{-2})	16.2 ± 3.5	10.3 ± 1.9	0.17	5.2 ± 1.2	4.7 ± 0.6	0.55
P (g m^{-2})	3.9 ± 1.2	2.1 ± 0.14	0.36	1.15 ± 0.60	1.24 ± 0.30	0.84

However, the comparison of higher biomass of macrophytes in this study with studies where plants grew under lower nutrient concentrations, allow us to suppose that this saturation point for *E. crassipes* should be below 0.26 mg L^{-1} nitrogen and 77 µg L^{-1} phosphorus, and for *S. molesta* below 0.19 mg L^{-1} nitrogen and 15.1 µg L^{-1} phosphorus.

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