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PRIMARY RESEARCH PAPER

# Growth of free-floating aquatic macrophytes in different concentrations of nutrients

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**Abstract** The objective of the study was to evaluate the effect of different concentrations of nitrogen and phosphorus on the growth of the free-floating aquatic macrophytes *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia molesta*. The plants were cultured in 2,000-l outdoor concrete tanks. Triplicate tanks, with a continuous flow of effluent from culture ponds containing Nile tilapia, were used for each plant type ($n = 3$). The plant material was collected monthly from 0.25 $m^2$ floating quadrats, at the two ends of the tanks (higher nutrient concentrations near the inflow and lower nutrient concentrations near the outflow). In low nutrient concentrations, the maximum relative growth rates (RGRs) for *E. crassipes* (0.016/day) and *P. stratiotes* (0.016/day) were significantly lower ($P \leq 0.05$) than for *S. molesta* (0.029/day). There were no significant differences between the RGRs of *S. molesta* in the different nutrient concentrations. *Eichhornia crassipes* and *P. stratiotes* had their growth limited by nitrogen and phosphorus concentrations. The increase in plant density during the experiment probably also affected the growth of these species. In this context, *E. crassipes* and *P. stratiotes* can cause problems in nutrient-rich waterbodies, but under these experimental conditions their growth was limited by nitrogen and/or phosphorus concentrations. The growth of *S. molesta* was not influenced by the different nutrient concentrations.

**Keywords** *Eichhornia crassipes* · *Pistia stratiotes* · *Salvinia molesta* · Relative growth rate · Nitrogen · Phosphorus

## Introduction

The free-floating aquatic macrophytes *Eichhornia crassipes* (Mart.) Solms, *Pistia stratiotes* L., and *Salvinia molesta* (Mitchell) are natives of tropical America and have a largely pan-tropical distribution. These species are considered the most important aquatic weeds worldwide (Cook, 1990). In Brazil, several species of floating aquatic macrophytes are abundant and widely distributed, occurring in both polluted and non-polluted aquatic ecosystems (Henry-Silva & Camargo, 2005; Thomaz & Bini, 1998).

In many countries, these plants interfere with the utilization of water resources: blocking water flow in

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irrigation channels, depleting oxygen in the water, interfering with generation of electrical power, obstructing the movement of boats, and limiting the discharge capacity of lowland rivers (Pieterse & Murphy, 1990; Vereecken et al., 2006). On the other hand, aquatic macrophytes play an important role in the structure and function of the aquatic environment (Chambers et al., 2008; Engelhardt & Ritchie, 2001). Aquatic ecosystems with well-developed macrophyte communities tend to support more diverse communities of zooplankton (Lansac-Toha et al., 2003), benthic macro-invertebrates (Van den Berg et al., 1997), and fish (Pelicice et al., 2005).

*Eichhornia crassipes*, *P. stratiotes*, and *S. molesta* have high growth rates and can rapidly colonize aquatic ecosystems. However, their excessive growth is a result of human activities, which create favorable conditions for their development (Brendonck et al., 2003; Caffrey et al., 2006; Finlayson, 1984). The productivity of aquatic macrophytes varies as a function of environmental conditions such as temperature, light, pH, water velocity, salinity, organic matter, flood pulse, plant density, availability of nutrients, composition of bottom substrates, and competition processes (Camargo et al., 2003; Daniel et al., 2006; Henry-Silva & Camargo, 2005; Joye et al., 2006; Milne et al., 2006; Sand-Jensen, 1989; Sharma & Sridhar, 1989). In several countries, some species of floating aquatic macrophytes are used in constructed wetlands, because of their capacity to absorb and store large quantities of nutrients, and their rapid growth (Costa-Pierce, 1998; Henry-Silva & Camargo, 2006; Ran et al., 2004; Redding et al., 1997). Although growth rates of floating macrophytes are expected to be very high in tropical areas, there are few investigations comparing different species in the tropics (e.g., Camargo et al., 2006; Thomaz et al., 2006).

Undesirable growth of *E. crassipes*, *P. stratiotes*, and *S. molesta* in many tropical aquatic ecosystems of different continents, impeding many uses of aquatic resources, has often been reported in recent decades (Adams et al., 2002; Bini et al., 1999; Fitzsimons & Vallejos, 1986; Mansor, 1996; Mbati & Neuenschwander, 2005). In this context, understanding the abiotic variables that influence the growth of these free-floating aquatic macrophytes is important, especially in regions in which these species are native and where there is a lack of studies.

In this study, we evaluated the effects of different concentrations of nitrogen and phosphorus on the growth of three free-floating species, *E. crassipes*, *P. stratiotes*, and *S. molesta,* under tropical climate conditions, in Brazil.

## Materials and methods

The study period extended from December 1999 to April 2000 (summer and autumn), at the Tropical Fish Research Center, Brazil (21°55′46″ S; 47°22′ 24″ W). *Eichhornia crassipes*, *P. stratiotes*, and *S. molesta* were grown in 2,000-l outdoor concrete tanks (4 $m^2$ surface area), under different nutrient concentrations. Eighty percent of the tank surfaces were covered with plants, to shade the water column and prevent the growth of phytoplankton that would compete for nutrients. The aquatic macrophytes were collected in lotic ecosystems of southern São Paulo State, Brazil (24°11′ S; 46°48′ W), and selected for similar size and appearance. Triplicate tanks, with a continuous flow of effluent from Nile tilapia culture ponds, were used for each plant species ($n = 3$). The two ends of the tanks (high and low nutrient concentrations) were separated by a net to prevent the wind from moving individuals from one end to the other (Fig. 1).

The plant material was collected monthly from 0.25 $m^2$ floating quadrats, at the two ends of the tanks (higher nutrient concentrations near the inflow and lower nutrient concentrations near the outflow), for measurement of the fresh mass (FM), and was then returned to the tanks. Initial densities used in each tank to cover 80% of the surface were *E. crassipes* 5,500 g FM/$m^2$ (238 g dry mass/$m^2$), *P. stratiotes* 3,700 g FM/$m^2$ (154 g dry mass/$m^2$), and *S. molesta* 3,370 g FM/$m^2$ (200 g dry mass/$m^2$).

The relative growth rates (RGRs) of the plants were determined from the equation of Mitchell & Tur (1975) as:

$$\mathrm{RGR} = (\ln x_2 - \ln x_1)/(T_2 - T_1).$$

where $x_1$ and $x_2$ are the dry mass (g) at times $T_1$ and $T_2$, respectively.

Inflow and outflow water samples were obtained and analyzed monthly. Water temperature was measured with a Horiba U-10 Water Quality Checker. Total Kjeldahl nitrogen, nitrate nitrogen, and nitrite

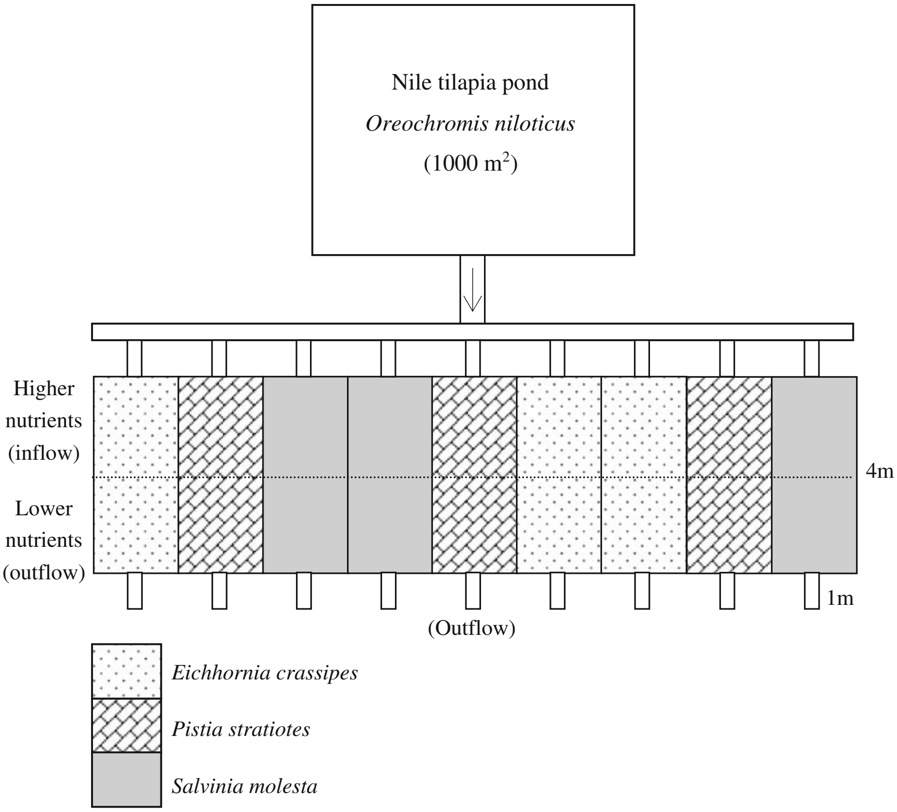

**Fig. 1** Schematic diagram of the experiment with three species of aquatic macrophytes grown at two nutrient levels

nitrogen were analyzed following the method of Mackereth et al. (1978). Ammonia nitrogen was determined according to Koroleff (1976). Total and dissolved phosphorus were determined according to Golterman et al. (1978).

ANOVA with repeated measures with subsequent mean separation by Tukey's honest significant difference test was applied to identify significant differences ($P < 0.05$) among RGRs of each species in the inflow (high nutrient concentrations) and outflow (low nutrient concentrations) and in different time periods (30, 60, 90 and 120 days). A *t*-test was applied in order to assess significant differences ($P \leq 0.05$) in nutrient concentrations and temperature in the inflow and outflow. Dry mass (DM) of aquatic macrophytes was estimated by a simple linear regression between fresh ($x$) and dry ($y$) mass of subsamples before incubation. A simple linear regression between DM and RGRs of each species grown in high and low nutrient concentrations was applied to assess the effect of plant density on growth of aquatic macrophytes.

## Results

Significantly higher concentrations of different forms of N and P were recorded in the inflow than in the outflow water of all the tanks. For example, the mean concentrations of total phosphorus in the first month of the experiment in inflow and outflow water were 45.6 and 8.9 $\mu g\ l^{-1}$, respectively. Water temperatures remained high (21.0–26.6°C) throughout the experimental period (Table 1).

The macrophyte species responded differently to nutrient treatments. The growth curves of the three species indicated differences in the increase of biomass for *E. crassipes* and *P. stratiotes* in different concentrations of the organic and inorganic forms of nitrogen and phosphorus (Fig. 2). Contrarily, *S. molesta* grew similarly under different nutrient contents (Fig. 2). *E. crassipes* gained more biomass than did *P. stratiotes* and *S. molesta*. At the end of the experiment, the total biomasses of *E. crassipes* (2887.2 g DM/m$^2$) and *P. stratiotes* (1005.7 g DM/m$^2$), grown in high nutrient concentrations, were

**Table 1** Means of total Kjeldahl nitrogen, total phosphorus, dissolved phosphorus, ammonia nitrogen and nitrite nitrogen contents, and temperature in inflow (high NP) and outflow (low NP) water

| Variable | Month 1 | | Month 2 | | Month 3 | | Month 4 | |
|---|---|---|---|---|---|---|---|---|
| | High NP | Low NP | High NP | Low NP | High NP | Low NP | High NP | Low NP |
| Total Kjeldahl nitrogen (mg $l^{-1}$) | 0.30 (a) | 0.24 (b) | 0.30 (a) | 0.16 (b) | 0.41 (a) | 0.18 (b) | 0.35 (a) | 0.16 (b) |
| Total phosphorus (μg $l^{-1}$) | 45.6 (a) | 8.9 (b) | 69.9 (a) | 20.9 (b) | 86.1 (a) | 12.2 (b) | 106.6 (a) | 18.2 (b) |
| Dissolved phosphorus (μg $l^{-1}$) | 17.6 (a) | 3.6 (b) | 39.2 (a) | 13.5 (b) | 31.6 (a) | 8.6 (b) | 32.2 (a) | 7.1 (b) |
| Ammonia nitrogen (μg $l^{-1}$) | 7.9 (a) | 4.7 (b) | 4.4 (a) | 2.2 (b) | 9.5 (a) | 2.9 (b) | 18.3 (a) | 7.2 (b) |
| Nitrite nitrogen (μg $l^{-1}$) | 7.2 (a) | 6.5 (b) | 8.5 (a) | 5.0 (b) | 12.4 (a) | 9.3 (b) | 12.3 (a) | 4.3 (b) |
| Nitrate nitrogen (μg $l^{-1}$) | 36.6 (a) | 22.6 (b) | 29.0 (a) | 18.9 (b) | 62.6 (a) | 15.9 (b) | 92.7 (a) | 25.7 (b) |
| Temperature (°C) | 26.1 (a) | 25.5 (a) | 26.6 (a) | 25.9 (a) | 26.0 (a) | 25.7 (a) | 21.5 (a) | 21.0 (a) |

Different letters indicate significantly different means ($P \leq 0.05$). Comparisons were made within the same month and individually for each variable

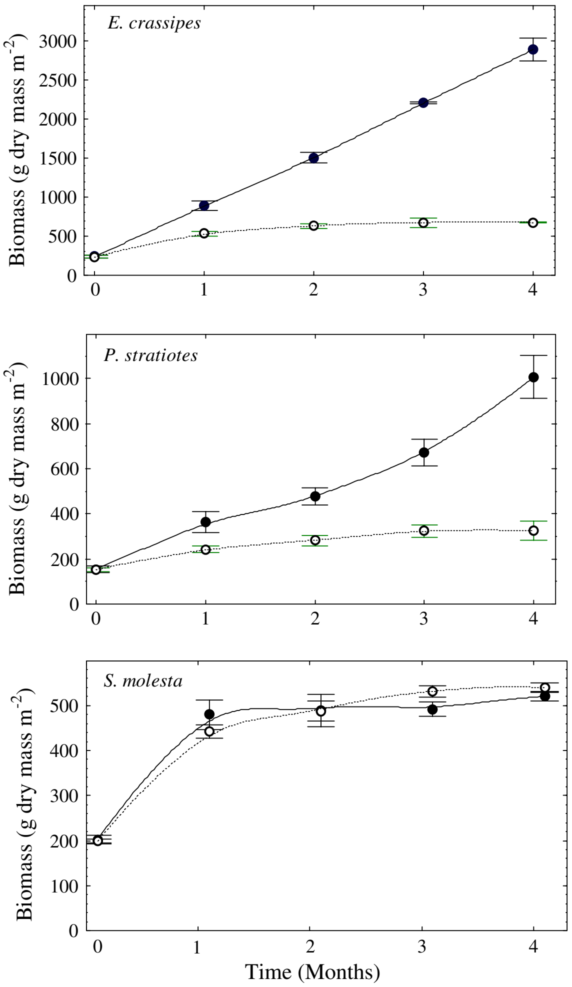

**Fig. 2** Growth curves of three species of aquatic macrophytes grown in high (●) and low (○) concentrations of N and P

higher than the total biomasses of the two species grown in low nutrient concentrations (590.2 and 324.7 g DM/m$^2$, respectively). The total biomass of *S. molesta* at the end of the experiment did not differ between the two concentration levels of the different forms of nitrogen and phosphorus (Fig. 2).

The ANOVA with repeated measures indicated significant differences between the growth rate of *E. crassipes* ($F = 435.69$; $P < 0.01$) and *P. stratiotes* ($F = 1788.39$; $P < 0.01$) at the two nutrient concentrations (Table 2). In addition, there were significant differences of RGR values between time periods (month) for both species ($F = 45.61$, $P < 0.01$ for *E. crassipes* and $F = 33.86$, $P < 0.01$ for *P. stratiotes*). In all months, RGRs were significantly higher (for both species) in high than in low nutrient concentrations (Tables 3 and 4). However, there was no significant difference between the RGRs of *S. molesta* grown in different nutrient concentrations ($F = 0.14$, $P = 0.72$). The RGRs for this species were significantly higher in the first month, in both nutrient concentrations ($F = 442.14$, $P = < 0.01$) (Table 5).

The highest RGRs of *E. crassipes* (0.025/day), *P. stratiotes* (0.031/day), and *S. molesta* (0.031/day) were observed at the beginning of the experiment (month 1), when plant densities were lower and nutrient concentrations in the water were higher. In both nutrient concentrations, when densities of the three species increased, RGR values decreased. In higher densities, *E. crassipes* (2,207–2,887 g DM/m$^2$), *P. stratiotes* (670–1,005 g DM/m$^2$), and *S. molesta* (491–521 g

**Table 2** Repeated measures ANOVA applied to relative growth rate of the *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia molesta* grown in high and low nutrient concentrations (treatment) and in four different time periods (month)

| Factors | df | MS | *F* | *P* |
|---|---|---|---|---|
| *E. crassipes* | | | | |
| Treatment | 1 | <0.01 | 435.69 | <0.01 |
| Month | 3 | <0.01 | 45.61 | <0.01 |
| Treatment × month | 3 | <0.01 | 0.92 | 0.46 |
| Error | 12 | <0.01 | | |
| *P. stratiotes* | | | | |
| Treatment | 1 | <0.01 | 1788.39 | <0.01 |
| Month | 3 | <0.01 | 33.86 | <0.01 |
| Treatment × month | 3 | <0.01 | 2.82 | 0.08 |
| Error | 12 | <0.01 | | |
| *S. molesta* | | | | |
| Treatment | 1 | <0.01 | 0.14 | 0.72 |
| Month | 3 | <0.01 | 442.14 | <0.01 |
| Treatment × month | 3 | <0.01 | 3.37 | 0.06 |
| Error | 12 | <0.01 | | |

**Table 3** Relative growth rates (RGR, day$^{-1}$) of *Eichhornia crassipes* grown in high and low concentrations of nitrogen (N) and phosphorus (P)

| Month | *Eichhornia crassipes* (RGR) | |
|---|---|---|
| | High NP | Low NP |
| 1 | 0.025 ± 0.001 (a) | 0.016 ± 0.001 (b,c) |
| 2 | 0.017 ± 0.003 (b) | 0.006 ± 0.004 (d,e) |
| 3 | 0.013 ± 0.002 (b,c) | 0.002 ± 0.002 (e) |
| 4 | 0.009 ± 0.002 (c,d) | 0.0002 ± 0.003 (e) |

Different letters indicate significantly different means ($P \leq 0.05$)

**Table 4** Relative growth rates (RGR, day$^{-1}$) of *Pistia stratiotes* grown in high and low concentrations of nitrogen (N) and phosphorus (P)

| Month | *Pistia stratiotes* (RGR) | |
|---|---|---|
| | High NP | Low NP |
| 1 | 0.031 ± 0.003 (a) | 0.016 ± 0.004 (b) |
| 2 | 0.009 ± 0.004 (bc) | 0.004 ± 0.002 (c) |
| 3 | 0.011 ± 0.002 (b) | 0.005 ± 0.003 (c) |
| 4 | 0.014 ± 0.003 (b) | 0.0004 ± 0.002 (c) |

Different letters indicate significantly different means ($P \leq 0.05$)

**Table 5** Relative growth rates (RGR, day$^{-1}$) of *Salvinia molesta* cultured in high and low concentrations of nitrogen (N) and phosphorus (P)

| Month | *Salvinia molesta* (RGR) | |
|---|---|---|
| | High NP | Low NP |
| 1 | 0.031 ± 0.004 (a) | 0.029 ± 0.001 (a) |
| 2 | 0.001 ± 0.001 (b) | 0.003 ± 0.001 (b) |
| 3 | 0.001 ± 0.001 (b) | 0.003 ± 0.001 (b) |
| 4 | 0.002 ± 0.001 (b) | 0.001 ± 0.001 (b) |

Different letters indicate significantly different means ($P \leq 0.05$)

DM/m$^2$) showed, respectively, RGRs of 0.009/day, 0.014/day, and 0.002/day (Fig. 3).

## Discussion

The growth curves of the three species in the two different concentrations of nutrients showed that nitrogen and phosphorus individually or nitrogen and phosphorus together limited the growth of *E. crassipes* and *P. stratiotes*. The growth of *S. molesta* was not limited by the nutrient concentrations used in this experiment. The use of mesocosms provided controlled conditions to test hypotheses and hence improve our understanding of the factors limiting the growth of aquatic macrophytes. In fact, few studies on aquatic macrophyte ecology have been undertaken in natural habitats, and relatively little is known about how these plants respond to management measures in natural conditions (Camargo et al., 2003; Thomaz et al., 2006).

Temperature is an important variable that influences the growth of aquatic macrophytes (Sand-Jensen, 1989). Temperatures above 30°C and below 15°C limit the growth of *S. molesta,* and *Eichhornia crassipes* and *P. stratiotes* do not tolerate temperatures above 35°C (Holm et al., 1977; Usha Rani & Bhambie, 1983). In the present study, water temperatures varied between 21.0 and 26.7°C and probably did not limit the RGRs of the three species.

Our results are congruent with some observations in natural tropical habitats. Junk & Piedade (1997), in a study of the ecology of aquatic macrophytes on the Amazon floodplain, demonstrated that *S. molesta* showed similar growth rates in blackwater (nutrient-poor) and whitewater (nutrient-rich) environments.

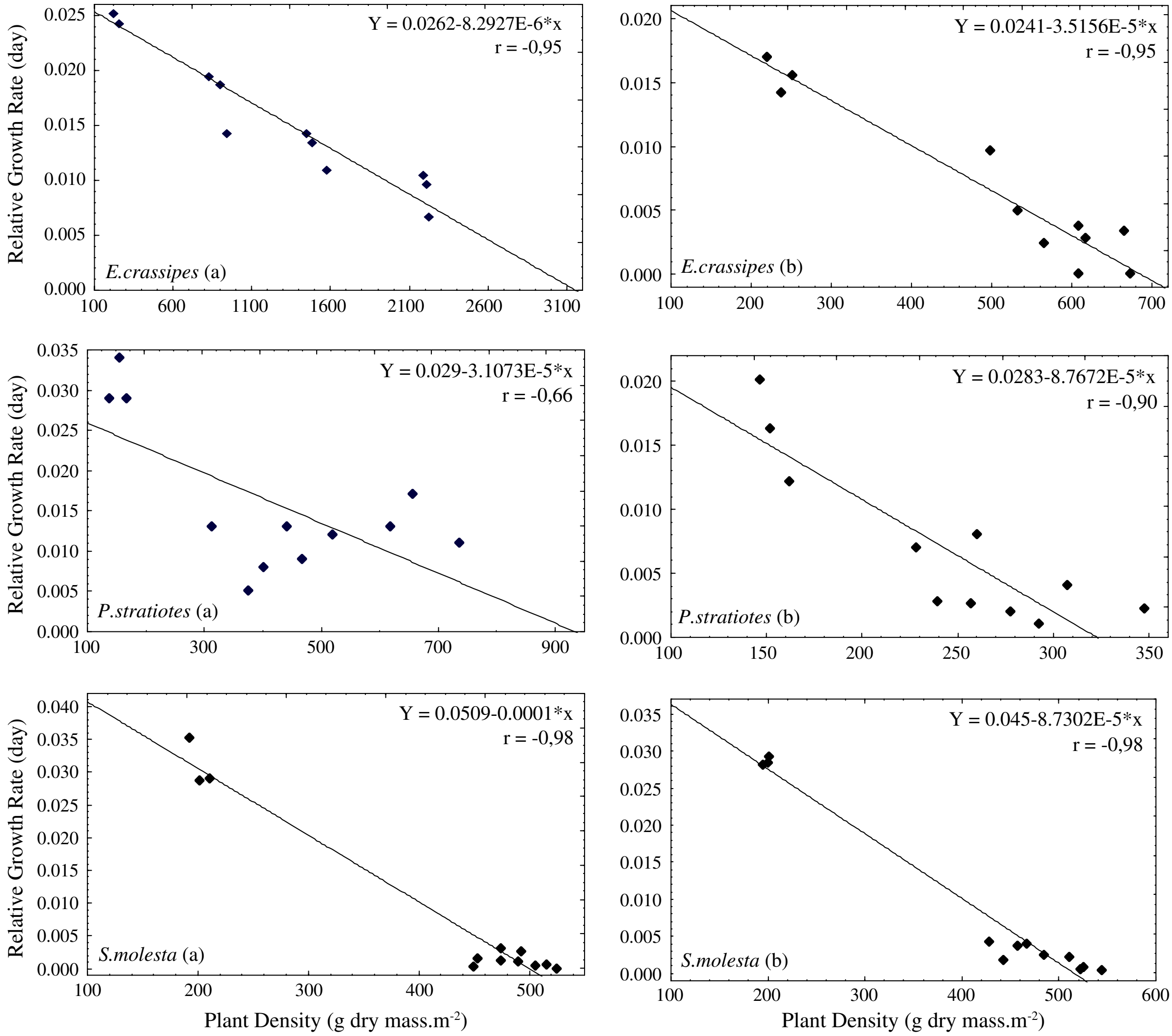

**Fig. 3** Effect of plant density on relative growth rate of *Eichhornia crassipes*, *Pistia stratiotes*, and *Salvinia molesta* (**a**) high nutrient concentrations; (**b**) low nutrient concentrations)

These authors also observed that *E. crassipes* and *P. stratiotes* showed monthly weight losses of up to 35% in water with low nutrient concentrations. These results indicated that these two species grow more slowly when nutrient concentrations are low, and the mortality rate may be higher than the growth rate. Thomaz et al. (2006) observed that in Itaipu Reservoir, a succession process occurred during the growth phase of floating macrophytes, with initial dominance by *Salvinia herzogii*. The authors concluded that the initial rapid growth of *S. herzogii* may be associated with its habit of horizontal growth.

The values of RGR of the three free-floating aquatic macrophytes in our experiment were similarly high in the first month in higher nutrient concentrations, indicating the nuisance potential of these species. However, there were no significant differences between the RGR of *S. molesta* in higher and lower nutrient concentrations, indicating that this species is probably capable of rapid growth in ecosystems with lower nitrogen and phosphorus concentrations. Rubim & Camargo (2001) also observed high growth rates (0.11–0.20/day) of *S. molesta* in water with nutrient concentrations of

14.2 µg/l total phosphorus and 0.14 mg/l total Kjeldahl nitrogen.

Although the three species had similar RGRs, the biomass at the end of the experiment in higher nutrient concentrations was very different for each species. The results showed that *E. crassipes* can potentially yield more biomass than *P. stratiotes* and *S. molesta*, when grown in higher nutrient concentrations. Probably, the higher biomass of *E. crassipes* in relation to *P. stratiotes* and *S. molesta* is due to the capacity of *E. crassipes* to grow vertically especially in higher densities (Henry-Silva & Camargo, 2006; Sale et al., 1985). Probably, the lower concentrations of nitrogen and phosphorus limit the biomass of *E. crassipes* and *P. stratiotes*. For *S. molesta* the biomass at the end of the experiment was similar in both nutrient concentrations, indicating that the higher or lower nutrient concentrations did not favor or limit its growth. Taheruzzaman & Kushari (1988) showed that the production rate of *E. crassipes* in the Ganges River was positively correlated with nitrogen and phosphorus concentrations.

The increase of plant densities during the experiment was another factor that affected the growth of the three species, but particularly for *S. molesta*, because this species reached the maximum biomass in the first month of the experiment, in both concentrations of nitrogen and phosphorus. Results obtained by Reddy & DeBusk (1984) also showed a decrease in RGR of *E. crassipes* and *P. stratiotes* as a function of an increase in plant density. The results for density of aquatic macrophytes obtained in this study indicate that the use of this variable alone did not furnish precise information about weed problems. In fact, the maximum biomass of *S. molesta* was 500 g DW/m$^2$ and that of *E. crassipes* was 3,000 g DW/m$^2$, but the RGR of *S. molesta* in both nutrient concentrations was higher than for *E. crassipes* in higher nutrient concentrations. Moreover, *S. molesta* had an RGR of zero with a density of 500 g DW/m$^2$ and *E. crassipes* had an RGR of 0.010 with a density between 2,000 and 2,500 g DW/m$^2$. Therefore, it is important to analyze both values of biomass per square meter (plant density) and growth rate, in order to obtain a more accurate view of the infestation potential of aquatic macrophytes.

We conclude that *E. crassipes* and *P. stratiotes* can cause problems in nutrient-rich waterbodies, but under these experimental conditions, their growth was limited by the lower nitrogen and/or phosphorus concentrations. On the other hand, the growth of *S. molesta* was not influenced by the different nutrient concentrations, and thus this species might be the first to cover the water surface during infestations in tropical aquatic ecosystems, even in conditions of low nutrient availability.

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