

# Relative Growth Rate and Doubling Time of the Submerged Aquatic Macrophyte *Egeria densa* Planch.

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**ABSTRACT:** Relative growth rate and doubling time of the submerged aquatic macrophyte *Egeria densa* Planch. *Egeria densa* is a species native of the South America that has been introduced in other continents. Its invasive capacity causes the reduction or elimination of native species of certain water bodies. In Brazil, *E. densa* has been growing abundantly in reservoirs, and having adverse effects on the hydroelectric power generation. The objective of this work was to determine the relative growth rate (RGR) and the doubling time (DT) of *E. densa* in laboratory experiment. Every 4 days, during 44 days, the length of shoots was measured. Along the experiment, physical and chemical variables of water and nitrogen and phosphorus concentrations from sediment were measured. The length of shoots was transformed in dry mass using a linear regression model between these two variables. In the firstly 20 days there are a rapid increase in dry mass (gain of 0.065 g) and between 20 and 44 days a slightly increase (gain of 0.025 g). Significant differences of RGR of *E. densa* were recorded between 4 days periods ( $F=31.46$ ,  $p<0.001$ ). The values of RGR were significantly higher in the period of 8-12 days ( $RGR=0.063 \text{ day}^{-1}$  and  $DT=12$  days) when compared with the values of the other periods. During the last period of experiment (20-44 days) we observed the minor values of RGR ( $0.009$  to  $0.016 \text{ day}^{-1}$ ) and highest DT values (90 to 194 days). This species growth in water with low availability of inorganic forms of nitrogen and phosphorus, and probably, the gradual reduction of the RGR of *E. densa* during the time is due to the increase of biomass and density. Our data showed that *E. densa* have higher growth capacity, similar to free-floating species considered some of most aquatic weed species of the world.

**Key-Words:** *Egeria densa*, relative growth rate, doubling time, submerged aquatic macrophyte.

**RESUMO:** Taxa de crescimento relativo e tempo de duplicação da macrófita aquática submersa *Egeria densa* Planch. *Egeria densa* é uma espécie nativa da América do Sul e tem sido introduzida em outros continentes. A rápida capacidade de colonização desta espécie tem causado a redução ou eliminação de espécies nativas em alguns corpos d'água. No Brasil *E. densa* tem crescido abundantemente em represas, causando prejuízos à geração de energia elétrica. O objetivo deste trabalho é determinar a taxa de crescimento relativo (TCR) e o tempo de duplicação (TD) de *E. densa* em um experimento de laboratório. A cada 4 dias durante 44 dias, foi medido o comprimento de ramos apicais. Durante o experimento foram medidas variáveis físicas e químicas da água e as concentrações de nitrogênio e fósforo do sedimento. O comprimento dos ramos foi transformado em peso seco utilizando uma equação de regressão linear. Nos primeiros 20 dias observou-se um rápido aumento de biomassa seca (ganho de 0,065 g) e entre 20 e 44 dias um aumento moderado (ganho de 0,025 g). Diferenças significativas da TCR de *E. densa* foram registradas entre os períodos de 4 dias ( $F=31,46$ ;  $p<0,001$ ). Os valores de TCR foram significativamente maiores no período de 8-12 dias ( $TCR=0,063 \text{ dia}^{-1}$  e  $TD=12$  dias) quando comparado com os valores dos outros períodos. Nos últimos períodos do experimento (20-44 dias) foram observados os menores valores de TCR ( $0,009$  a  $0,016 \text{ dia}^{-1}$ ) e os maiores valores de TD (90 a 194 dias). Esta espécie pode crescer em água com pequena disponibilidade de nitrogênio e fósforo inorgânico e, provavelmente a redução da TCR de *E. densa* ao longo do experimento foi devido ao aumento da biomassa e da densidade. Os resultados demonstraram que *E. densa* tem grande capacidade de crescimento, similar a espécies flutuantes livres consideradas daninhas em várias regiões do mundo.

**Palavras-chave:** *Egeria densa*, taxa de crescimento, tempo de duplicação, macrófita aquática submersa.

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## Introduction

The excessive growth of aquatic macrophytes is a result of human activities, which create favourable conditions for their growth (Seshavatharam, 1990). The biological and physiological characteristics of the species that enable them to exploit the aquatic environment in an opportunistic way, growing and reproducing in sub-optimal conditions, even when in competition with other species (Spencer & Bowes, 1990). The submerged species that cause most problems (affecting navigation, hydroelectric power generation, water supply, recreation and flood control) in United States are *Hydrilla verticillata* (L.f.) Royle, *Myriophyllum spicatum* L. and *Egeria densa* Planch, know as Brazilian elodea (Madsen et al., 1998).

The traditional methods of control (chemical, mechanical and biological) of aquatic macrophytes, frequently have low efficiency due to the lack or few knowledge about their ecology (Thomaz & Bini, 1999). Accordingly to Cary & Weerts (1983) for the development of efficient methods of control is necessary to know the growth rate of aquatic macrophytes in different environmental conditions. Many authors (Seshavatharam, 1990; Khedr & EL-Demersdach, 1997; Bini et al., 1999; Lenssen et al., 1999; Maine et al., 1999) reported that various environmental factors, such as, transparency, depth, temperature, type of sediment and availability of nutrients in water, acting together to determine the growth of aquatic macrophytes.

*Egeria densa* is a submerged rooted aquatic macrophyte, native of the South America, that has been introduced in other continents and frequently can rapidly occupy water bodies and interfere with the use of water resources, especially in North America and New Zealand (Ashton & Mitchell, 1989). Its invasive capacity causes the reduction or elimination of native species of certain water bodies (Coffey & Clayton, 1987; Haramoto & Ikusima, 1988; Nakanishi et al., 1989; Tanner et al., 1990a, 1990b). In Brazil *E. densa* had been growing abundantly in reservoirs, and so having adverse effects on the hydroelectric power generation (Thomaz et al., 1999; Barreto et al., 2000).

The objective of this work was to determine the relative growth rate and the doubling time of *E. densa* in laboratory conditions, in an aquarium with water and sediment from the natural habitat of the species.

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## Material and methods

*E. densa* was collected in River Branco (Itanhaém River Basin, South Coast of São Paulo State, Brazil) and transported to the Laboratory of Aquatic Ecology (UNESP-Rio Claro). Fifteen apical shoots of ca. 6.0 cm length were placed in the aquarium (60 cm length x 30 cm width x 40 cm height) with 52 L of water from the river and cultivated in individual pots with sediment of the stands. Every 4 days (except in day 28), during 44 days in summer (February/March), the length of the shoots were measured and the values of temperature (°C), pH, electrical conductivity (mS.m<sup>-1</sup>), turbidity (NTU) and dissolved oxygen (mg.L<sup>-1</sup>) were obtained with a water quality checker (Horiba, U-10). Photosynthetically-active radiation (PAR) (μmol.m<sup>-2</sup>.s<sup>-1</sup>) was measured with a LiCor light meter (Model 189) coupled to an underwater LiCor quantum sensor (Model 192) at 0.0 and 0.2 m, and the coefficient of vertical attenuation (k) was calculated according Wetzel (1975).

To transform the growth in length of *E. densa* into dry mass, others 95 shoots between 2 and 38 cm, in quadruplicate, was dried (60° C) until constant mass. Using a linear regression between these two variables (DM=0.0196+0.0047 \* BL, where DM = dry mass and BL = shoot length; R<sup>2</sup>=64.9%) the dry mass of the 15 shoots cultivated in the aquarium was calculated. The relative growth rate (RGR) was calculated using the following equation described in Jackson (1980):

$$RGR = (\ln M_2 - \ln M_1) / (T_2 - T_1)$$

Where, RGR = relative growth rate; T = time (days); M<sub>1</sub> = the dry mass of the shoot at T<sub>1</sub>; M<sub>2</sub> = the dry mass of the shoot at T<sub>2</sub>.

The doubling time was obtained using the formula described by Mitchell (1974):

$$DT = \ln(2)/RGR,$$

Where DT = doubling time.

Water samples were collected (triplicate) in the beginning and at the end of the experiment. Concentrations of ammoniacal nitrogen (ammoniacal-N;  $\text{mg.L}^{-1}$ ) (Koroleff 1976), nitrite ( $\text{NO}_2^- \text{ N}$ ;  $\text{mg.L}^{-1}$ ), nitrate ( $\text{NO}_3^- \text{ N}$ ;  $\text{mg.L}^{-1}$ ), total dissolved N (TDN;  $\text{mg.L}^{-1}$ ) were determined according to Mackereth et al. (1978). Total dissolved P (TDP;  $\text{mg.L}^{-1}$ ) and orthophosphates ( $\text{PO}_4^- \text{ P}$ ;  $\text{mg.L}^{-1}$ ) were determined by the method described in Golterman et al. (1978), using filtered water samples (Whatman GF/C filter). The non-filtered water samples were used to determine total nitrogen (TN;  $\text{mg.L}^{-1}$ ) (Mackereth et al., 1978) and total phosphorus (TP;  $\text{mg.L}^{-1}$ ) concentrations (Golterman et al., 1978). In the beginning of the experiment, concentrations of total nitrogen (TN; %DM) in sediment were determined using Kjeldahl method (Allen et al., 1974) and total phosphorus (TP; %DM) according Esteves (1980). At the end of the experiment, the sediment of the 15 pots with *E. densa* were mixed to determine TN and TP concentrations.

The analysis of variance (ANOVA-one way) was used in order to check for significant differences ( $p < 0.05$ ) in RGR between 4 days periods (Statistica, 2000). A Tukey post-hoc test was used for mean-separation with statistically-significant relationships.

## Results

Fig. 1 shows the growth curve (dry mass) of *E. densa* during the 44 days of the experiment. In the firstly 20 days there are a rapid increase in dry mass (gain of 0.065 g) and between 20 and 44 days a slightly increase (gain of 0.025 g). The analysis of variance indicated significant differences of RGR of *E. densa* between 4 days periods ( $F=31.46$ ,  $p < 0.001$ ).

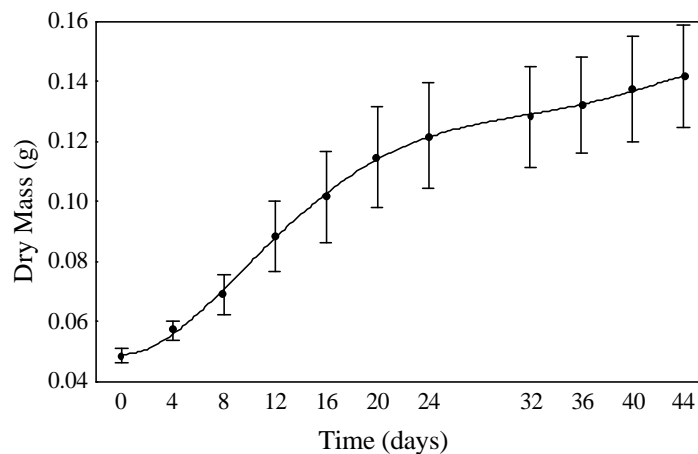


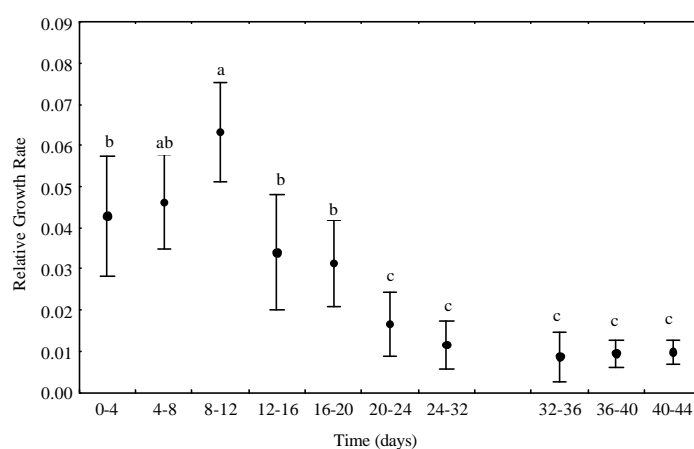
Figure 1: Growth curve of *E. densa* during the 44 days of experiment. Bars indicate standard error of dry mass of the shoots.

The values of RGR were significantly higher in the period of 8-12 days ( $RGR=0.063 \text{ day}^{-1}$  and  $DT=12 \text{ days}$ ) when compared with the values of the others periods. Intermediary values of RGR and DT were obtained in the periods of 0-4, 4-8, 12-16 and 16-20 days. During the last period of experiment (20-44 days) we observed the minor values of RGR ( $0.009$  to  $0.016 \text{ day}^{-1}$ ) and highest DT values (90 to 194 days) (Fig. 2).

Tab. I shows mean values and standard deviation of the physical and chemical variables of the water. The water temperature variation (range  $27.5 - 29.5^\circ \text{ C}$ ) was small during the experimental period. Electrical conductivity oscillated between  $4.3$  and  $6.3 \text{ mS.m}^{-1}$  and the coefficient of vertical attenuation ( $k$ ) between  $4.9$  and  $7.5 \text{ m}^{-1}$ . The mean PAR values at the surface was  $123.3$  and at the bottom  $36.7 \text{ mmol.m}^{-2}.\text{s}^{-1}$ .

**Table I: Mean and standard deviation of physical and chemical variables of water.**

Variables	Mean $\pm$ SD
pH	7.50 $\pm$ 0.85
Conductivity (mS.m <sup>-1</sup> )	5.30 $\pm$ 1.02
Turbidity (NTU)	8.40 $\pm$ 4.36
DO (mg.L <sup>-1</sup> )	9.80 $\pm$ 1.05
Temperature (°C)	28.40 $\pm$ 1.08
PAR – surface (mmol.m <sup>-2</sup> .s <sup>-1</sup> )	123.30 $\pm$ 4.39
PAR – bottom (mmol.m <sup>-2</sup> .s <sup>-1</sup> )	36.70 $\pm$ 2.96
K (m <sup>-1</sup> )	6.23 $\pm$ 1.30



**Figure 2: Mean and standard error of the relative growth rate of *E. densa* in the different periods (intervals of 4 days) along the experiment. Different letters indicate significant differences ( $p < 0.05$ ) among periods.**

The concentration of dissolved N and dissolved P in the water were similar at the beginning and at the end of experiment (Tab. II). The higher variation was obtained for total phosphorus (17.8 to 38.7 mg.L<sup>-1</sup>) at the beginning and at the end of the experiment, respectively. The values of total P in the sediment showed little differences between the beginning and end of experiment (mean of 0.022 %P).

**Table II: Concentrations of the different forms of nitrogen and phosphorus of water and sediment in the beginning and at the end of the experiment.**

Variables	beginning	end
NO <sub>2</sub> -N (mg.L <sup>-1</sup> )	6.23	11.23
NO <sub>3</sub> -N (mg.L <sup>-1</sup> )	77.55	55.00
NH <sub>4</sub> -N (mg.L <sup>-1</sup> )	18.52	3.15
DN (mg.L <sup>-1</sup> )	0.13	0.15
TN (mg.L <sup>-1</sup> )	0.16	0.24
PO <sub>4</sub> -P (mg.L <sup>-1</sup> )	3.49	4.90
DP (mg.L <sup>-1</sup> )	17.18	19.96
TP (mg.L <sup>-1</sup> )	20.87	33.69
P-sediment (% DM)	0.02	0.03
N-sediment (% DM)	0.40	0.48

## Discussion

The values of RGR were significantly different during the time of experimentation, higher at the beginning and lower at the end. Although the concentrations of nitrogen and phosphorus in the water were low, submerged aquatic macrophytes in general take nutrients from the sediment (Carpenter & Lodge, 1986; Barko & Carpenter, 1991) and the contents of N and P in the sediment do not decrease at the end of the experiment. The results also indicate that *E. densa* growth in water with low availability of inorganic forms of nitrogen and phosphorus. The lower range of temperature, probably do not limit the growth, because *E. densa* is native of tropical region (Cook & Urmi-Konig, 1984) and adapted to high water temperatures. Light controls the growth of submerged aquatic macrophytes (Camargo et al., 2003). However, Pezzato (2002) in a laboratory experiment do not observed reduction on photosynthetic rate of *E. densa* between 36.7 and 123.3  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of photosynthetic-active radiation. Probably the gradual reduction of the RGR of *E. densa* at the end of experiment is due to the increase of biomass and density. In fact, at the beginning the mean shoot biomass was of 0.05 g DM (mean length = 6.0 cm) and the total biomass 0.75 g DM/ aquarium. At the end, the mean shoot biomass was of 0.14 g DM (mean length = 16.8 cm) and the total biomass 2.1 g DM/ aquarium. Henry-Silva et al. (2002) in an experiment of growth of free-floating aquatic macrophytes observed that the increase of plant densities reduce the growth. As plants grow they occupy more space and interfere in the growth of other by the access to resources (Silverston & Doust, 1993). Hence, in order to know the growth potential of a species of aquatic macrophyte, it is important to measure the growth rate in low density, because in high densities the growth rate approximate to zero. Density of *E. densa* in the experimental conditions was always low. In Itaipu Reservoir (Brazil-Paraguay) Thomaz et al. (1999) obtained biomass values varying from 98 to 166  $\text{gDM}\cdot\text{m}^{-2}$  and in River Branco (Itanhaém River Basin, São Paulo State) Camargo & Esteves (1995) measure mean values of 157  $\text{gDM}\cdot\text{m}^{-2}$ .

The values of RGR and DT obtained in the beginning of the experiment show that *E. densa* can grow quickly in low density and in similar condition of free-floating weed species. Tab. III presents a comparison of some values of RGR and DT of *Salvinia molesta*, *Eichhornia crassipes*, and *Pistia stratiotes* and *E. densa*. These three free-floating species are considered some of most aquatic weed species of the world (Pieterse & Murphy 1990) due to their capacity of growth quickly. The RGR ( $0.063\text{ day}^{-1}$ ) and DT (12 days) of *E. densa* are similar to *E. crassipes* and *S. molesta*. Although Esteves (1998) reported that submerged species present low production when compared with emergent and free-floating, our data show that *E. densa* have higher growth capacity.

In conclusion, higher growth capacity of *E. densa* favours it to occupy large areas in short time. When the environmental characteristics are favourable, growth of this species can cause problems to the multiple uses of some aquatic ecosystems.

Table III: Values of relative growth rate (RGR) and doubling time (DT) of different species of aquatic macrophytes. F = field, L = laboratory experiment.

Species	RGR	DT	Local	Authors
<i>S. molesta</i>	0.059	8.6	F	Mitchell & Tur (1975)
<i>S. molesta</i>	0.5	1.4	F	Finlayson (1984)
<i>S. molesta</i>	0.20 - 0.11	3.5 - 7.1	F	Rubim & Camargo (2001)
<i>S. molesta</i>	0.07 - 0.01	9.9 - 69.3	L	Usha Rani & Bhambie (1983)
<i>S. molesta</i>	0.031 - 0.01	22.4 - 69.3	L	Henry - Silva et al (2002)
<i>E. crassipes</i>	0.025 - 0	27.7	L	Henry - Silva et al (2002)
<i>E. crassipes</i>	0.06 - 0.01	11.6 - 69.3	L	Reddy & DeBusk (1984)
<i>P. stratiotes</i>	0.18 - 0.003	3.9 - 231	L	Reddy & DeBusk (1984)
<i>P. stratiotes</i>	0.031 - 0	22.4	L	Henry - Silva et al (2002)
<i>E. densa</i>	0.063 - 0.009	12 - 194	L	This study

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