

USE OF GRASS CARP (*Ctenopharyngodon idella*) AS A BIOLOGICAL CONTROL AGENT FOR SUBMERGED AQUATIC MACROPHYTES¹

Utilização da Carpa-capim (Ctenopharyngodon idella) como Agente de Controle Biológico de Macrófitas Aquáticas Submersas

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ABSTRACT - This study aimed to evaluate feed preference and control efficacy of grass carp (*Ctenopharyngodon idella*) on the aquatic macrophytes *Ceratophyllum demersum*, *Egeria densa* and *Egeria najas*. An experiment was carried out at mesocosms conditions with 2,000 liters capacity and water residence time of 2.8 days. *C. demersum*, *E. densa* e *E. najas* biomasses were offered individually with sixty g and coupled in similar quantities of 30 g of each species, evaluated during 81 days, involving 6 treatments. (1 - *C. demersum*, 2 - *E. najas*, 3 - *E. densa*, 4 - *C. demersum* + *E. najas*, 5 - *C. demersum* + *E. densa* and 6 - *E. najas* + *E. densa*). When offered individually, *E. najas* and *C. demersum* presented the same predation rate by grass carp, which was higher than *E. densa* predation rate. When plants were tested in pairs, the order of feed preference was *C. demersum* > *E. najas* > *E. densa*. *E. najas* and *C. demersum* percentage control ranged from 73 to 83%. No relation between biomass consumption and grass carp body weight gain was observed, probably due to differences in nutritional quality among macrophyte species according to fish necessities. Therefore, it is concluded that the use of grass carp is one excellent technique to control submersed macrophytes in Brazil.

Keywords: feed preference, *Egeria najas*, *Ceratophyllum demersum*, *Egeria densa*.

RESUMO - O objetivo deste estudo foi avaliar a preferência alimentar e a eficácia da carpa-capim (*Ctenopharyngodon idella*) no controle das macrófitas aquáticas submersas ***Ceratophyllum demersum*, *Egeria densa* e *Egeria najas***. Foram instalados mesocosmos de 2.000 L, com tempo de residência da água de 2,8 dias. As macrófitas foram oferecidas individualmente: ***C. demersum*, *E. densa* e *E. najas*** com 60 gramas de biomassa e associadas duas a duas em quantidades iguais de 30 g de biomassa de cada espécie durante 81 dias, com total de seis tratamentos (1 - ***C. demersum***, 2 - ***E. najas***, 3 - ***E. densa***, 4 - ***C. demersum* + *E. najas***, 5 - ***C. demersum* + *E. densa*** e 6 - ***E. najas* + *E. densa***). Quando ofertada isoladamente, a carpa-capim consumiu quantidades similares as de ***E. najas*** e de ***C. demersum*** e menor quantidade de ***E. densa***. Quando oferecida em conjunto, a ordem de preferência alimentar pela carpa-capim estabelecida foi: ***C. demersum* > *E. najas* > *E. densa***. O controle de ***E. najas*** e ***C. demersum*** variou entre 73 e 83%. Não foi observada relação entre o consumo de biomassa e ganho de peso pela carpa-capim, provavelmente devido a diferenças na qualidade nutricional das plantas em relação às necessidades do peixe. Com isso, conclui-se que a carpa-capim é uma excelente técnica para controle de macrófitas aquáticas submersas no Brasil.

Palavras-chave: controle, *Egeria najas*, *Ceratophyllum demersum*, *Egeria densa*.

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INTRODUCTION

Macrophytes are important components in water bodies, playing a fundamental role in energy and carbon storage at the bases of food pyramids, promoting spatial and temporal heterogeneity, which favor biodiversity, acting as protection and reproduction refuges for many organisms, as well as young fish, and its' submerged parts allow the development of periphyton communities, which are fundamental as food sources for fish fingerlings, tadpoles and others (Pitelli, 1998). In water collections under strong anthropogenic influence some plant species are favored, while others are disadvantaged, often resulting in large and dense poorly diversified communities which causes damages to the environment and the multiple uses of the water bodies (Pitelli, 1998; Pompeo, 2008). In this situation, a need of control is established (Corrêa et al., 2003).

Submerged macrophyte began to cause serious problems in Brazilian hydropower reservoirs after the 80's, specifically with *Egeria densa*, *Egeria najas* and *Ceratophyllum demersum* (Rocha & Martins, 2011). *Ceratophyllum demersum* is a submerged macrophyte from Ceratophyllaceae family and is native to tropical America. This plant is propagated by seeds and mainly by stem fragmentation, forming dense populations, hampering the water flow and affecting other water uses as well (Cross et al., 2003). *Egeria densa* e *E. najas* belong to Hydrocharitaceae family and are also native of South America. *Egeria densa* is found in almost all tropical and subtropical regions, having been introduced as an ornamental plant for aquarium (Kahara & Vermaat, 2003; Pelicice & Agostinho, 2006). *Egeria najas* has not been reported as an important invader in other parts of the world, but is quite frequent in hydroelectric reservoirs in Brazil (Rocha & Martins, 2011).

The control of submerged aquatic weeds can be accomplished through (1) physical control by simple manual collect, by shading, with barriers and by changing the water level (Pompêo, 2008); (2) mechanical control with boats, dredgers and treadmill (Pompêo, 2008); (3) chemical control, by applying herbicides

based on copper, imazamox, diquat, fluridone and penoxulam (Gettys et al., 2009); and (4) biological control through the use of plant pathogens (Cuda et al., 2008), snails (Cowie, 2001), insects (Junjiao et al., 2010) and fish (Miyazaki & Pitelli, 2003; Gettys et al., 2009).

Among fishes, grass carp (*C. idella*) has been the most researched and used organism in biological control of submersed aquatic weeds. This fish is native from the large rivers of Asian east coast, located between 20 and 50 degrees of latitude north and 100 to 140 degrees of longitude east (Fisher & Lyakhnovich, 1973). This fish was introduced in Europe, America and Africa for aquatic plants control and for fish production through polyculture (Schoonbee, 1991; Opuszynski & Schireman, 1995; Catarino et al., 1997; Cudmore & Mandrak, 2004; Hermes et al., 2007).

At the beginning of this process, there were concerns about grass carp natural reproduction which could turning this organism into an exotic invader, and also with the introduction of parasites and diseases of other species of fish (Sutton, 1986). However, this fish spawns and reproduces in large, turbid and turbulent rivers with sideline lagoons highly vegetated and under the influence of some abiotic factors mentioned by Stanley et al. (1978) and Cyrino et al. (2004).

Therefore, grass carp natural reproduction is limited to a few water bodies that must be frequently found only in its site of origin. Grass carp prefers submersed aquatic plants without long fibers and tender tissues, such as filamentous algae and plants from Lemnaceae family. The more fibrous emerging plants and the ones with toxic compounds are rejected (Gettys et al., 2009). Grass carp consumes only the edges of the leaves or the younger tissues of the less preferred species. There are some plants which are not consumed, but only tasted (Stanley et al., 1978). By using grass, submerged macrophytes kept their populations stable and under control.

Other organisms with potential for aquatic weeds biocontrol agents are: *Eiccritotarsus catarinesis* for *Eichhornia crassipes* (Stanley & Julien, 1999); *Piaractus mesopotamicus* for *C. demersum*, *E. densa*



and *E. najas* (Miyazaki & Pitelli, 2003); *Mycolectodiscus terrestris* for *Hydrilla verticillata* (Shearer & Jakson, 2006); *Agasicles hygrophila* for *Alternanthera phyloxeoides* and *A. sessilis* (Junjiao et al., 2010); and *Cyrtobagous salviniae* for *Salvinia molesta* (Sullivan et al., 2011).

However, beyond the large potential control area due to its movement in the water body, grass carp use as a control agent is widespread worldwide. Therefore, this project aimed to evaluate feeding preference and consumption effectiveness of diploid grass carp on plants of *C. demersum*, *E. densa* and *E. najas* offered individually and combined in pairs. This study is justified as a previous analysis of grass carp feeding behavior on these weed species important in Brazil, where they are native and, theoretically, have the most efficient defense mechanisms against predators.

MATERIAL AND METHODS

The trials were conducted at Paulista State University (UNESP), campus of Jaboticabal, Sao Paulo State. Tests were conducted in conditions of 2,000 liters mesocosms, supplied with water cistern pit, with 2.8 days residence time, during 81 days and starting date at August 18, 2011. The submersed macrophytes *Egeria najas*, *Ceratophyllum demersum* and *Egeria densa* were grown in sufficient quantity to perform the whole study.

The animals, purchased at Santa Candida fish farming, were 4.80 ± 0.46 cm long and weighted 4.47 ± 0.62 g. Fish were distributed in number of fifteen individuals ($n = 15$) per mesocosm.

Before offering the macrophytes, the fishes were kept in the mesocosms for one week and fed during the first four days with commercial food for carp. The floating macrophyte *Lemna* sp. was provided on the last three days as food until satiation.

The experimental was conducted in a randomized block design (RBD) with three replications. The treatments were: (1) *C. demersum* (CERDE), (2) *E. densa* (ELLDE), (3) *E. najas* (ELDNA), (4) CERDE + ELLDE, (5) CERDE + ELDNA and (6) ELLDE + ELDNA. In treatments with isolated plants, 60 g of

biomass were offered in each tank. When tanks were supplied with two species, 30 g of each plant were added. Treatments 4, 5 and 6, based on combined plants, were divided into subplots for a better elucidation, as follows: CERDE/D and ELLDE/C for treatment 4 (CERDE + ELLDE); CERDE/N and ELDNA/C for treatment 5 (CERDE + ELDNA); and ELLDE/N and ELDNA/D for treatment 6 (ELLDE + ELDNA).

Evaluations were performed every three days (72 hours) during the experimental period. After the plants biomasses were supplied (SB), they remained in the tank for 72 hours. At the end of this period, the biomass was again removed and evaluated for the remaining biomass (RB) and then discarded. A new patch of macrophytes biomass (always similar to the previous one) was then available to fish for a new foraging period.

The amount of aquatic macrophytes consumed was calculated by the difference between the biomass offered and the biomass remaining: $CB = SB - RB$ (g), where CB = consumed biomass, SB = supplied biomass, RB = remaining biomass.

The percentage of macrophyte control (%C) exerted by *C. idella* was calculated by the following formula: $\%C = CB/SB * 100$.

Other performance indicators of submersed aquatic macrophytes biological control were also calculated:

- Macrophyte biomass consumed per *C. idella* individual (BCI)

$$BCI = CB / n^{\circ} \text{ of fishes per mesocosms}$$

- Macrophyte biomass consumed per initial fish biomass allocated in the mesocosm (CBPI)

$$CBPI = CB / \text{initial fish biomass allocated in the mesocosm}$$

- Gain in total length (GTL)

$$GTL = \text{final length} - \text{initial length}$$

- Gain in total biomass (GTB)

$$GTB = \text{final biomass} - \text{initial biomass}$$

- Percentage of length gain (GLP)

$$GLP = GTL * 100 / \text{final length}$$



- Percentage of biomass gain (GBP)

$$GBP = GTB * 100 / \text{final biomass}$$

Data were subjected to variance analysis with F test at 5% probability ($p < 0.05$), and the average values compared using Fisher's statistical test. All statistical data were analysed using the Statistica 5.0 software.

RESULTS AND DISCUSSION

The descending order of cumulative consumption per mesocosm during the trial period for grass carp was ELDNA (1241.92 g), CERDE (1209.12 g), ELLDE (880.3 g), CERDE/N (672.29 g), ELDNA/D, (625.82 g), CERDE/D (622.65 g), ELDNA/C (590.57 g), ELLDE/N (422.99 g) and ELLDE/C (300.09 g) (Figure 1A). These results demonstrate that, when plants were offered isolated, similar *E. najas* and *C. demersum* consumption occurred, which was higher than *E. densa* consumption.

When *C. demersum* and *E. najas* were offered at the same time, the Ceratophyllaceae consumption was higher (672.3 g for CERDE/N and 590.57 g for ELDNA/C). However, both have been more consumed when given together with *E. densa*. In mesocosms with *E. najas* and *E. densa*, the consumption was 625.82 g for ELDNA/D and 422.99 g for ELLDE/N. In situations where *C. demersum* and *E. densa* were supplied, the consumption was 622.65 g for CERDE/D and 300.09 g for ELLDE/C.

Concerning the remaining biomass variable, it was found that the cumulative values for ELLDE (739.70 g) was the highest, followed by ELLDE/C (498.80 g), CERDE (410.88 g), ELLDE/N (387.01 g), ELDNA (379.26 g), ELDNA/C (215.59 g), CERDE/D (176.24 g), ELDNA/D (176.24 g) and CERDE/N (133.87 g) (Figure 1B).

Results from treatments where two species were given at the same time showed

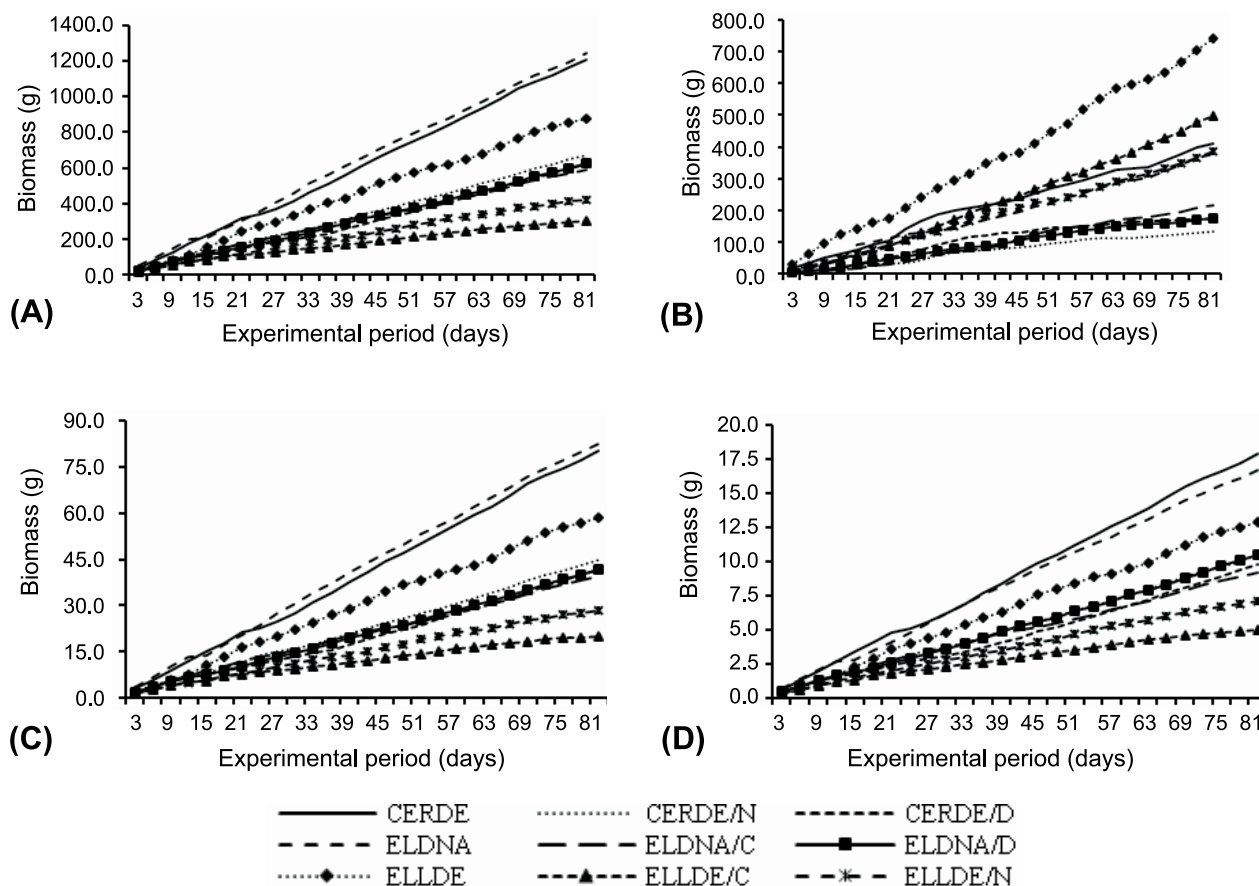


Figure 1 - Accumulated value per mesocosmos of macrophytes consumption (A), remained biomass (B), individual biomass consumption (C) and biomass consumption per initial fish weight (D).



that the grass carp's food preference is: *C. demersum* > *E. najas* > *E. densa*, at the experimental conditions used. These results disagree with those obtained by Cassani & Caton (1983), Pine & Anderson (1991) and Cudmore & Mandrak (2004), that showed a higher preference for *E. densa* compared to *C. demersum*, but are nevertheless similar to the work realized in Brazil by Miyazaki & Pitelli (2003), evaluating young pacu individuals (*Piaractus mesopotamicus*). The low *E. densa* predation in this study may be due to the fact that the fish are young and have small and weak pharyngeal teeth, preferring immature plant parts and tender plants. Under the conditions these studies were conducted, *C. demersum* plants showed a higher growth of shoots when compared to *E. densa*

The highest values of individual accumulated consumption were for ELDNA (82.79 g), CERDE (80.61 g) and ELLDE (58.69 g), and the lowest for ELLDE/C (20.01 g), ELLDE/N (28.20 g) and ELDNA/C (39.37 g) (Figure 1C). For the variable accumulated biomass consumption per fish initial weight, the highest values were obtained for CERDE (17.95 g), ELDNA (16.73 g) and ELLDE (12.86 g), and the lowest for ELLDE/C (4.97 g), ELLDE/N (7.06 g) and ELDNA/C (9.19 g) (Figure 1D).

Considering the individual consumption and the consumption by unit of fish weight at the beginning of the experimental period

(Figure 1B), the results are similar to those of total consumption, because the allocated fish size was similar in all experimental plots. There were small inversions, as for example, the consumption of *C. demersum* was a little higher than *E. najas* when offered isolated.

Beyond harming the water multiple uses, extensive populations of submerged macrophytes compromise its quality; as an example, we have the decline in natural mixing of the surface layer, increasing the temperature during summer and interfering with the gas dynamics of the water column (oxygen and carbon dioxide) which causes unpleasant odors and high mosquitoes populations (Gettys et al., 2009).

Concerning the remaining macrophytes biomass average (spares), the highest was ELLDE (27.40 g) and the lowest CERDE/N (4.96 g); for consumption, the highest average value was 44.78 g (CERDE), and the lowest, 11.11 g (ELLDE/C) (Table 1). The highest plant consumption per individual was in treatment ELDNA (3.07 g) and the lowest in ELLDE/C (0.74 g) (Table 1).

The highest average value of consumption biomass per fish initial weight was CERDE (0.66 g), and the lowest was ELLDE/C (0.18 g). Also, the highest control obtained was for CERDE/N (83.00%) and the lowest for ELLDE/C (37.05%) (Table 1).

Table 1 - Remained biomass, biomass consumption, consumption by individual, average biomass consumption values per initial fish weight and *C. demersum*, *E. najas* and *E. densa* on the percentage control of macrophytes offered individually and in pairs after 81 days of experimental period

Treatment	Remained biomass (g)	Consumption (g)	Consump.by individual (g)	Consump. by initial fish weight (g)	Control (%)
CERDE	15.22 b	44.78 a	2.99 a	0.66 a	74.64 ab
CERDE/N	4.96 c	24.90 c	1.66 c	0.39 bc	83.00 a
CERDE/D	6.53 c	23.06 c	1.54 c	0.36 cd	76.87 ab
ELDNA	14.05 b	46.00 a	3.07 a	0.62 a	76.66 ab
ELDNA/C	7.98 c	21.87 c	1.46 c	0.34 cd	72.91 b
ELDNA/D	6.43 c	23.18 c	1.55 c	0.39 bc	77.26 ab
ELDDE	27.40 a	32.60 b	2.17 b	0.48 b	54.34 c
ELLDE/C	18.47 b	11.11 d	0.74 d	0.18 e	37.05 d
ELLDE/N	14.33 b	15.67 d	1.04 d	0.26 de	52.22 c
F	13.30	46.04	46.04	17.28	23.56
p	<0.05	<0.05	<0.05	<0.05	<0.05

P values below 0.05 indicate that there was significance in treatments comparison by F Test. Averages followed by the same letter do not statistically differ among themselves by Fisher's statistical test.



Results show that, statistically, *C. demersum* and *E. najas* consumptions per mesocosm were similar and higher than *E. densa*, as it occurred per individual and per unit of fish initial weight. These comparisons are possible because in the three conditions 60 g of biomass were provided per mesocosm. When plants were offered in pairs, the amount of biomass for each species was 30 g only and these treatments only are being compared in the following discussion.

E. najas and *C. demersum* consumption values were similar ($p < 0.05$) when provided with either other two other species, but higher than *E. densa* consumption. There was no difference on *E. densa* consumption when offered with either one of the two other species.

The control *C. demersum* provided by the grass carp ($p < 0.05$) was statistically similar in any given situation. The *E. najas* control was similar ($p < 0.05$) to *C. demersum* when provided alone or with *E. densa*. However, comparing the three treatments with *E. najas*, there was no statistical difference in its percentage of control.

E. densa control was much lower when compared with the other species and negatively influenced when exposed along with *C. demersum* and when associated to *E. najas*, once the fish had a choice, which reduced the control value in the less preferred species.

The biological control of submersed aquatic plants is a practice that requires a rigorous risk/benefit analysis, due to the great importance of these plants in the aquatic environment dynamics, and the risk of predation of non-target plants (Milstein, 1992). The total removal of the aquatic vegetation generally results in changes on water quality, since the grass carp consumes much of the surrounding vegetation, causing changes in the preexisting flora and fauna composition (Pípalová, 2002; Gettys et al., 2009). This risk justifies further studies, relating fish biomass to aquatic weeds consumption and also the relative preference among non-target species.

Santos et al. (2006) indicated that the feeding of *Plagioscion squamosissimus* fish was

75% with cinnamon shrimp (*Macrobrachium amazonicum*) in environments with macrophytes presence, but in environments without macrophyte, there was 100% of *M. amazonicum* consumption in the diet. This observation corroborates the fact that macrophytes in a balanced environment play a fundamental role, promoting spatial and temporal heterogeneity, and serving as habitat and refuge for many organisms (Pitelli, 1998).

The increases in fish average length varied from 0.5 to 1.25 cm (Figure 2A). The statistical analysis detected a significant difference ($p > 0.05$) between the grass carp gain in length when fed only with *E. najas* (ELDNA) and the one obtained with *E. densa* (ELLDE). This behavior can probably be explained by the lower consumption of this latter.

Fish weight gain was greater when grass carp was fed with *E. najas* and *E. densa* at the same time (ELDNA/ELLDE) at 33.6 g, which was statistically higher ($p < 0.05$) to the gain observed in other treatments. The lowest weight gain was observed when fish was fed with ELLDE (5.3 g) and only *C. demersum* and *E. najas* associated (CERDE/ELDNA) at 4.0 g. Intermediate weight gains were observed for *C. demersum* and *E. najas* offered individually as well as *C. demersum* and *E. densa* offered together (CERDE/ELLDE).

The highest percentage of length gain occurred for ELDNA (19.09%) and CERDE/ELLDE (19.05%), and the lowest for ELLDE (9.27%) and CERDE/ELLDE (15.01%) (Figure 2C), with $p > 0.05$. Concerning the percentage of biomass gain variable, the highest values were for ELLDE associated with ELDNA (57.24%) and CERDE/ELLDE (28.08%), and the lowest for ELLDE/ELDNA (5.86%) and ELLDE (7.55%) (Figure 2D), with $p < 0.05$.

It is quite interesting to observe that situations where a greater grass carp predation occurred were not necessarily those in which there were higher gains in fish size. This behavior is probably linked to the macrophytes nutritional quality related to the fish needs. Corrêa et al. (2003) reported higher crude fiber in *E. najas* and higher crude protein and minerals in *C. demersum*, results that do not explain the grass carp growth behavior in the present experiment.

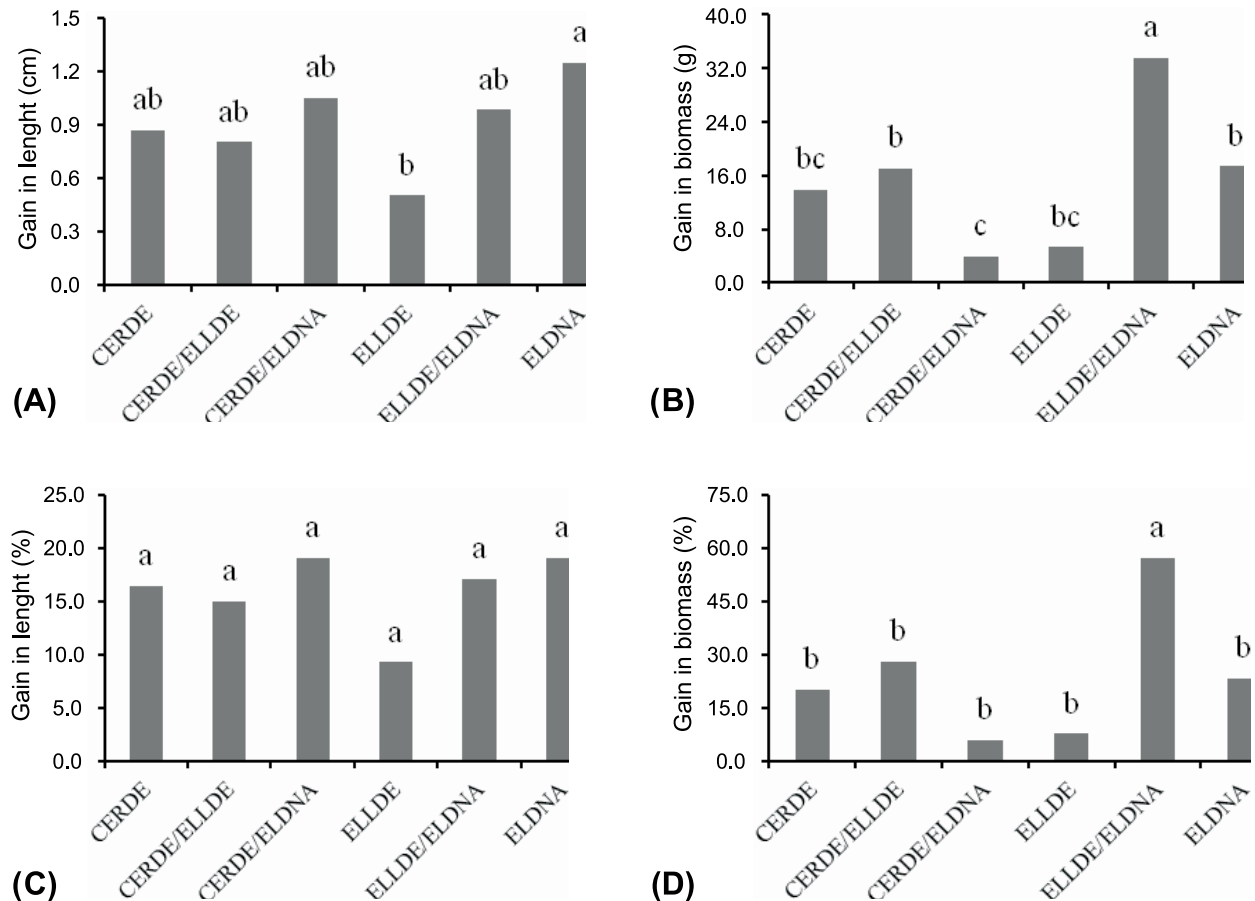


Figure 2 - Total length gain average values (A), overall biomass gain (B), percentage of length gain (C) and percentage of biomass gain (D) for *C. idella* individuals fed with *C. demersum*, *E. densa* and *E. najas* offered individually and in pairs (CERDE/ELDNA, CERDE/ELLDE and ELDNA/ELLDE) in 81 days experiment.

According to Cyrino et al. (2004), the low growth and weight gain of fishes can be explained by a deficiency in essential aminoacids, which may have caused a reduction in proteins use, resulting in reduced growth, weight gain and feed efficiency. Therefore, a source of animal protein is also important (Gettys et al., 2009), with zooplankton being partially replaced by benthos and more specifically by periphytic fauna (Adámek et al., 1996). These arguments may explain the reduced growth of carps in mesocosm condition, where there was no chance for periphytic and benthic communities to develop during the trial period.

The grass carp has many advantages in the aquatic plants management prog. However, the introduction of exotic species requires careful observation to avoid changes in the

native fish community structure (Hrabik et al., 1998), as habitat, competition, hybridization changes as well as pathogens and diseases introduction (Hall & Miils, 2000). Grass carp should not be treated as an exotic invasive organism, once its reproduction is very unlikely to occur in Brazilian conditions (Cyrino et al., 2004; Piedraz et al., 2006). New researches involving biological control of submersed macrophytes and growth conditions of grass carp must be accomplished, since, in integrated management of submerged vegetation prog, its use in commercial polycrops is an important incentive (Opuszynski & Schireman, 1995; Hermes et al., 2007).

This study revealed that grass carp is an excellent tool for the integrated management of submerged aquatic plants, preventing their



populations to expand in a high rate and interfering with the multiples uses of water bodies.

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