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Inhibition effects of daughter ramets on parent of clonal plant *Eichhornia crassipes*

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ABSTRACT

The aquatic invasive clonal *Eichhornia crassipes* was selected to study the effect of nutrients and stolon severing on clonal plants in less heterogenous aquatic environments. Under high nutrient conditions, parent ramets produced more daughter ramets and leaves with higher biomass, and, subsequent to severing daughter ramets, especially those in early stages; parents produced more daughter ramets and leaves with higher biomass. However, leaf number and biomass of individual daughter ramets did not differ from individuals with severed stolons and the control treatment. The biomass and leaves of the daughter ramets were lower than the control and other severing treatments that were carried out at later stages only when the nutrient level was low and the stolons of the daughter ramets were severed at early stages of growth. For this reason, the total number of ramets and leaves were lower in early stage severing treatments than severing treatments at later stages. After severing the connection with the daughter ramets, the root/shoot biomass ratio of the parent ramets increased, indicating that water hyacinths display morphological plasticity after stolon severing by increasing root growth. This study indicates that in aquatic environments where the nutrient distribution is less heterogenous in the same layer of water, daughter ramets of the water hyacinth can inhibit the growth and clonal reproduction of the parent, and this inhibition was strongest at early stages of ramet growth.

1. Introduction

The responses of clonal plants to heterogeneous resources have been extensively researched since the 1980s. In a heterogeneous habitat, clonal plants have two strategies, one is physiological integration and the other is phenotypic plasticity. Physiological integration can buffer clonal plants against spatial heterogeneity by greater resource availability through structures such as rhizomes and stolons (Pitelka and Ashmun, 1985). Phenotypic plasticity is the ability of a genotype to modify its growth and development in response to changes in the environment (Dorken and Barrettp, 2004).

The role of clonal integration and plasticity among clonal plants has been studied extensively in terrestrial environments. However, despite clonal growth being an important aspect of population biology in many aquatic and wetland plants (van Groenendael et al., 1996) studies focusing on clonal integration in aquatic plants are few (Tomasko and Dawes, 1989; Nielsen and Pedersen, 2000). Studies have shown that the vertical distribution of nutrients in aquatic microenvironments may not be uniform, but nutrient distribution in the same layer of open water is less heterogeneous than in soil environments (Sculthorpe, 1967; Cook, 1985; Les, 1988; Barrett et al., 1993; Santamaría, 2002). Ramets of submerged clonal plants may be distributed in different water layers under varying nutrient, light and other biological (e.g., microbial) conditions (Wallsten and Forsgren, 1989; Weisner et al., 1997; Van den Berg et al., 1998; Cronk and Fennessy, 2001; Scheffer, 2004; Liu et al., 2012). Due to these differences physiological integration occurs between ramets the leaf size and number of a submerged clonal plant, *Potamogeton malaianus* Miq., have changed significantly for physiological integration in different water sediments (Liu et al., 2007). Similarly, in different water CO_2 concentrations, *Potamogeton crispus* also has significant physiological integration in leaf size, number and mass (Xie et al., 2003). However, in plants that only grow in the same water layer, the physiological integration of clonal plants may not be found in uniform aquatic habitats, because it cannot improve the fitness of ramets in horizontal directions (Pitelka et al., 1985; Caraco and Kelley, 1991; Alpert, 1999). Studies have shown that clonal plants in homogeneous environments, often via stolon fragmentation among ramets to disseminate propagules,

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because in homogeneous environments, maintaining the connection among ramets and sharing resources among ramets have little significance (deKroon et al., 1996). In addition, when daughter ramets mature, fragmentation among ramets can greatly improve fitness (Zhang et al., 2002, 2003). The extent of ramet interdependence vs. independence may potentially be mediated by resource availability in specific habitats (Derner and Briske, 1998). Caraco and Kelley (1991) proposed that intraclonal ramets may be integrated to a lesser extent in environments with high resource availability compared to those with low resource availability. In heterogeneous habitats, the combined growth of connected ramets is greater than the sum of the growth of the two ramets when the stolon is severed (Friedman and Alpert, 1991; Hutchings and Wijesinghe, 1997), whereas in homogeneous habitats, the potential benefits of an intact clonal system may be nil or negative. In aquatic environments, the connection between mature ramets through rhizomes or stolons ("spacers") may restrict the amount of water-surface ramets can occupy. When the ramets were connected with parent, the older parts of the parent plants are rapidly abandoned in favour of new ramets (Noble et al., 1979; Schmid and Harper, 1985; Schmid and Bazzaz, 1987). The stolons between the parent and the daughter of aquatic clonal plants tend to be broken in uniform aquatic environments, and favour clonal growth and the occupation of more water space (Crowley et al., 2005), however a number of studies have produced contrasting effects on the artificial severing of stolons. A study by Hutchings and Wijesinghe (1997), for example, showed that severing the stolons between all the ramets of clonal plants had no impact on yield in homogeneous environments, whereas Teng et al. (2011) showed that after splitting the stolons or rhizomes, a clonal plant can grow and regenerate more vigorously. In addition, because aquatic environments have a fluid character, and their chemical and physical factors change easily, severing the stolons of floating aquatic clonal plants facilitates the spread of ramets over long distances in rivers or lakes (van Groenendael et al., 1996). In this way, aquatic clonal plants can avoid mortality by loss of physiological integration between ramets or by fragmentation (Hutchings, 1999). Many observations have also showed that if connected ramets all lie within a homogeneous patch of habitat, the morphology of the whole plant alters to enhance acquisition of the most limiting resource (Noble and Marshall, 1983; Slade and Hutchings, 1987; Friedman and Alpert, 1991; Stuefer et al., 1994). When nutrients or other soil-based resources are scarce, the Root/Shoot ratio increases, and when light is scarce the root/shoot ratio decreases (Hunt and Nicholls, 1986). However, it is not clear what this connectedness means for the growth and clonal reproduction of aquatic plants. Much of the relevant information on physiological integration comes from clonal plants, and physiological integration inevitably occurs early on in the ontogeny of ramets, because older ramets provide resources to support the establishment of their successors (Alpert, 1999; Hutchings, 1999). The level of support declines as new ramets acquire the capacity to obtain their own resources, but significant resource transfer can often be detected over very long periods (Jónsdóttir and Callaghan, 1989). However, most of these investigations have evaluated the relations between parent and daughter ramets only for terraneous clonal plants with few focusing on aquatic clonal plants. We test whether, in an aquatic environment, the daughter ramet restricts the growth and clone of the parent, and whether this restriction will continue after the stolon is severed.

We examined the responses in plasticity and integration to severing stolons in an aquatic clonal plant, *Eichhornia crassipes* (Mart.) Solms-Laubach, a perennial floating aquatic clonal species with the popular name "water hyacinth". Under greenhouse conditions, we considered whether: (1) in a uniform aquatic habitat, the daughter ramet can inhibit the growth of the parent via the stolon; (2) severing the stolon promotes the growth and clonal reproduction of the parent; (3) the inhibition of the parent ramet by the daughter changes with the growth of the daughter ramet, and a juvenile ramet exerts stronger inhibition than a mature ramet; and (4) this inhibition effect differs in response to nutrient levels after the stolons are severed.

2. Materials and methods

2.1. Study species

E. crassipes originated in the state of Amazonas, Brazil, spread to other regions of South America, and was carried by humans throughout the tropics and sub-tropics. It now occurs in at least 62 countries and causes extremely serious problems between 40°N and 45°S. It is the most damaging floating aquatic weed worldwide and much money has been spent in attempts to control it (Harley, 1994). *E. crassipes* is usually a free-floating species, whereas other species of *Eichhornnia* are rooted in the substrate (Wright and Purcell, 1995). It is an erect perennial herb, which reproduces from stolons and by seed. The leaves have either bulbous or slender petioles. The flowers are bluish-purple with yellow centres and are arranged spirally in spikes. The roots are fibrous, with many laterals trailing freely in the water to a depth of 1 m or more. With an average annual productivity of 50 dry (ash-free) tonnes per hectare per year, *E. crassipes* is one of the most productive (perhaps the most productive) plants in the world (Abbasi and Nipaney, 1986). *E. crassipes* ramets are root-bearing rosettes that form new rosettes in the leaf axil. Each new rosette is produced at the end of a stolon, which elongates with age and can eventually carry the rosette over 50 cm away from the parental ramet. The morphology and architecture of leaves, rosettes, and clonal groups are highly plastic (Richards, 1982; Watson and Cook, 1982).

2.2. Experimental procedures

E. crassipes was cultivated in a greenhouse. When enough ramets were produced, we chose ramets without new daughter ramets as the material for the experiment. Each ramet had 5–6 leaves. The compound fertilizer for water cultivation was produced by the Scotts Company of USA, trade name Osmocote I. The main elements of the fertilizer are total nitrogen 14% (ammonium nitrogen 6.8%, nitrate nitrogen 7.2%), water-soluble phosphoric acid 14%, water soluble potash 14%, and the valid microelements contain Fe 0.15%, Mn 0.16%, Cu 0.05%, B 0.02%, Mo 0.015%, and Zn 5%. We quantified the compound fertilizer at four levels: 25 g (Nutrient 1, N1), 50 g (Nutrient 2, N2), 100 g (Nutrient 3, N3), and 200 g (Nutrient 4, N4), together with a blank control (only tap water, without fertilizer) (N0). Each nutrient level was represented by 16 samples. Each fertilizer sample was put into a cubic plastic barrel (70 cm × 50 cm with 50 cm deep). Tap water was added to all the plastic barrels to a depth of 20 cm. Therefore, according to the above nutrient level design, the concentrations of nitrogen, phosphorus and potassium in different treatments were about N: 50 mg/L, P: 50 mg/L and K: 50 mg/L in N1 treatment, and accordingly, N: 100 mg/L, P: 100 mg/L, K: 100 mg/L in N2 treatment, N: 200 mg/L, P: 200 mg/L and K: 200 mg/L in N3 treatment, N: 400 mg/L, P: 400 mg/L and K: 400 mg/L in N4 treatment. However, the fertilizer of Osmocote is a kind of slow release fertilizer, and various nutrients will not immediately be released into the water.

Each selected ramet was then grown in a plastic barrel and regarded as a parent ramet. Severing treatments were carried out once daughter ramets were established. The stolon between the parent and the daughter ramet was severed at one of three levels: (1) severing treatment 1 (S1), the ramet was severed just after it had grown from the base of the bulbiform petiole, with

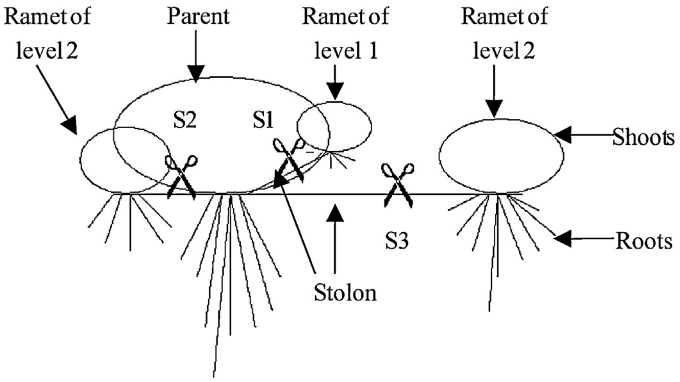

Fig. 1. Schematic diagram of stolon severing, showing the daughter ramets at three different stages of growth. S1–S3 represent the three levels of stolon severing.

Table 1
The two-factor ANOVA of the effects of nutrient and severing treatments on ramets, leaves and biomass of *E. crassipes*. NTR (number of total ramets), NTL (number of total leaves), NDR (number of daughter ramets). NPL (number of parent leaves), DMP (dry mass of parent ramet), RSRP (root/shoot ratio of parent ramet), NDL (number per unit daughter ramet leaves), RMD (root mass of daughter ramet), SMD (shoot mass of daughter ramet).

Source	Nutrient		Severing		Nutrient × severing	
	$F_{(4, 60)}$	p	$F_{(3, 60)}$	p	$F_{(12, 60)}$	p
NTR	19.43	<0.001	2.26	0.09	0.69	0.75
NTL	24.72	<0.001	2.57	0.06	0.83	0.62
NDR	24.02	<0.001	5.35	0.002	1.87	0.06
NPL	11.34	<0.001	3.35	0.03	0.87	0.58
DMP	26.57	<0.001	10.36	<0.001	1.74	0.08
RSRP	1.198	0.32	3.60	0.02	1.34	0.22
NDL	15.28	<0.001	1.94	0.13	1.3	0.24
RMD	29.85	<0.001	1.67	0.18	0.88	0.57
SMD	32.17	<0.001	3.82	0.014	1.69	0.093

2–3 fully expanded leaves and the new roots enveloped by the stipule sheath; (2) severing treatment 2 (S2), the stolon was severed after it had elongated considerably, the new roots had grown into the water, but the new daughter ramet was still partly sheltered by the parent ramet, with 3–4 fully expanded leaves; (3) severing treatment 3 (S3), the daughter ramet was severed after it had grown 5–6 fully expanded leaves, and the stolon had elongated sufficiently for the photosynthesis of the new daughter ramet to be unaffected by the shade of the parent (Fig. 1). In the control treatment, nothing was severed (S0). For statistical analysis, we used four repetitions at every cutting level. Thus, the experiment had set (4 nutrient levels + 1 control) × (3 severing level + 1 control) × 4 = 80 cubic plastic barrels. All the barrels were labelled randomly before severing treatments.

Every second day, we recorded the number of ramets and leaves. We also severed the stolons during the cultivation period when they had grown to the level described on the barrel label.

To compute the coverage of the water surface by the *E. crassipes*, we laid a 10 cm ruler on the water's surface and took vertical platform photographs of the ramets in each plastic barrel with a digital camera. All the photographs were printed on graph paper divided into 1 cm × 1 cm squares. We calculated the number of grid units that were more than half occupied by the *E. crassipes* image. The area of the grids was then converted into the *E. crassipes* coverage of the water surface by dividing the square of the ratio of the ruler length on the paper to 10 cm. The *E. crassipes* was cultivated for 50 days and photographs were taken four times in that period.

After 50 days, we weighed the fresh mass (without any water) of the parent, the daughter ramets, and all the generation ramets. We also calculated the numbers of leaves (live and dead), shoot heights, and root lengths of the parent and daughter ramets (three daughter ramets from each barrel were sampled randomly). All plant material was then placed in an oven at 80 °C for 48 h, after which the dry mass of the roots and shoots of all groups were weighed.

2.3. Data analysis

All datasets were checked for normality before analysis and none deviated significantly from a normal distribution. Repeated two-factor ANOVA was used to examine the effects of severing the stolons and of nutrient treatments on the total ramet and leaf number along with cultivation days. Post hoc tests (Fisher's least significant difference [LSD] test) were also performed to compare the mean values for each parameter examined at each nutrient (N0–N4) and severing level (S0–S3), and the LSD between the means was estimated at the 95% confidence level. Differences between nutrient levels or severing levels were deemed significant at $P < 0.05$. All analyses were conducted using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results

As expected, both nutrient level and severing treatments profoundly affected clonal growth (Table 1). These influences also differed between all the generation ramets, parent, and daughter ramets only.

3.1. Effects on total ramet number and leaves

During the cultivation period, total *E. crassipes* ramet numbers (all the generation ramets of parent) increased with cultivation time, particularly in the later period of cultivation due to the increase of mature generation ramets, which can also produce new ramets (Table 2). There were also significant differences in total ramet number between different nutrient treatments, where the highest nutrient level (N4) produced the most ramets (LSD tests, $N4 > N3 \geq N2 \geq N1, N0$).

However, in the early period of cultivation, there were no differences in total ramet number among different stolon severing treatments but differences between severing treatments were detected after 19 days In particular the total ramet number in the S3 treatment was significantly higher than in the S1 and S2 treatments, but there were no significant differences between any severing treatments and the control (LSD tests, $S3 \geq S0 \geq S2, S1$). In the last period of cultivation, because more mature generation ramets continue to produce new generation ramets, total ramet number differences caused by severing the daughter ramets were can not be found. Further analysis found that, after 19 days, the total ramet number in the S3 treatment was significantly higher than that of the S1 treatment only at low nutrient level (N1), and, there were no significant differences with the control treatment (S0). This suggests that, the effect of severing stolons on ramet

Table 2
Multivariate tests (Wilks' Lambda tests) of the effects of cultivation days on total ramet number and leaf number of *E. crassipes* in the treatments.

Source	Total ramet no.			Total leaf no.		
	df	F	p	df	F	p
Days	18	16.07	<0.001	17	27.17	<0.001
Days × nutrient	72	2.52	<0.001	68	2.83	<0.001
Days × severing	54	1.4	0.065	51	1.81	0.04
Days × nutrient × severing	216	1.03	0.408	204	1.14	0.126

number was significantly expressed only at particular nutrient concentration, moreover, the effects of different severing treatments on ramet number were different.

Similar to total ramet production above, total leaf number was greatest under the S3 treatment and significantly different from the S1 and S2 treatments but not the control. From the severing experiment, we suggest that the number of leaves produced in *E. crassipes* is not a plastic trait.

3.2. *Effects on parent*

Under the three severing treatments, the number of daughters produced by parents was somewhat dependent on nutrient concentrations. A two-factor ANOVA showed that, at higher nutrient levels (N3, N4) parents produced more daughter ramets, more leaves, and a higher biomass in the three treatments (S1–S3) than in the control (S0) (Table 3). Further analysis showed that, at higher nutrient concentrations (N3, N4), severing daughter ramets earlier (S1, S2) increased parent production of daughter ramets than later-stage severing (S3) (Table 3). Leaf number and biomass of parental individual also increased greatly in S1 and S2 treatments with similar differences and patterns. These results indicate that, at earlier stages, the impact on parents was stronger, and with the continuous growth of the daughter ramet, the influence on parent growth and clonal reproduction will weaken.

The parent root and shoot (leaf) biomass analysis showed that under the different nutrient treatments, the root/shoot ratio of parents did not change significantly, but after stolons were severed, the root/shoot ratio increased greatly indicating an increase in root length. This suggests that the parent of the *E. crassipes* displays phenotypic plasticity after severing the connection with the daughter ramets.

3.3. *Effects on daughter ramets*

Similar to parents, leaf number, root dry mass, shoot dry mass, and size of daughter ramets significantly increased with nutrient concentration (Table 4). But, the effects of severing treatments on the daughter ramets were different from those on the parents, where leaf number, root dry mass and shoot dry mass of the daughter ramets were all significantly lower in the S1 stolon severing treatment than in other treatments (Table 4). This illustrates that severing of daughter ramets at early stages will influence future growth. Further analysis showed that, at the N1 nutrient level, leaf number, root and shoot dry mass of the daughter ramets were lower in the S1 treatment than in the S3 or control treatments. This shows that early stage severing at low nutrient levels hinders the growth of juvenile daughter ramets. However, where nutrients were not available (i.e., N0 – control treatment), there were no significant differences among severing treatments, with very little growth observed in all severing treatments.

Based on analysis of the root and shoot dry mass, the root/shoot ratio did not differ with severing treatment in daughter ramets, indicating that the relationship between root and shoot size in *E. crassipes* is not a plastic trait.

4. Discussion

The experimental results showed that different severing treatments have different impacts on *E. crassipes* growth. After severing the early stage ramets (S1), growth and clonal reproduction of daughter ramets were affected and declined greatly. Leaf number and biomass of daughter ramet individuals in the S1 treatment were lower than in the S2 and S3 treatments, and consequently the output of total number of ramets and leaves from the S1 treatment was also reduced. In view of this, the growth of early stage daughter ramets needs a maternal nutrient supply. But at the same time, daughter ramets inhibited the growth and reproduction of the parent, and after the daughter ramets were severed, the growth and reproduction of parents were enhanced. This inhibition on the parent was especially significant in the early stages of daughter ramet growth. With the continuous growth and elongation of stolons, the functions of roots and leaves of daughter ramets improved and developed constantly, and the dependence on the parent was gradually weakened. The inhibitory effects on the parent were also gradually weakened, therefore, after severing the later stage ramets, the number of daughter ramets and leaf number of parents were higher than in the control but lower than the severing of early stage ramets. This inhibitory effect on the parent is not difficult to understand, because in the aquatic environment, the distribution of nutrients is relatively uniform among parent and daughter ramets (Sculthorpe, 1967; Cook, 1985; Les,

Table 3
The effects of nutrient and severing treatments on parent ramet of *E. crassipes*, including means ± SE. Letters (a–c) indicate statistically significant differences (LSD tests) between severing levels in every nutrient treatment level. NDR (number of daughter ramets), NPL (number of parent ramet leaves), DMP (dry mass of parent ramet), RSRP (root/shoot ration of parent ramet).

Nutrient	Severing	NDR	NPL	DMP	RSRP
N0	S0	1.5 ± 0.96	12.0 ± 0.41	0.4 ± 0.10^{a}	1.1 ± 0.16
	S1	4.3 ± 1.75	17.8 ± 2.29	2.1 ± 0.69^{ab}	1.0 ± 0.06
	S2	3.8 ± 1.80	16.8 ± 3.95	2.7 ± 0.85^{b}	1.2 ± 0.09
	S3	3.3 ± 1.18	16.3 ± 2.29	1.9 ± 0.66^{ab}	0.9 ± 0.13
N1	S0	3.3 ± 0.48	13.8 ± 1.89	1.1 ± 0.54^{a}	1.1 ± 0.11
	S1	2.8 ± 0.48	13.3 ± 1.18	1.1 ± 0.09^{a}	1.0 ± 0.09
	S2	3.0 ± 1.42	15.5 ± 2.47	2.7 ± 1.29^{ab}	1.3 ± 0.17
	S3	6.3 ± 1.70	18.3 ± 1.55	3.9 ± 0.81^{b}	1.3 ± 0.05
N2	S0	5.0 ± 1.42	16.8 ± 4.13	2.3 ± 0.89	1.0 ± 0.15^{ab}
	S1	4.5 ± 1.94	19.0 ± 0.41	3.9 ± 0.71	1.0 ± 0.12^{a}
	S2	4.8 ± 1.89	18.8 ± 2.40	4.0 ± 1.20	1.2 ± 0.08^{ab}
	S3	5.0 ± 1.36	16.0 ± 1.08	2.4 ± 0.30	1.6 ± 0.28^{b}
N3	S0	3.3 ± 1.65^{a}	18.0 ± 0.41^{a}	3.3 ± 0.23^{a}	1.0 ± 0.08^{a}
	S1	11.3 ± 3.12^{b}	24.0 ± 0.71^{b}	8.9 ± 0.96^{c}	1.3 ± 0.10^{ab}
	S2	10.3 ± 1.70^{b}	25.0 ± 2.28^{b}	7.5 ± 1.45^{bc}	1.5 ± 0.17^{b}
	S3	7.8 ± 1.93^{ab}	22.5 ± 1.56^{b}	6.0 ± 0.40^{ab}	1.2 ± 0.16^{ab}
N4	S0	7.5 ± 0.65^{a}	21.0 ± 2.49	3.8 ± 0.41^{a}	1.0 ± 0.15
	S1	18.0 ± 3.11^{b}	25.0 ± 3.19	9.9 ± 2.55^{b}	1.2 ± 0.12
	S2	16.5 ± 2.37^{b}	24.0 ± 1.47	8.1 ± 0.87^{b}	1.2 ± 0.11
	S3	12.3 ± 1.11^{ab}	20.0 ± 1.29	7.1 ± 0.59^{b}	1.1 ± 0.10

Table 4
The effects of nutrient and severing treatments on daughter ramet of *E. crassipes*, including means ± SE. Letters (a–c) indicate statistically significant differences (LSD tests) between severing levels in every nutrient treatment level. NDL (number per unit daughter ramet leaves), RMD (root mass of daughter ramet), SMD (shoot mass of daughter ramet).

Nutrient	Severing	NDL	RMD	SMD
N0	S0	3.3 ± 1.93	0.03 ± 0.02	0.03 ± 0.02
	S1	6.8 ± 2.99	0.13 ± 0.10	0.19 ± 0.15
	S2	7.8 ± 2.09	0.23 ± 0.12	0.30 ± 0.14
	S3	7.8 ± 2.81	0.11 ± 0.06	0.08 ± 0.03
N1	S0	11.8 ± 2.06b	0.52 ± 0.27^{ab}	0.48 ± 0.22^{ab}
	S1	5.0 ± 0.83a	0.04 ± 0.01a	0.46 ± 0.01a
	S2	8.3 ± 2.96^{ab}	0.27 ± 0.17^{ab}	0.36 ± 0.23^{ab}
	S3	13.3 ± 1.21b	0.79 ± 0.23b	0.84 ± 0.21b
N2	S0	14.5 ± 2.29	0.78 ± 0.30b	0.88 ± 0.28b
	S1	8.7 ± 1.76	0.12 ± 0.05a	0.17 ± 0.07a
	S2	10.3 ± 3.03	0.40 ± 0.22^{ab}	0.48 ± 0.23^{ab}
	S3	11.2 ± 0.91	0.32 ± 0.07^{ab}	0.31 ± 0.09^{ab}
N3	S0	14.1 ± 1.89	1.22 ± 0.29	1.06 ± 0.27
	S1	13.1 ± 1.04	1.12 ± 0.33	0.93 ± 0.12
	S2	15.8 ± 1.27	1.45 ± 0.31	1.20 ± 0.18
	S3	14.7 ± 2.02	1.28 ± 0.31	1.14 ± 0.22
N4	S0	15.4 ± 0.55	1.45 ± 0.05	1.21 ± 0.20^{ab}
	S1	15.3 ± 0.92	1.28 ± 0.28	0.99 ± 0.12^{a}
	S2	15.7 ± 1.08	1.28 ± 0.15	1.29 ± 0.15^{ab}
	S3	15.8 ± 0.54	1.34 ± 0.15	1.60 ± 0.09^{b}

1988; Barrett et al., 1993; Santamaría, 2002), and there is almost no nutrient foraging and competition among ramets in aquatic environments. However, because ramets shelter each other, and the distribution of sunlight among the ramets is not uniform, water space will become the object of competition. *E. crassipes* displays phenotypic plasticity and integration responses to light quality and quantity (Richards and Lee, 1986; Méthy et al., 1990). The daughter ramets can acquire more water space through inhibiting the continuous growth of the parent ramet, and at the same time, the daughter ramet obtains nutrients from the parent for rapid growth of itself, especially at early stages. Therefore, the behaviour of daughter ramets can be considered selfish (Alpert, 1999). Many studies have confirmed that the relationship between parent and daughter ramets of clonal plant is generally beneficial for young ramet settlement (Pitelka et al., 1985; Alpert and Mooney, 1986; Lau and Young, 1988; Jónsdóttir and Callaghan, 1989; Alpert, 1996). When maintaining ramet connection and sharing resources are of little significance, ramets will quickly fracture and separate from the parent (deKroon et al., 1996; Dong, 1996). Fragmentation of clonal plants is a common phenomenon in herbal clonal plants (Wihalm, 1995; Alpert and Stuefer, 1997), and clonal plants are often separated by the fragmentation of asexual reproduction to form an independent individual, in order to achieve more long-distance spread (Crowley et al., 2005). Light gaps in the canopy might increase clonal growth in *E. crassipes*, and the separation of stolons increases the success of ramets in potentially favourable microsites (Méthy and Roy, 1993). Furthermore, after separating from the daughter ramets, the parent can also float freely on the water's surface and gain more space, so the parent can produce further ramet generations and also increase in biomass, leaf number, root length, and shoot height. The tissue of *E. crassipes* also favours the fragmentation of daughter ramets separating from the parent, because the stolon of *E. crassipes* has few fibres, and is easily broken by waves, wind, and other external forces (Barrett, 1980; Langeland and Burks, 1998). The easy fracturing characteristic greatly enhances the adaptability and invasive capacity of *E. crassipes* in aquatic environments.

From the experimental results, we can see that nutrient concentration has a significant influence on the growth and clonal reproduction of *E. crassipes*, where the total ramet number, leaf number, and each parent and offspring individual increased with nutrient concentration. The effect of severing treatments differed with nutrient concentration. At higher nutrient concentrations (N3, N4), because *E. crassipes* grows quickly, the impact of severing stolons on daughter ramets was soon restored, so the leaf number, and biomass of daughter ramets did not differ among different severing treatments. In contrast, at lower nutrient concentrations (N1, N2), the impact of the S1 severing treatment was significantly lower than other severing treatments, and especially the control (S0) treatment. This suggests that when nutrients are lacking, nutrients are transported from parent to daughter ramets, and after severing the connection, the daughter ramets, especially those at early stages of growth, will grow slowly, and when nutrients are adequate, this nutrient transport will become less important. But, the effects of inhibition on parent ramets were obvious at higher nutrient levels (N3, N4), and this further illustrates that *E. crassipes* can acquire more water space by inhibiting the growth of the parent when there are sufficient nutrients.

In clonal plants, the maintenance of physiological integration between ramets is dependent on the income of resources transported between ramets. If the distribution of environmental resources is homogeneous, the fitness decline of the ramet that acts as nutrient exporter caused by integration will be greater than the fitness increase of the nutrient importer, and the integration will not occur among ramets (Alpert, 1999). Obviously, in a homogeneous aquatic environment, physiological integration cannot significantly increase the fitness of *E. crassipes* clonal group. But when nutrients are deficient, the nutrient output of the parent to the early daughter ramet is still necessary, because it can increase the fitness of the daughter ramet, allowing it to mature quickly, and thus enhancing its competitive capacity in water space. In a homogenous environment, the physiological integration of *E. crassipes* belongs to the Splitter Strategy of physiological integration (Oborny and Kun, 2001), where the longevity of connections (C) is less than the longevity of ramets (R) (Jónsdóttir and Watson, 1997). When the daughter ramet is mature and settles, physiological integration will stop, and the stolon will disconnect. This experiment showed that, except in the one-way physiological integration in the early stage, the daughter ramet inhibits the parent, because the inhibition allows for acquisition of resources, significantly increasing fitness. The severing treatments did not induce changes to the root/shoot ratio of daughter ramets, and the root size did not increase, suggesting that in a homogenous aquatic environment *E. crassipes* does not undergo phenotypic plasticity related to foraging. In contrast, due to nutrient inhibition, the size of the parents was small, but once the stolons were severed, root/shoot ratio, leaf number and biomass of the parent increased quickly. The increased root/shoot ratio enables increased absorbtion of nutrients from the water. This further illustrates that under the inhibition of daughter ramets, the parent does not compensate for the decline of fitness by increasing root size, otherwise, the compensation of increasing roots would have offset the effects of inhibition by daughter ramets.

The ecological strategies of *E. crassipes* in the aquatic environment and its ability to invade new habitats quickly are of significance. Because of the inhibitory effects by the daughter ramets on the parent, more new daughter ramets quickly occupy a greater space in the water, *E. crassipes* can even take up an entire river, forming a single grass mat (OgutuOhwayo et al., 1997). In addition, because stolons are easily fractured, the mature ramets quickly separate from the parent, and spread far away with water flow, and the parent can also reproduce further ramets after separating from mature ramets.

E. crassipes is a clonal floating plant, and due differing aquatic environmental factors, the adaptation strategies of *E. crassipes* are different from submerged plants. Submerged plants adapt to the differing distribution of light, nutrients, and biological factors at different water depths through phenotypic plasticity (Hara et al., 1993; Xie et al., 2005, 2007; Xiao et al., 2006, 2007a, b; Liu et al., 2007; Wolfer and Straile, 2012). To clonal floating plants, the physiological integration among ramets is not obvious on the water nutrients absorption, but, to other factors, such as, due to the mutual shadowing between ramets, the distribution of sunlight will be uneven among ramets, or due to the differences of water depth caused by different distances to the shore, the clonal floating plants can undergo phenotypic plasticity changes to achieve physiological integration between ramets, or adjust the allocation between sexual reproduction and clonal growth. Our study looked at the adaptation strategies of an aquatic clonal plant simply from the foraging to the nutrients of water. At present the researches of aquatic clonal plant on water environment adaptability are relatively short, but clonal plants dominate aquatic communities (Grace, 1993; Cronk and Fennessy, 2001), understanding the adaptation strategies of aquatic clonal plants in response to aquatic conditions may give more insights into the evolutionary significance of clonality in aquatic conditions.

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