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Clonal integration increases growth performance and expansion of *Eichhornia crassipes* in littoral zones: A simulation study

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ABSTRACT

Clonal integration can improve the spread and growth of invasive plants in response to various disturbances. However, little is known about its role in floating aquatic clonal plants that expand from aquatic into terrestrial habitats in littoral zones. Thus, in this study, we simulated the expansion of the invasive clonal aquatic plant *Eichhornia crassipes* from aquatic to terrestrial habitats through two modes of clonal integration. We subjected *E. crassipes* parent plants and offspring ramets to three levels of natural light in terrestrial habitats: 100%, 60%, and 10%. The stolon connections were either severed or kept intact. Our findings showed that clonal integration had positive effects on plants exposed to shade in the terrestrial habitats and produced negative effects on plants in the aquatic habitats. Overall, clonal integration significantly increased whole-plant growth performance. Parent plants and offspring ramets in the terrestrial environments can enhance their adaptability to shade by increasing the maximum quantum yield of photosystem II and chlorophyll content. Clonal integration can support the expansion of *E. crassipes* from aquatic into terrestrial habitats with limited light conditions through significantly elevated growth traits. Thus, *E. crassipes* has a high ability for clonal integration and may be a potential threat to littoral zone ecosystems.

1. Introduction

Invasive species have become a major threat to global biodiversity, ecosystem functioning, and economic development (Van Kleunen et al., 2010, 2015; Rejmanek, 2015). Many invasive aquatic plants have the potential for prolific clonal propagation or expansion (Santamaría, 2002; Liu and Yu, 2009); they produce rhizomes or stolons that spread over a relatively large area, while maintaining a physical and physiological connection between the parent and its ramet (Wolfer and Straile, 2004; Xiao et al., 2007; Waters and Watson, 2015). Clonal plants can share photosynthates, mineral nutrients, or water among individual subunits through clonal integration, which increases the survival and performance of clonal plants when individual subunits experience different conditions (Alpert, 1996; Hutchings and Wijesinghe, 1997; Dong et al., 2015; Lyu et al., 2016). For example, when part of a clone is damaged or limited by local environmental stress, unaffected ramets may aid individual ramet survival or escape from unfavourable habitat by translocation of resources (Hellström et al., 2006; Wang et al., 2009; Lyu et al., 2016). Resource translocation may be acropetal (from parent plant to offspring ramet) and basipetal (from offspring ramet to parent plant) (Hertefeldt and Jonsdottir, 1999). However, clonal integration may not always be positive for the clonal plant, because pathogens can spread more easily among interconnected individuals (Stuefer et al., 2004).

Clonal integration can enhance the expansion of alien clonal plants; for example, physiological integration might support the expansion of *Alternanthera philoxeroides* or *Paspalum paspaloides* from terrestrial into aquatic environments because established ramets of the amphibious clonal plants in terrestrial habitats can support new ramets in aquatic habitats (Wang et al., 2009; Luo et al., 2017). In addition, unlike clonal fragmentation (when the connected stolons are severed), clonal integration can increase the competitive ability of *Eichhornia crassipes* and *Pistia stratiotes* (Wang et al., 2016a). Thus, clonal integration may increase the competitive advantage of invasive clonal plants over natives (Wang et al., 2017).

Notorious invasive aquatic plants such as *E. crassipes*, *P. stratiotes*, or *A. philoxeroides* can form dense offspring ramets that move with the surface of water while connected through stolons (Liu and Yu, 2009;

Abbreviations: ANOVA, analysis of variance; 2°, secondary ramets; F_v/F_m, maximum quantum yield of photosystem II; O-P, offspring ramets grown in aquatic environment and connected parent plants in terrestrial environment; P-O, parent plants grown in aquatic environment and connected offspring ramets in terrestrial environment

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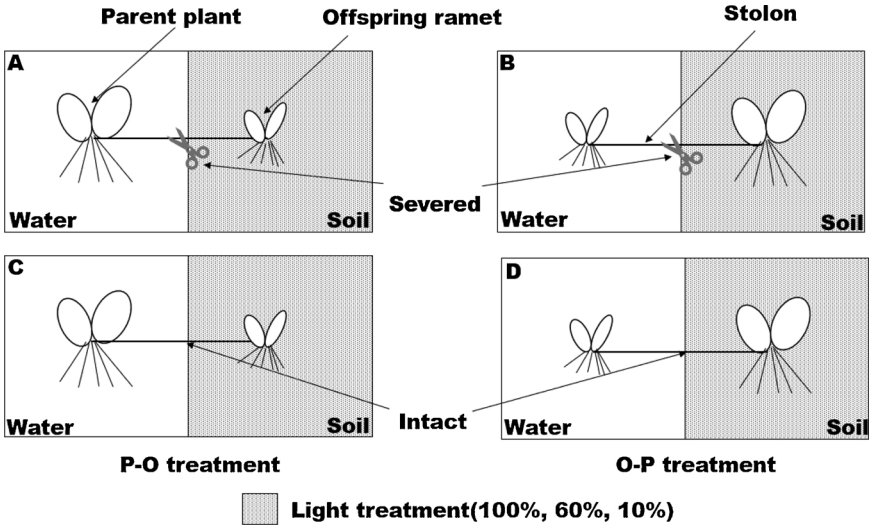

Fig. 1. Schematic representation of the experimental design. Containers (diameter = 70 cm and height = 42 cm) were divided into two equal parts: clay (height = 30 cm) and water (depth = 28 cm). Each clonal plant of *Eichhornia crassipes* consisted of one parent plant and one offspring ramet. We simulated two treatments of resource translocation: (1) the parent plant was grown in water and the connected offspring ramets were grown in soil (**P-O treatment**; **A, C**); (2) the offspring ramets were grown in water and the connected parent plants were grown in soil (**O-P treatment**; **B, D**). Clonal integration: stolon connected (allowing integration) (**C, D**) or not connected (preventing integration) (**A, B**). Light conditions: part of the soil was selectively shaded by a net to establish three natural light concentrations: 100%, 60%, and 10%. A total of 72 plants were randomly assigned to the 12 treatments, with six replicates per treatment.

Villamagna and Murphy, 2010; Adebayo et al., 2011). Clonal integration aids connected ramets by sharing resources and transferring signals for dispersal (Alpert, 1996; Jelínková et al., 2012). For example, *E. crassipes* can benefit from clonal integration in response to defoliation (Lyu et al., 2016), and the parent plant of *P. stratiotes* provides photosynthates to offspring ramets when they are connected (Wang et al., 2014). Thus, invasive clonal plants can benefit from clonal integration when resources are heterogeneously distributed (Wang et al., 2016a; You et al., 2016) as clonal plants produce more ramets in nutritionally favourable patches to efficiently exploit the resources. These specialized independent subunits have specific tasks (Stueffer et al., 1996; Hutchings and Wijesinghe, 1997; Li et al., 2018).

Eichhornia crassipes (Mart.) Solms (Pontederiaceae) is an aquatic vascular plant with floating and rooted forms; it is a perennial herb native to South America that mainly relies on asexual reproduction to produce new ramets and form dense mats (Villamagna and Murphy, 2010; Rezania et al., 2015). This invasive clonal aquatic plant has had significant negative ecological and socio-economic effects (Villamagna and Murphy, 2010). For example, *E. crassipes* can decrease phytoplankton production, light intensity, and the level of dissolved oxygen (Mangas-Ramirez and Elias-Gutierrez, 2004; Perna and Burrows, 2005) and significantly affect the native community composition and food-web structure (Mitchell, 1985; Grenouillet et al., 2002). To enhance its potential for light acquisition, *E. crassipes* increases the light interception area through horizontal growth of the stolons or rhizomes and by the placement of new ramets in resource-rich patches (Méthy et al., 1990). Field observation and experimental research revealed that these amphibious clonal plants are able to survive in wetlands by using their complex root structure to directly absorb nutrients from the soil (Mitchell, 1985; You et al., 2013; Liu et al., 2016). In the littoral region, the light conditions are usually distributed heterogeneously among plant individuals and within the community (Howard-Williams and Lenton, 1975). Therefore, they are more likely to affect littoral zone ecosystems, and they are subjected to resource heterogeneity often found in littoral zones, especially suffer from the shortage of light resources. However, the mechanisms for growth and invasion performance of *E. crassipes* in the littoral zone remain unknown.

Here, we investigated the effects of clonal integration on the growth and physiological responses of *E. crassipes* ramets under variable light conditions in a simulated littoral zone. Specifically, we tested the following two hypotheses:

(1) Clonal integration significantly increases growth performance of *E. crassipes* under various natural light concentrations.
(2) Clonal integration can increase the expansion of *E. crassipes* in the littoral zone.

2. Materials and methods

2.1. Plant material

Plants were collected from monocultures in Liangzi Lake (30°05′–30°18′N, 114°21′–114°39′E) in January 2015 and placed in aquariums filled with Liangzi Lake water in a greenhouse. On April 20, 2015, we selected 82 similarly sized plants in which the parent plant was connected to only one offspring ramet. Ten *E. crassipes* plants were randomly selected and dried at 70 °C for 72 h to determine the initial biomass (mean ± SE; parent plant = 1.43 ± 0.07 g; offspring ramet = 0.58 ± 0.04 g; stolon length = approximately 14 cm). The 72 remaining plants were selected for the experiment.

2.2. Experimental design

To simulate the littoral zone, containers (diameter = 70 cm and height = 42 cm) were divided into two equal parts (Fig. 1): (i) clay, to simulate the terrestrial environment (mean ± SE; 0.66 ± 0.06 mg total N g^{-1} and 0.85 ± 0.08 mg total P g^{-1}; height = 30 cm); and (ii) water, to simulate the aquatic environment (mean ± SE; 0.59 ± 0.05 mg total N L^{-1} and 0.03 ± 0.007 mg total P L^{-1}; water depth = 28 cm).

The experiment was a three-way factorial design, with direction of resource transportation, clonal integration, and light conditions as treatment factors. For the direction of resource transportation, 36 parent plants were grown in the aquatic environment and connected offspring ramets were grown in the terrestrial environment (P-O treatment; Fig. 1A, C), and 36 offspring ramets were grown in the aquatic environment and connected parent plants were grown in the terrestrial environment (O-P treatment; Fig. 1B, D). For clonal integration, the stolon remained connected (allowing integration) (Fig. 1C, D) or was severed (preventing integration) (Fig. 1A, B). For light conditions, the soil patches in each terrestrial container were selectively shaded with a net to establish a bright-light area (100% natural daylight), medium-light area (60% natural daylight), and poor-light area (10% natural daylight). A total of 72 plants were randomly assigned to the 12 treatments, with six replicates per treatment.

2.3. Treatments and measurements

The experiment began on April 27, 2015. Seven days after planting, half of the stolons were severed (18 plants in each of the P-O and O-P treatments). Daily illumination was recorded with a Digital Luxmeter (ZDS-10 W-2D; JiaDingXueLian, Co., Ltd., Shanghai, China) every 8 h

Table 1
Analysis of variance of the effects of severance (S) and illumination (I) on the growth performance of *Eichhornia crassipes* offspring ramets (terrestrial environment), parent plants (aquatic environment), and whole plants.

P-O treatment	Offspring ramet			Parent plant			Whole plant		
	S	I	S × I	S	I	S × I	S	I	S × I
Total mass	110.380^{***}	151.144^{***}	0.578^{ns}	6.721^{*}	15.443^{***}	20.704^{***}	16.011^{***}	96.885^{***}	14.453^{***}
Leaf mass	23.005^{***}	31.441^{***}	1.665^{ns}	8.401^{**}	2.544^{ns}	6.321^{**}	27.359^{***}	12.543^{***}	2.793^{ns}
Shoot mass	52.054^{***}	74.136^{***}	0.640^{ns}	6.857^{*}	1.809^{ns}	7.210^{**}	4.024^{ns}	32.820^{***}	5.571^{**}
Root mass	23.321^{***}	32.175^{***}	2.991^{ns}	8.991^{**}	18.400^{***}	27.812^{***}	0.213^{ns}	28.673^{***}	16.862^{***}
Leaf area	44.726^{***}	176.383^{***}	5.955^{**}	18.542^{***}	34.136^{***}	17.328^{***}	3.705^{ns}	36.115^{***}	3.768^{*}
Ramet number	5.213^{*}	76.782^{***}	4.495^{*}	2.248^{ns}	4.450^{*}	5.000^{*}	5.668^{*}	51.375^{***}	4.897^{*}
Stolon length	14.403^{**}	25.822^{***}	0.082^{ns}	5.809^{*}	8.111^{**}	4.040^{*}	14.305^{**}	21.667^{***}	1.153^{ns}
Chlorophyll content	3.968^{ns}	6.027^{**}	0.323^{ns}	0.417^{ns}	0.878^{ns}	2.109^{ns}	3.584^{ns}	2.135^{ns}	0.637^{ns}
Fv/Fm	21.856^{***}	90.885^{***}	85.620^{***}	0.054^{ns}	506.156^{***}	1.907^{ns}	23.329^{***}	523.512^{***}	100.407^{***}

Values are *F*; significant *P* values ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{ns}P \geq 0.05$).

(mean ± SE; 100% natural daylight = 5081.67 ± 543.96 μmol m^{-2} s^{-1}; 60% natural daylight = 3002.83 ± 341.25 μmol m^{-2} s^{-1}; and 10% natural daylight = 591.67 ± 80.57 μmol m^{-2} s^{-1}). Soil temperature and humidity were measured with a soil moisture probe (SIN-TN8; Liance Instrument, Co., Ltd., Hangzhou, China) (mean ± SE; temperature = 29.94 ± 1.56 °C and humidity = 33.30 ± 2.60%); and the physicochemical characteristics of the water were measured with a Professional Plus Multiparameter Instrument (YSI Incorporated, Yellow Springs, OH, USA) (mean ± SE; pH = 8.24 ± 0.27; temperature = 30.06 ± 0.55 °C; dissolved oxygen = 2.11 ± 0.34 mg·L^{-1}; total dissolved solids = 80.86 ± 16.63 mg·L^{-1}; conductivity = 134.73 ± 26.72 μS·cm^{-1}; and salinity = 0.06 ± 0.01 ppt).

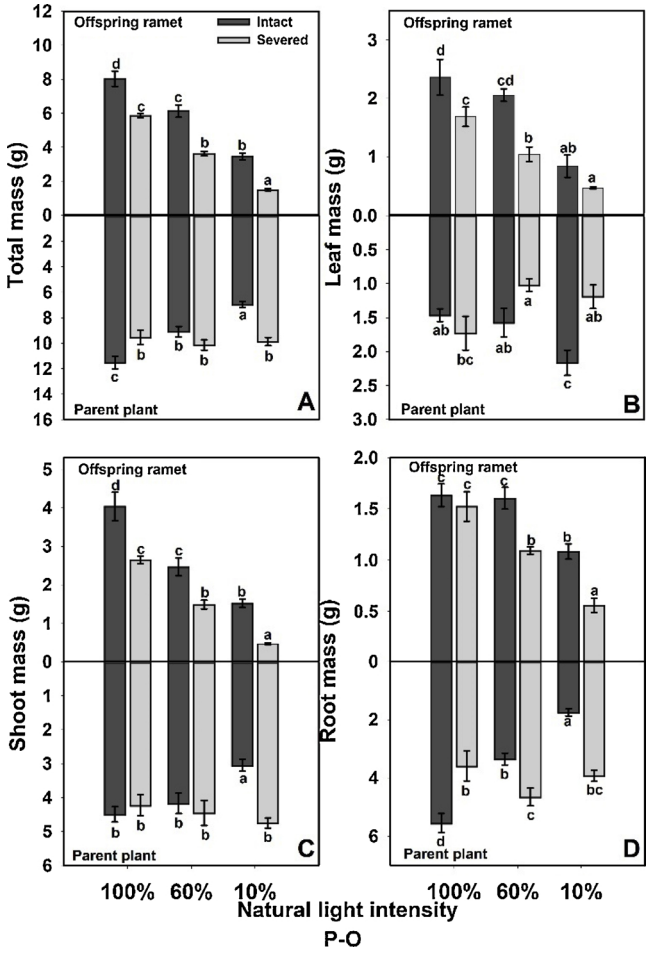

Fig. 2. Effects of illumination and severance on total mass (A), leaf mass (B), shoot mass (C), and root mass (D) of *Eichhornia crassipes* in the P-O treatment. The data indicate the mean ± SE.

The plants were harvested on July 10, 2015, after 82 days of growth. Before harvest, the plants were adapted to natural dark conditions until 04:00 h to ensure sufficient time for photosystem II reaction centres to remain open. The minimum (F_0) and the maximum (F_m) fluorescence yield were measured on fully formed, healthy leaves (for parent and offspring) using a portable chlorophyll fluorometer (DIVING-PAM; Walz, Effeltrich, Germany). The maximum quantum yield of photosystem II (F_v/F_m) was calculated as $(F_m\text{-}F_0)/F_m$ (Schreiber et al., 1998).

The plant materials were divided into parent and offspring parts and then measured or recorded for leaf area (LI-COR, LI-3100 AREA METER, USA), stolon length, number of secondary (2°) ramets, and expansion direction (recorded the plants that produced 2° ramets towards the water or soil by branching angle in the terrestrial environment). In addition, we selected fresh and healthy leaves to measure chlorophyll content by a spectrophotometer (UV-1800; SHIMADZU, Tokyo, Japan). Leaf tissue (0.2 g fresh weight) was cut into pieces, ground, and placed in 10-mL centrifuge tubes. The chlorophyll was extracted with 80% acetone for 48 h in the dark, until the leaf was fully dissolved. The absorbance was measured at 645 and 663 nm against 80% acetone as a blank. The total chlorophyll content was calculated according to Lichtenthaler and Wellburn (1983), and then each part was separated into roots, shoots (including stolon and stem), and leaves, dried at 70 °C for 72 h, and weighed.

2.4. Statistical analysis

Two-way analysis of variance (ANOVA) was implemented to test for the effects of stolon connection and light condition on plant traits. The P-O treatment data regarding total mass and root mass of the parent plant and the O-P treatment data regarding the leaf area of the offspring ramets were $\log_{10}$ transformed before analysis. The other data required no transformation to meet requirements for homoscedasticity and normality. Post-hoc pair-wise comparisons of the means were performed to examine differences between the treatments using the Duncan's test for multiple comparisons. Statistical significance was assigned at $P < 0.05$. All data analyses were performed using SPSS 22.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Growth performance of E. crassipes in the P-O treatment

Except for chlorophyll content, severance significantly affected ramet morphology and physiology (Table 1). All biomass traits, leaf

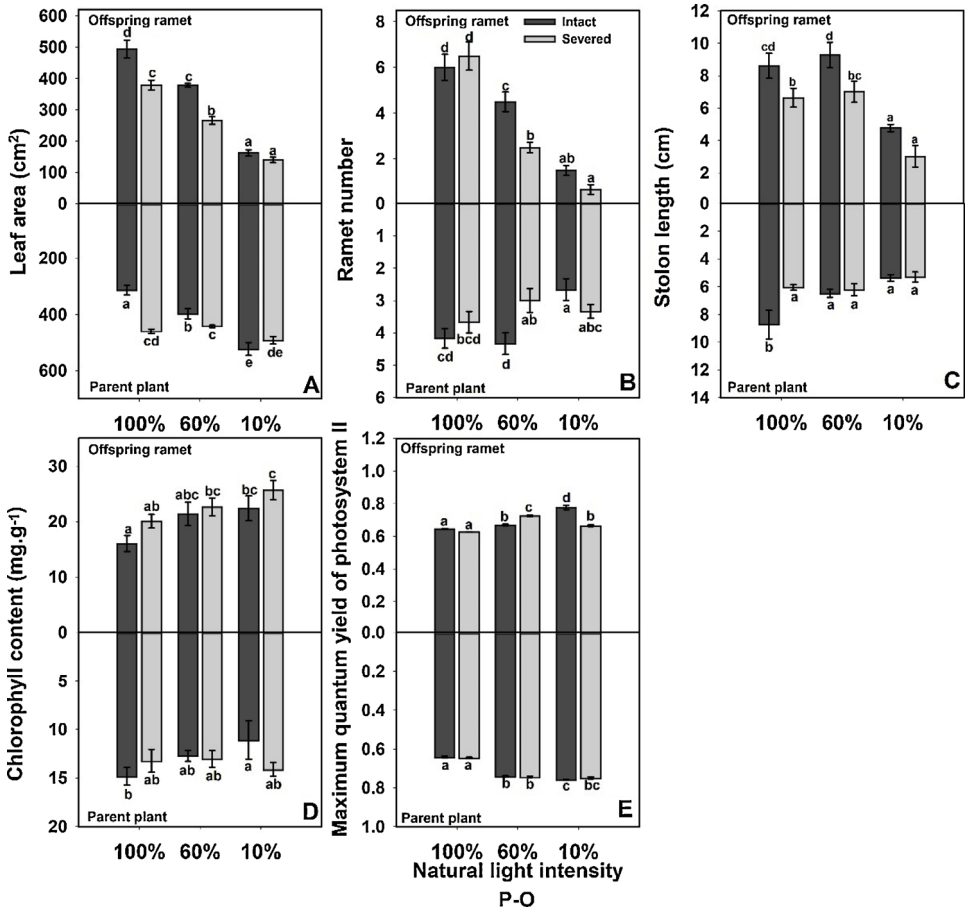

Fig. 3. Effects of illumination and severance on leaf area (A), ramet number (B), stolon length (C), chlorophyll content (D), and maximum quantum yield of photosystem II (*Fv/Fm*) (E) of *Eichhornia crassipes* in the P-O treatment. The data indicate the mean ± SE.

area, and stolon length were affected by severance; total mass, root mass, leaf area, ramet number, stolon length, and *Fv/Fm* were affected by light (Table 1). Total mass, leaf mass, ramet number, stolon length, and *Fv/Fm* of the whole plant were affected by severance (Table 1). Except for chlorophyll content, all the whole-plant traits were affected by light (Table 1, Fig. 4H). The interactive effects of severance and light condition on leaf area, ramet number, and *Fv/Fm* of offspring ramets were significant (Table 1).

Except for chlorophyll content and *Fv/Fm*, the interactive effects of severance and light condition on all parent plant traits were significant (Table 1). Excluding leaf mass, stolon length, and chlorophyll content, the interactive effects of severance and light condition on all whole-plant traits were significant (Table 1). Regarding offspring ramets, severance significantly decreased all the mass traits, except the leaf mass under 10% natural light and root mass under 100% natural light (Fig. 2). Under 10% natural light, severance significantly increased the total mass, shoot mass, and root mass and decreased the leaf mass of the parent plant (Fig. 2). Conversely, Under 100% natural light, severance decreased the total mass and root mass of the parent plant (Fig. 2A, D). Under 60% natural light, except for higher root mass, severance had no significant effects on total mass, leaf mass, or shoot mass (Fig. 2).

Under 100% natural light, severance significantly decreased the leaf area of offspring ramets, while it significantly increased the leaf area of parent plants (Fig. 3A). Severance had no significant effects on ramet number under 100% and 10% natural light, while it significantly decreased the ramet number under 60% natural light (Fig. 3B). Severance led to a significantly lower stolon length in offspring and parent plants under 100% and in offspring plants under 60% natural light (Fig. 3C). The severance led to a significantly higher and lower *Fv/Fm* of offspring plants under 60% and 10% natural light, respectively, but had no effect on *Fv/Fm* under 100% natural light and the chlorophyll content (Fig. 3D, E).

All ramet traits, except for chlorophyll content and *Fv/Fm* under 10% natural light, were significantly reduced (Figs. 2 and 3). Total mass, shoot mass, root mass, ramet number, stolon length, and chlorophyll content of the parent plant were significantly reduced by lower light values, while leaf mass, leaf area, and *Fv/Fm* of the parent plant were significantly increased (Figs. 2 and 3).

Both severance and light limitation led to lower whole-plant mass and decreased morphological trait values (Fig. 4A–G). Significantly lower values were found in severed plants for: total mass, shoot mass, root mass, and stolon length under 100% natural light; total mass, leaf mass, leaf area, and ramet number under 60% natural light; and leaf mass under 10% natural light (Fig. 4A–G). Light had no significant effects on the chlorophyll content (Fig. 4H). The *Fv/Fm* of the intact whole plants gradually improved with decreasing light (Fig. 4I).

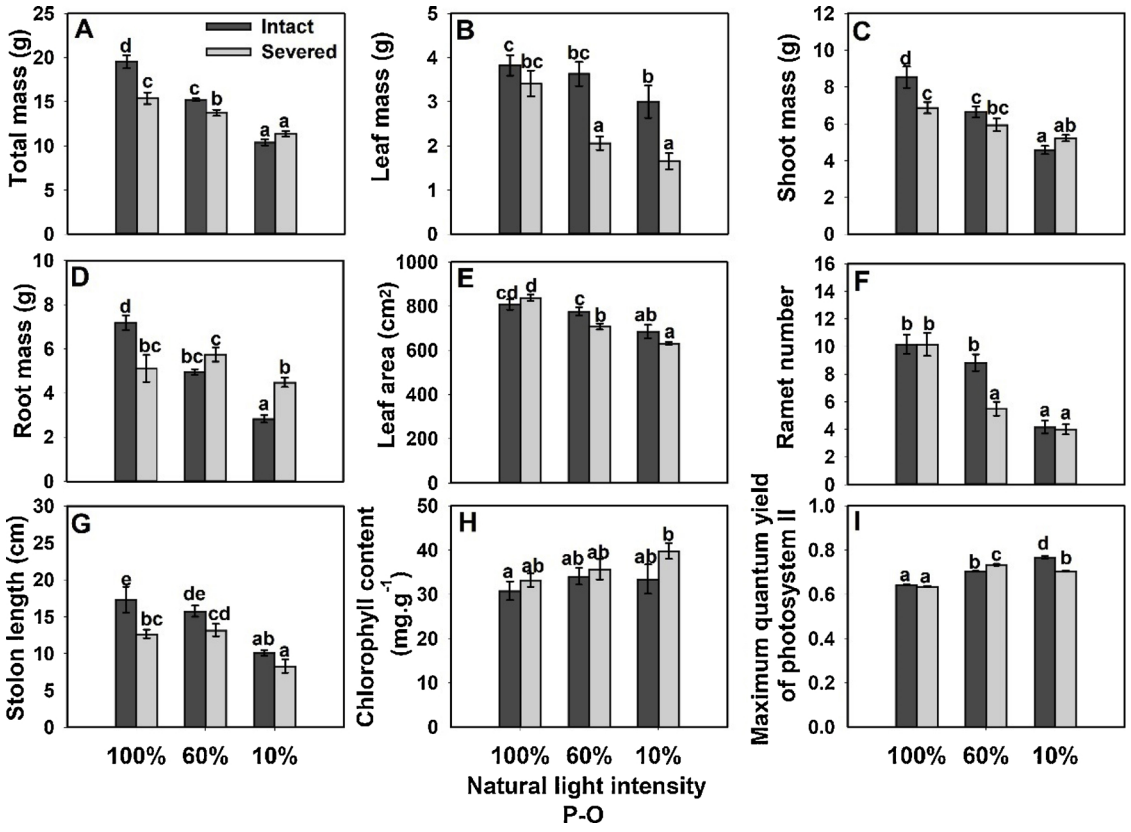

Fig. 4. Effects of illumination and severance on final mass, biomass allocation, morphology, and physiology of whole *Eichhornia crassipes* plants in the P-O treatment. The data indicate the mean ± SE.

Table 2
Analysis of variance of the effects of severance (S) and illumination (I) on the growth performance of *Eichhornia crassipes* in offspring ramets (aquatic environment), parent plants (terrestrial environment), and whole plants.

O-P treatment	Offspring ramet			Parent plant			Whole plant		
	S	I	S × I	S	I	S × I	S	I	S × I
Total mass	25.684^{***}	2.124^{ns}	0.964^{ns}	96.870^{***}	136.087^{***}	2.645^{ns}	25.134^{***}	83.789^{***}	1.394^{ns}
Leaf mass	9.292^{**}	3.590^{*}	1.350^{ns}	4.899^{*}	59.405^{***}	0.067^{ns}	0.956^{ns}	43.684^{***}	0.675^{ns}
Shoot mass	121.276^{***}	1.553^{ns}	2.972^{ns}	59.921^{***}	56.295^{***}	1.257^{ns}	7.193^{*}	46.349^{***}	1.848^{ns}
Root mass	0.263^{ns}	1.297^{ns}	0.641^{ns}	42.022^{***}	31.590^{***}	3.212^{ns}	25.801^{***}	17.107^{***}	0.879^{ns}
Leaf area	12.826^{**}	39.676^{***}	8.628^{**}	66.282^{***}	188.111^{***}	26.711^{***}	81.751^{***}	115.442^{***}	9.902^{***}
Ramet number	0.000^{ns}	2.395^{ns}	0.711^{ns}	17.784^{***}	49.569^{***}	5.013^{**}	17.357^{***}	20.827^{***}	11.625^{***}
Stolon length	7.971^{**}	1.505^{ns}	0.395^{ns}	5.469^{*}	3.053^{ns}	1.454^{ns}	0.221^{ns}	2.167^{ns}	0.137^{ns}
Chlorophyll content	0.773^{ns}	12.303^{***}	2.675^{ns}	0.010^{ns}	14.793^{***}	2.116^{ns}	0.559^{ns}	27.782^{***}	0.113^{ns}
Fv/Fm	0.946^{ns}	24.037^{***}	0.398^{ns}	24.781^{***}	65.402^{***}	3.552^{*}	19.984^{***}	25.474^{***}	3.207^{ns}

Values are *F*; significant *P* values ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{ns}P \geq 0.05$).

Severance significantly increased and decreased the whole-plant *Fv/Fm* under 60% and 10% natural light, respectively (Fig. 4I).

3.2. Growth performance of E. crassipes in the O-P treatment

Severance significantly affected the total mass, leaf mass, shoot mass, leaf area, and stolon length of the offspring ramets, while light significantly affected only the leaf traits (mass, area, chlorophyll content, and *Fv/Fm*) (Table 2). Severance had no significant effects on the root mass, ramet number, chlorophyll content, and *Fv/Fm* of the offspring ramets (Table 2; Figs. 5D and 6 B, D, E). Except for chlorophyll content and stolon length, severance and light significantly affected all the parent plant growth parameters (Table 2). Severance had no significant effects on leaf mass, stolon length, and chlorophyll content, while light significantly affected the whole-plant growth traits (except the stolon length) (Table 2; Fig. 7B, G, H). The interaction

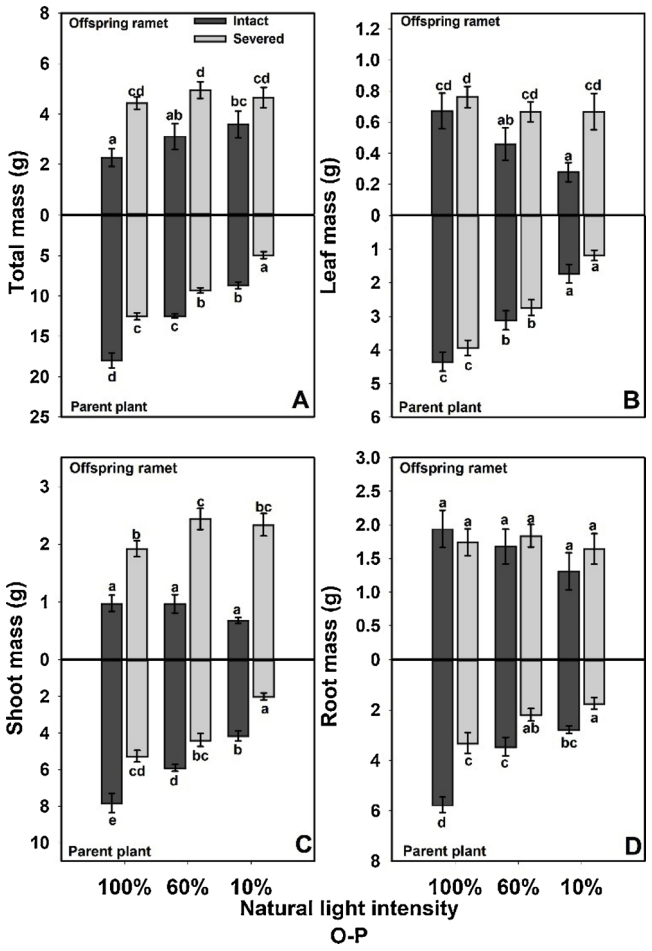

Fig. 5. Effects of illumination and severance on total mass (A), leaf mass (B), shoot mass (C), and root mass (D) of *Eichhornia crassipes* in the O-P treatment. The data indicate the mean ± SE.

between severance and light condition had no significant effects on all the ramet growth traits, except leaf area; the leaf area, ramet number, and *Fv/Fm* of the parent plant; and all the traits, excluding leaf area and ramet number, of the whole plants (Table 2).

Severance increased the total mass, leaf mass, and shoot mass of the offspring ramets, but severed stolons greatly decreased the leaf area and stolon length of the offspring ramets under 10% natural light (Figs. 5A–C and 6 A, C). The leaf area and chlorophyll content of the offspring ramets gradually increased while their leaf biomass gradually decreased in response to severance (Figs. 5B, 6 A, D). Reducing the natural light to 60% significantly increased the *Fv/Fm* of offspring ramets (Fig. 6E).

Most growth features were larger when the stolon connection was intact than when it was severed (Figs. 5 and 6). Significantly higher values were found in the total mass, shoot mass, root mass, leaf area, and *Fv/Fm* of intact parent plants (Figs. 5A, C, D and 6 A, E), while severance greatly increased the stolon length of parent plants under 10% natural light (Fig. 6C). Limited light resources notably decreased the parent plant biomass accumulation and leaf area (Figs. 5 and 6A). The minimum ramet number was found in both the intact and severed parent plants under 10% natural light (Fig. 6B). The chlorophyll content of parent plants markedly increased with increasing light (Fig. 6D). Compared with 100% natural light, 10% natural light led to significantly higher chlorophyll content and lower *Fv/Fm* of the parent plants (Fig. 6E).

Severance significantly decreased the biomass accumulation, leaf area, ramet number, and *Fv/Fm* of the whole plants under restricted light conditions (Fig. 7 A–F, I). The intact stolon supported a maximum leaf area and ramet number at 60% natural light (Fig. 7E, F). Except for stolon length under various light intensities, the whole-plant growth performance gradually decreased, while chlorophyll content increased (Fig. 7).

3.3. The expansion of new ramets (2°)

As light intensity decreased, the number of 2° ramets gradually decreased in the terrestrial environment and increased in the aquatic environment for the O-P treatment (Table 3; Fig. 8). Severance significantly decreased the number of 2° ramets when plants were grown in the terrestrial environment with 60% natural light, while it significantly increased 2° ramets when plants were grown in water with 100% natural light in the P-O treatment (Table 3; Fig. 8). The intact stolon significantly increased 2° ramets in the terrestrial environment (Table 3; Fig. 8).

4. Discussion

4.1. Clonal integration significantly increases growth performance of *E. crassipes* under various natural light concentrations

We found that clonal integration between connected individuals in different habitats increased the growth performance and clonal reproduction of *E. crassipes*, particularly when individuals were subjected to restricted light conditions. This is consistent with other studies that have shown that clonal integration can be ameliorated when connected ramets are exposed to different resource restrictions (Dong et al., 2015; Lyu et al., 2016; Luo et al., 2017).

Compared with severed stolons, clonal integration notably promoted *E. crassipes* growth performance. First, well-established parent plants supported the growth of the interconnected offspring ramets under reduced light intensities, which was likely due to the acropetal resource translocation via clonal integration (Wang et al., 2008; You et al., 2014). Second, we found that basipetal resource translocation also exists in *E. crassipes*: intact offspring ramets effectively utilized local resources to assist the parent plant when it encountered environmental stress. This showed that flexible resource transmission direction via clonal integration supports the survival and reproduction of *E. crassipes*; particularly, resources were distributed differently among donor and recipient ramets (Roiloa and Retuerto, 2007; Xu et al., 2010; Chen et al., 2015).

Many experiments have shown that clonal integration can increase the growth performance of parent plants when connected offspring ramets are exposed to nutrient or water deficiency, shading, or herbivory (Roiloa and Retuerto, 2007; Wang et al., 2008; Luo et al., 2017). However, in the present study, clonal integration had negative effects on intact-plant growth in the aquatic environment. For example, clonal integration significantly reduced parent plant biomass accumulation and leaf area in the aquatic P-O treatment (Table 1; Figs. 2 and 3A). Moreover, clonal integration greatly decreased the biomass of offspring ramets in the aquatic O-P treatment (Table 2; Fig. 5). However, clonal integration significantly increased whole-plant growth. Our results were consistent with those for *P. paspaloides* (Luo et al., 2017) and *A.*

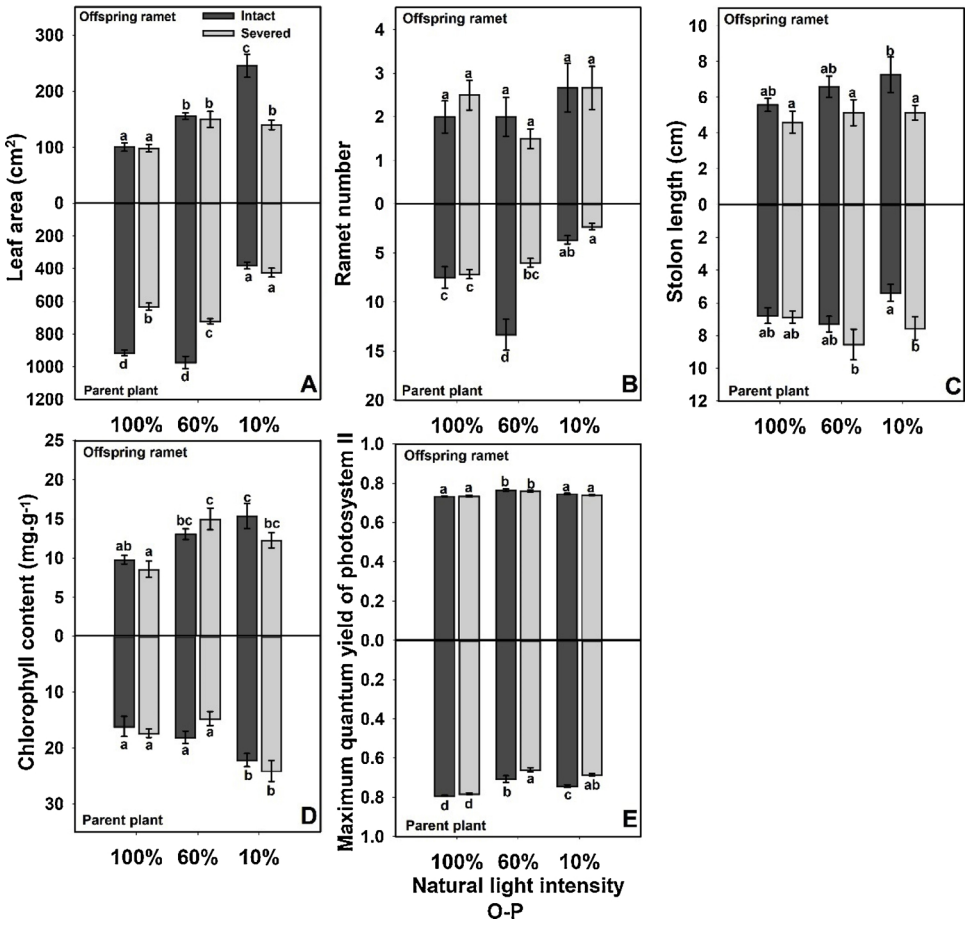

Fig. 6. Effects of illumination and severance on leaf area (A), ramet number (B), stolon length (C), chlorophyll content (D), and maximum quantum yield of photosystem II (*Fv*/*Fm*) (E) of *Eichhornia crassipes* in the O-P treatment. The data indicate the mean ± SE.

philoxeroides (Wang et al., 2009; Dong et al., 2015). Because the income of recipient ramets is markedly more than the cost of donor ramets, the growth performance of the whole plant is significantly enhanced by clonal integration, particularly when connected ramets differ in uptake ability and external resources are distributed heterogeneously (Alpert, 1999; Vermeulen et al., 2009; Dong et al., 2015).

Clonal integration significantly modified the biomass allocation of *E. crassipes*, which concurs with previous studies on other clonal plants (Stueffer et al., 1996; Roiloa and Retuerto, 2007; Wang et al., 2008). For the P-O treatment, clonal integration greatly increased the parent plant biomass allocation to leaves, at the expense of that to roots and shoots. This is most likely because parent plants need to enhance photosynthetic capacities to help the offspring ramets when exposed to shading. For the O-P treatment, clonal integration increased the offspring ramet biomass allocation to roots and decreased that to leaves and shoots. This is probably because it is more important for offspring ramets to maintain stable nutrient absorption and share nutrients with the parent plant when subjected to shading. Therefore, biomass allocation of *E. crassipes* under the two treatments was consistent with the theory of 'labour division' in clonal plants. For example, ramets can produce functional specializations that are physiologically or morphologically conducive to the absorption of specialized resources (Hutchings and Wijesinghe, 1997; Sergior et al., 2007; Rodríguez et al., 2018).

For the P-O treatment, the offspring ramets could enhance their adaptability to shade by increasing the photosynthetic performance (Fig. 3E). 'Shade tolerance strategy' enables plants to develop shade-adapted leaf physiological or morphological traits such as increased leaf area, chlorophyll content, and maximum quantum yield (Givnish, 1988; Valladares and Niinemets, 2008). Furthermore, when connected to the parent plant in the aquatic environment, the offspring ramets and whole plants had significantly higher photosynthetic capacities (particularly with 10% natural light) and leaf area compared to severed plants (Figs. 3D, E and 4 H, I). Furthermore, in the O-P treatment, the parent plants and whole plants also benefited from unlimited offspring ramets in the aquatic environment through clonal integration (Figs. 5–7). These results support the hypothesis that clonal integration is an additional compensatory mechanism for clonal plants, and it improves the survival of connected ramets that may be affected by various stress factors (Liu et al., 2009; Lyu et al., 2016).

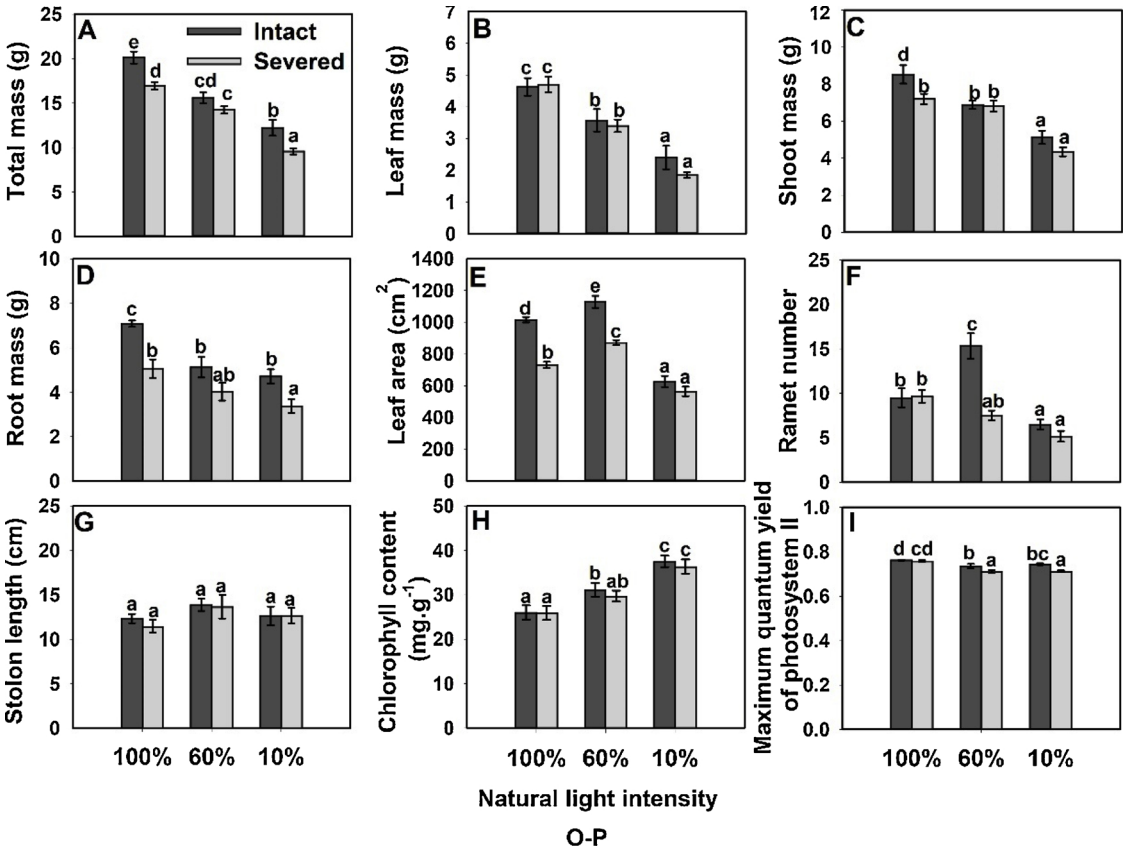

Fig. 7. Effects of illumination and severance on final mass, biomass allocation, morphology, and physiology of whole *Eichhornia crassipes* plants in the O-P treatment. The data indicate the mean ± SE.

Table 3
Two-way analysis of variance of the effects of severance (S) and illumination (I) on the distribution of new secondary ramets (2°) of *Eichhornia crassipes* from the offspring ramets or parent plants (terrestrial environment) under P-O or O-P treatment.

	Severance (S)	Illumination (I)	S × I
'P-O'			
New 2° ramets of 'offspring ramet'			
2° ramets (towards aquatic environment)	0.298^{ns}	22.384^{***}	7.550^{**}
2° ramets (towards terrestrial environment)	6.914^{*}	18.397^{***}	1.746^{ns}
'O-P'			
New 2° ramets of 'parent plant'			
2° ramets (towards aquatic environment)	1.181^{ns}	21.691^{***}	4.898^{*}
2° ramets (towards terrestrial environment)	12.011^{**}	5.291^{*}	4.769^{*}

Values are *F*; significant *P* values ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{ns}P \geq 0.05$).

4.2. Clonal integration can increase the expansion of E. crassipes in littoral zones

The severed *E. crassipes* in the terrestrial environment produced 2° ramets mainly towards the aquatic environment (Table 3; Fig. 8). This is likely because clonal plants tend to obtain more light resources from aquatic environments (Wang et al., 2016b). Clonal plants usually place more nutrient-absorbing organs (e.g., roots and ramets) in resource-rich microsites when experiencing patchy environments (Hutchings and Kroon, 1994; Xiao et al., 2006; Gao et al., 2012). In the present study, we found that clonal integration supports the spread of *E. crassipes* into the terrestrial environment with reduced light conditions by producing more 2° ramets (Fig. 8). In addition, the stolon length of offspring ramets was significantly increased by clonal integration in the P-O treatment. Greater elongation of the stolon length may be a clonal plant response to light resource limitation (Bell and Galloway, 2008), and the change in branching angle and position of offspring ramets the plant's tactic to escape unfavourable patches (Cain et al., 1996; Xiao et al., 2006; Wang et al., 2016b).

4.3. Conclusions

Clonal integration most likely enables *E. crassipes* to expand from aquatic to terrestrial habitats. Clonal integration may improve the growth and spread of ramets subjected to various environmental stresses in littoral zones. Thus, invasive aquatic plants with a high ability for clonal integration may be a potential threat to littoral zone ecosystems. Future studies should focus on how diversified ecological factors, such as temperature or competition, affect the invasive performance of *E. crassipes*

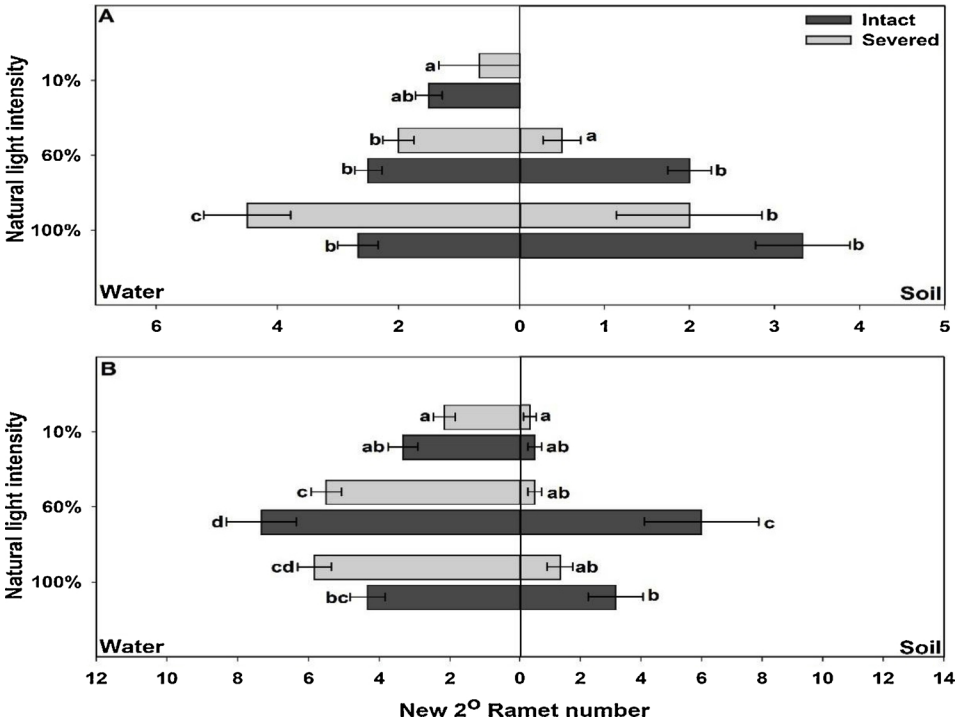

Fig. 8. Effects of illumination and severance on target plant (offspring ramets in the P-O treatment [A] or parent plants in the O-P treatment [B]) production of new secondary (2°) ramets of *Eichhornia crassipes* under different natural light concentrations. The data indicate the mean ± SE.

Declaration of interest

None.

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