

# Developmentally-programmed division of labour in the clonal invader *Carpobrotus edulis*

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**Abstract** Invasive species are one of the main causes for the loss of global biodiversity. However, the mechanisms that explain the success of invasive species remain unsolved. Clonal growth has been pointed out as an attribute that could contribute to the invasiveness of plants, however little research has been conducted to determine the importance of clonal traits in successful invaders. One of the most interesting attributes related to clonal growth is the capacity for division of labour. In this experiment we investigated the capacity for division of labour in the aggressive invader *Carpobrotus edulis*, and how clonal integration can contribute to the expansion of this species. Division of labour was determined by studying the degree of morphological and physiological specialization of individual ramets to a specific activity: acquisition of soil or aboveground resources and aboveground expansion. Our results showed that there is division of labour in the clonal fragments, with older ramets increasing the biomass allocated to roots (specialization in the uptake of belowground resources) and younger ramets increasing the

chlorophyll content and aboveground biomass (specialization in the uptake of aboveground resources). Physiological integration allows division of labour, and as consequence the overall performance of the clonal fragment was enhanced, with connected clonal fragments showing a higher total biomass than severed clonal fragments. Division of labour increased the aboveground growth of apical ramets of *C. edulis*, and therefore could contribute to an effective colonization of the surrounding area by this aggressive invader. Our study is the first exploring the role of division of labour in the expansion of an invader, and supports the idea that clonal traits could increase the invasiveness of plant species.

**Keywords** Biological invasions · Biomass allocation · Chlorophyll fluorescence · Clonal growth · Physiological integration · Spectral reflectance

## Introduction

The study of biological invasions is a rapidly developing field in ecology. Nowadays, biological invasions represent one of the most serious environmental risks worldwide because alien invasive species can modify the stability and functioning of local communities, and displace native plants with the consequent loss of biodiversity (Vitousek et al. 1996; Mack et al. 2000). However, the mechanisms that explain the success of invasive species remain unsolved

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(Rejmánek and Richardson 1996; Alpert et al. 2000; Levine et al. 2003, Blackburn et al. 2011). Some plant characteristics, such as clonal growth, could explain the success of invasive species (Pyšek 1997; Liu et al. 2006; Wang et al. 2008; Roiloa et al. 2010). In fact, clonal plants are usually dominant species in terrestrial ecosystems, and play an important role in the dynamics of many plant communities and ecosystem functioning (Schmid 1990; Oborny and Bartha 1995; Kliměš et al. 1997). Key attributes related to clonal growth—such as physiological integration, habitat selection and division of labour—allow clonal plants to compete successfully in a wide range of habitats (Hartnett and Bazzaz 1983; Alpert and Stuefer 1997; Saitoh et al. 2002; Roiloa and Retuerto 2006; Roiloa et al. 2007). Despite that many of the most aggressive invasive plant species show clonal growth, little research has been conducted to determine the role of clonal traits in successful invaders (Pyšek 1997; Amsberry et al. 2000; Peltzer 2002; Liu et al. 2006, 2008; Otfinowski and Kenkel 2008; Wang et al. 2008; Roiloa et al. 2010).

Clonal growth is characterised by the asexual production of offspring (ramets) that can remain attached to the parent for a variable period of time (Pitelka and Ashmun 1985; Price and Marshall 1999). Connection allows the transport of resources between ramets. This phenomenon has been called physiological integration, which allows the clonal system to compensate heterogeneous distribution of essential resources that occurs in natural habitats. This is, to say physiological integration allows clones to act as cooperative systems, which can buffer differences in resources availability due to environmental heterogeneity (Hartnett and Bazzaz 1983; Slade and Hutchings 1987; Klimes et al. 1997; Alpert 1999). One of the most interesting attributes related to clonal growth is the capacity for division of labour under certain conditions. Division of labour in plants seems to be exclusive for clonal species and it has been traditionally defined as the capacity of specialization to acquire locally abundant resources to increase the overall performance of the clone (Alpert and Stuefer 1997; Hutchings and Wijesinghe 1997; Stuefer 1998). When the availabilities of two resources are negatively correlated, connected ramets may specialize to acquire the resource that is more abundant for each ramet. The subsequent reciprocal resource sharing between the connected ramets will increase their overall

performance (Friedman and Alpert 1991; Birch and Hutchings 1994; Stuefer et al. 1996; Alpert and Stuefer 1997). In spite of the benefits of division of labour of clonal plants, no previous research has been conducted to determine the importance of ramets specialization for the invasiveness of clonal species.

In this experiment we investigated the capacity for division of labour in the aggressive invader *Carpobrotus edulis*, and how division of labour and physiological integration can contribute to the invasiveness of this species. We hypothesized that clonal fragments of *C. edulis* work as a cooperative system allowing its expansion into new habitats. To test this hypothesis we determined the capacity for division of labour in connected clonal fragments, and we compared performance between connected and severed clonal fragments. Division of labour was determined by estimating the degree of morphological and physiological specialization of individual ramets to a particular activity: acquisition of soil or aboveground resources, and aboveground growth. The capacity of division of labour has been studied both at morphological (Alpert and Stuefer 1997; Hutchings and Wijesinghe 1997; Stuefer et al. 1996; Stuefer 1998) and physiological level (de Kroon et al. 1996; Roiloa et al. 2007; Wang et al. 2011). This research contributes to determine role of clonality in plant invasions, which has been scarcely explored before (Pyšek 1997; Peltzer 2002; Otfinowski and Kenkel 2008; Roiloa et al. 2010). In addition, as far as we know, this is the first research pointing out division of labour as a potential trait favoring the expansion of clonal invaders.

Our specific hypotheses were: (1) Connection allows division of labour between ramets of the clonal fragment. Considering that in clonal plants acropetal transport of soil-based resources (i.e. from the older to the younger ramet) generally exceeds basipetal transport (i.e. from the younger to the older ramet) (Alpert and Mooney 1986; Price and Hutchings 1992; Alpert 1996), we predict a greater biomass allocation to roots (as an indicator of the energy allocated to harvest below-ground resources) in older compared to younger ramets of the connected clonal fragments. Also, we expect higher chlorophyll content (as an indicator of the energy allocated to harvest above-ground resources) in younger than in older ramets of the connected clonal fragments. (2) Physiological integration induces division of labour and increases the

overall performance of the clonal fragment. We expect higher photochemical efficiency and biomass production in connected than in severed clonal fragments. As a result, connected clonal fragments will have a higher growth capacity than severed clonal fragments. Because an increase in growth rates can be associated with a higher expansion capacity in clonal invaders (Wang et al. 2008; Roiloa et al. 2010), we expect that physiological integration, and more specifically capacity for division of labour, will increase the invasive capacity of *C. edulis*.

## Materials and methods

### Study species

*Carpobrotus edulis* (L.) N.E. Br. is a mat-forming succulent plant with perennial finger-shaped leaves, with showy flowers and succulent fruits. The plant grows clonally by stolons that spread along the soil surface, producing a new ramet at every other node (Wisura and Glen 1993). New ramets can take root after direct contact with soil and can survive if disconnected with the mother ramet. Clonal propagation allows *C. edulis* effectively colonize the surrounding area. *C. edulis* is native to the Cape Region (South Africa) and is an aggressive invader in many coastal habitats around the world, having a negative effect on diversity of the native flora (D'Antonio and Mahall 1991; D'Antonio 1993; Traveset et al. 2008; Vilà et al. 2008).

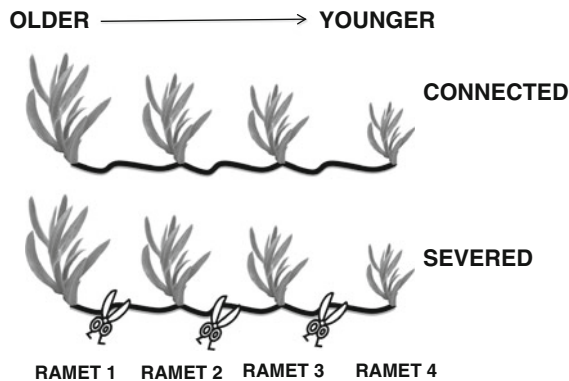
### Experimental material

Similarly-sized four-ramet clonal fragments of *C. edulis* were used in the experimental treatments. The clonal fragments were obtained by excising connected groups of unrooted ramets from clumps of *C. edulis* invading a sand dune system at the Mata Nacional das Dunas Quiaios (Portugal) (40°13'N, 8°53'W) (see Costa et al. 2000 for a detailed description of the vegetation at the collection site). Eight clumps of similar size were selected for the fragments collection. At the edge of each clump two clonal fragments were collected. All the clonal fragments contained the first, second, third and fourth ramets from the apices, and therefore we ensured that fragments in all the treatments had ramets of the same age, size and

developmental stage. Initial size of clonal fragments was determined using length ( $21.90 \pm 0.70$  cm, mean  $\pm$  SE,  $n = 16$ ) and fresh mass ( $60.22 \pm 3.82$  g, mean  $\pm$  SE,  $n = 16$ ). Preliminary analysis showed no differences in initial sizes between the clonal fragments assigned to the different treatments (ANOVA for initial length of clonal fragments  $F_{1, 14} = 0.017$ ,  $P = 0.899$ ; ANOVA for initial fresh mass of clonal fragments  $F_{1, 14} = 0.291$ ,  $P = 0.598$ ). To avoid potential confounding effect of orientation in the field, all the clonal fragments were collected in the east–west direction. At the start of the experiment, each of the unrooted clonal fragments was placed in individual expanded polystyrene containers (50 cm long  $\times$  30 cm width  $\times$  10 cm depth) filled with sand from a coastal dune system where *C. edulis* inhabits. *C. edulis* is a plant with succulent leaves and thick stolons that can act as a reservoir of resources. Unrooted ramets of *C. edulis* can survive healthy for several weeks before taking roots (personal observation). The size of the containers used in the experiment was enough to avoid confinement of the roots and to allow aboveground expansion.

### Experimental design

The experiment includes “ramet age” (four levels) and “connection” (connected, severed) as main factors. The “ramet age” factor comprises four stages: ramet 1, ramet 2, ramet 3 and ramet 4, corresponding with the fourth, third, second and first ramets from the apice, respectively. Therefore, ramet 1 is the oldest and ramet 4 is the youngest ramet of the fragment (see Fig. 1). In the “connection” treatment, ramets within each clonal fragment were either left connected (division of labour is allowed) or disconnected from each other (division of labour is prevented) (see Fig. 1). Immediately after the transplantation we cut the stolon halfway between every two adjacent ramets. This realistically reflects the fact that natural and human disturbance frequently break clonal fragments into smaller groups (Stuefer and Huber 1999; Latzel and Klimešová 2009). We did not observe any immediate negative effect of cutting the stolon (as sudden death or diseases). Therefore, we discarded any interference in our results derived from an initial trauma of cutting the stolon in the severed treatment. New ramets produced during the experiment were not allowed to root. Each treatment was replicated eight



**Fig. 1** Diagram of the experimental design showing the factor “connection” with two states (connected or severed) and the factor “ramet age” with four states (*ramet 1*, *ramet 2*, *ramet 3* or *ramet 4*). The age (older to younger) of the ramets within the clonal fragment is shown. See text for details

times ( $n = 8$ ). *C. edulis* fragments from each original clump were represented equally in each treatment.

The experiment was carried out in an open-end greenhouse at the University of Santiago de Compostela (Spain) under a natural day/night light cycle. Plants were watered regularly to prevent water stress. The experiment began on 29 April 2011 and continued for 90 days.

## Measurements

### Leaf spectral reflectance

We determined spectral reflectance parameters using a portable spectrometer (UniSpec Spectral Analysis System, PP Systems, Haverhill, MA). Measurements were done on a new fully formed and healthy leaf that had developed during the experiment for each individual ramet of all the clonal fragments. Reflectance spectra were calculated as the spectral radiance of the leaf divided by the radiance of a reflective white standard (Spectralon Reflectance Standard, Labsphere, North Sutton, NH). The wavelength range employed was 300–1,100 nm. Spectral reflectance parameters were determined throughout the experiment at 30, 60 and 90 days from the start of the experiment. Repeated measurements allow us to integrate the effect of time into our treatments. The spectral reflectance indices determined were: (1) PRI, the photochemical reflectance index. PRI was calculated as  $(R_{531} - R_{570}) / (R_{531} + R_{570})$ , where R refers to reflectance and the

subscripts to a specific spectral wavelength, in nanometers. Changes in xanthophyll cycle epoxidation state are reflected at 531 wavelength, and 570 is a reference wavelength unaffected by xanthophyll activity (Guo and Trotter 2004). PRI is significantly correlated with both net  $\text{CO}_2$  uptake and the photosynthetic radiation-use-efficiency ( $\text{mol CO}_2 \text{ mol}^{-1} \text{ photons}$ ) (Peñuelas et al. 1995; Gamon et al. 1997). PRI has also been correlated to the levels of de-epoxidised (photoprotective) xanthophyll cycle pigments (Sims and Gamon 2002; Stylinski et al. 2002). (2) CHL, chlorophyll content index, was determined as  $R_{750}/R_{700}$ , and is correlated to the chlorophyll content of leaves (Wood et al. 1993; Lichtenthaler et al. 1996).

### Chlorophyll fluorescence

At the same dates and on the same leaves where the spectral reflectance parameters were recorded, we determined chlorophyll fluorescence parameters by the saturation pulse method (Schreiber et al. 1998), using a portable fluorometer (MINI-PAM photosynthesis yield analyzer; Walz GmbH, Effeltrich, Germany). Measuring light and saturating light pulses ( $>4,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , 0.8 s pulse length, actinic white light) were applied through a fiberoptic at a  $60^\circ$  angle relative to the sample and at 12 mm distance from the sample. We recorded the following chlorophyll fluorescence parameters: (1)  $F_v/F_m$ , the maximum quantum yield of photosystem II (PSII), was determined as  $(F_m - F_0)/F_m$  (Bolh ar-Nordenkampf et al. 1989), where  $F_0$  and  $F_m$  are, respectively, the minimal and maximal fluorescence yield of a dark-adapted sample, with all PSII reaction centres fully open (i.e. all primary acceptors oxidized). This parameter was measured after a 30-min period of dark adaptation, which is considered to be enough for opening all PSII reaction centers (Roiloa and Retuerto 2005).  $F_v/F_m$  estimates the efficiency of excitation energy capture by open PSII reaction centres (Butler and Kitajima 1975). (2)  $\Phi_{\text{PSII}}$ , the effective quantum yield of PSII, was calculated as  $(F'_m - F_t)/F'_m$  (Genty et al. 1989), where  $F'_m$  is the maximal fluorescence yield reached in a pulse of saturating light with an illuminated sample (i.e. fluorescence reached when the pool of the primary quinone electron acceptor,  $Q_A$ , of the PSII reaction centers is fully reduced and hence photochemistry impeded), and  $F_t$  is the measured steady-state fluorescence under a given photosynthetic photon flux density (PPFD). This parameter was measured under

the prevailing ambient light ( $172.04 \pm 11.54 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; mean  $\pm$  SE,  $n = 192$ ).  $\Phi\text{PSII}$  measures the fraction of the light absorbed by chlorophyll that is photochemically converted in PSII (Maxwell and Johnson 2000).

### *Growth and biomass allocation*

The four individual ramets on each of the clonal fragments were numbered consecutively, from the oldest (1) to the youngest (4). At the end of the experiment, each of these individual ramets was harvested separately. Each of the individual ramets was divided into aboveground mass (leaves, the stolon internode connecting it to the next youngest ramet in the clonal fragment, and new ramet produced) and roots. Each of these fractions was dried to constant mass at  $60^\circ\text{C}$  and weighed. Total mass (aboveground mass + root mass) was calculated for individual ramets. Proportional allocation of biomass to roots, determined as the root mass ratio (RMR = root mass/total mass), was calculated for individual ramets. Aboveground mass and root mass were determined for each clonal fragment by adding up the data of the four individual ramets. Total mass and the RMR were also calculated for each clonal fragment.

### Statistical analysis

Prior to analyses, as a requirement for the parametric tests, normality and homoscedasticity of the data were checked using Kolmogorov–Smirnov and Levene tests, respectively. Neither root mass nor RMR of individual ramets, or their transformations, met the requirements of parametric tests. Differences between treatments in aboveground mass and total mass of individual ramets were analysed using two-way analysis of variance (ANOVA) with “ramet age” and “connection” as main factors. We used the Scheirer–Ray–Hare test (Dyham 1999) (the non-parametric equivalent of two-way ANOVA) to examine differences in root mass and RMR of the individual ramets, with “ramet age” and “connection” as main factors. Differences in chlorophyll fluorescence (Fv/Fm and  $\Phi\text{PSII}$ ) and spectral reflectance (PRI and CHL) parameters for individual ramets were analyzed by two way analysis of variance with repeated measures (ANOVAR), with “ramet age” and “connection” as between-subject effects, and “time” as the within-subject effect.

Differences in aboveground mass, root mass, total mass and RMR for clonal fragments were tested by two-way ANOVAs, with “ramet age” and “connection” as main factor.

Significance level was set at  $P < 0.05$ . Statistical test were performed with IBM SPSS Statistics 19.0 (IBM Corporation, Armonk, NY, USA).

## Results

### Individual ramets

#### *Leaf spectral reflectance and chlorophyll fluorescence*

The chlorophyll content index (CHL) was significantly affected by “ramet age”. CHL was higher in younger ramets than in older ramets (Table 1; Fig. 2a). The interaction “ramet age”  $\times$  “connection” for CHL was not significant. However, additionally, we examined the effect of “ramet age” on CHL separately for the connected and severed treatment by one-way ANOVAs, with “ramet age” as main factor. These separate ANOVAs confirmed different responses of the connected and severed treatment. While CHL values were significantly higher in younger ramets of the connected treatment (ANOVA  $F_{3, 92} = 6.114$ ,  $P = 0.001$ ), there were no significant differences in the severed treatment (ANOVA  $F_{3, 92} = 1.436$ ,  $P = 0.237$ ).

The remaining physiological parameters measured, photochemical reflectance index (PRI) and effective ( $\Phi\text{PSII}$ ) and maximum (Fv/Fm) quantum yield of PSII were significantly affected by “connection”. Connected ramets showed a higher PRI,  $\Phi\text{PSII}$  and Fv/Fm than severed ramets (Table 1; Figs. 2b–d, 3b–d).

“Time” had a significant effect on CHL, PRI and  $\Phi\text{PSII}$  (Table 1; Fig. 3). There was a significant effect of the interaction between “connection” and “time” on maximum quantum yield of PSII (Fv/Fm), with an increase of Fv/Fm in connected ramets and a decrease in severed ramets (Table 1; Fig. 3d).

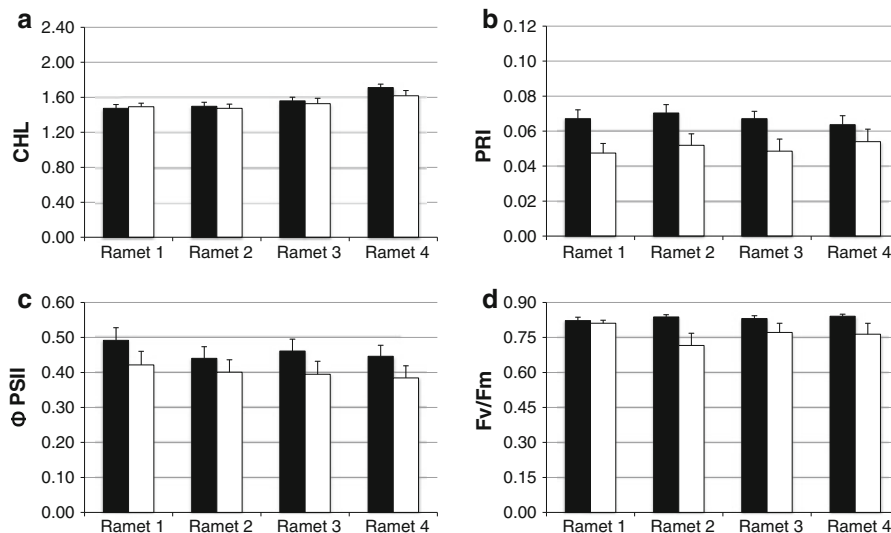
### *Growth and biomass allocation*

Aboveground and total mass increased significantly with “ramet age” (Table 2; Fig. 4). Aboveground and total mass was significantly higher in connected than in severed ramets (Table 2; Fig. 4). For root mass and RMR, the oldest ramets showed significantly higher

**Table 1** Results of the repeated-measure ANOVA for chlorophyll content index (CHL), photochemical reflectance index (PRI), effective ( $\Phi$ PSII) and maximum (Fv/Fm) quantum yield of photosystem II of individual ramets

	CHL			PRI			$\Phi$ PSII			Fv/Fm		
	df	F	P	df	F	P	df	F	P	df	F	P
Between-subject effect												
Ramet age	3	4.607	<b>0.006</b>	3	0.151	0.929	3	0.776	0.512	3	0.369	0.776
Connection	1	0.676	0.415	1	14.199	<b>&lt;0.001</b>	1	8.075	<b>0.006</b>	1	6.196	<b>0.016</b>
Ramet age x connection	3	0.337	0.798	3	0.273	0.844	3	0.109	0.955	3	0.714	0.548
Error	56			56			56			56		
Within-subject effect												
Time	2	3.144	<b>0.047</b>	2	40.234	<b>&lt;0.001</b>	2	80.601	<b>&lt;0.001</b>	2	0.267	0.776
Ramet age $\times$ time	6	1.587	0.157	6	1.316	0.256	6	0.065	0.999	6	1.080	0.379
Connection $\times$ time	2	0.183	0.833	2	0.325	0.723	2	0.289	0.750	2	3.674	<b>0.028</b>
Ramet age $\times$ connection $\times$ time	6	0.724	0.631	6	0.736	0.621	6	0.104	0.996	6	0.693	0.656
Error	112			112			112			112		

$P < 0.05$  are in bold. See Figs. 2, 3 for data



**Fig. 2** Spectral reflectance (CHL and PRI, *top panels a, b*) and chlorophyll fluorescence ( $\Phi$ PSII and Fv/Fm, *bottom panels c, d*) parameters for connected (*filled bars*) and severed (*empty bars*)

individual ramets (mean  $\pm$  SE). Average values of the three dates of measurements (30, 60, 90 days) are represented. See Table 1 for ANOVA results

values than younger ramets only in the connected treatment (Table 2; Fig. 4).

#### Clonal fragments

##### *Growth and biomass allocation*

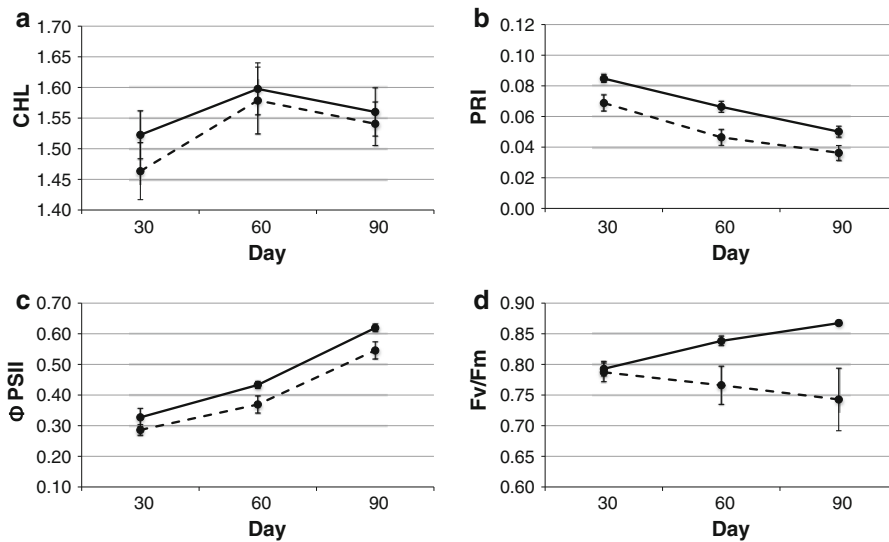
At clonal fragment level, aboveground mass ( $F_{1, 14} = 4.618$ ,  $P = 0.050$ ), root mass ( $F_{1, 14} = 6.296$ ,  $P = 0.025$ ) and total mass ( $F_{1, 14} = 5.149$ ,  $P = 0.040$ ) were

significantly higher for connected clones than for severed clones (Fig. 5a–c). However, no differences between connected and severed clones were found for the RMR ( $F_{1, 14} = 3.841$ ,  $P = 0.070$ ; Fig. 5d).

#### Discussion

We found specialization of older ramets in root production and younger ramets in chlorophyll content.





**Fig. 3** Time course of mean values ( $\pm$ SE) of spectral reflectance (CHL and PRI, *top panels a, b*) and chlorophyll fluorescence ( $\Phi$ PSII and Fv/Fm, *bottom panels c, d*) parameters

for connected (*solid lines*) and severed (*dashed lines*) treatments. Average values of the four ramets within each clonal fragment are represented. See Table 1 for ANOVA results

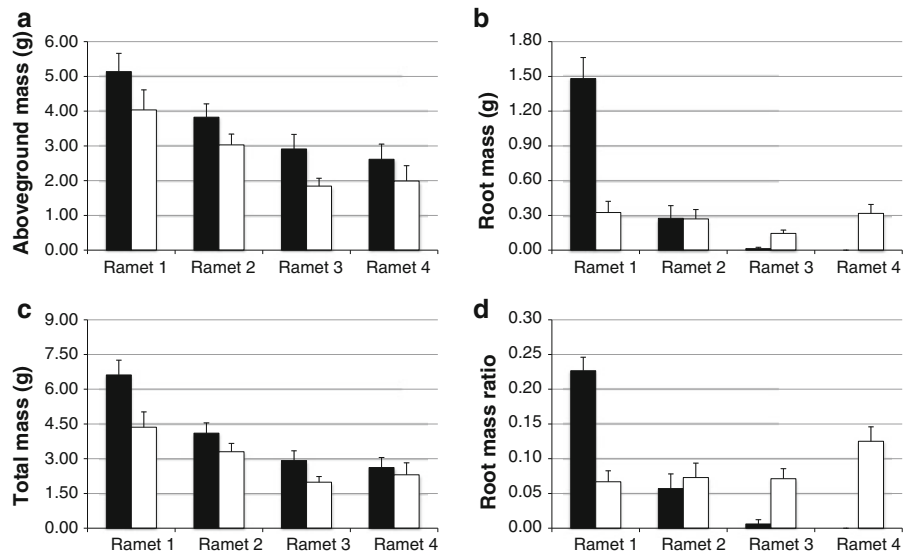
**Table 2** Results of the two-way ANOVA and Scheirer–Ray–Hare test to check for the effect of ramet age and connection on aboveground mass and total mass (ANOVA), and root mass and root mass ratio (SRH test) of individual ramets

	Above-ground mass			Total mass			Root mass			Root mass ratio		
	df	F	P	df	F	P	df	SS/MS <sub>total</sub>	P	df	SS/MS <sub>total</sub>	P
Ramet age	3	12.561	<b>&lt;0.001</b>	3	17.681	<b>&lt;0.001</b>	3	20.207	<b>&lt;0.001</b>	3	10.964	<b>0.012</b>
Connection	1	8.828	<b>0.004</b>	1	9.985	<b>0.003</b>	1	1.347	0.246	1	2.214	0.137
Ramet age $\times$ connection	3	0.141	0.935	3	1.497	0.225	3	17.180	<b>0.001</b>	3	20.127	<b>&lt;0.001</b>
Error	56											

$P < 0.05$  are in bold. See Fig. 4 for data

This supports our first hypothesis that connection allowed division of labour between ramets of the clonal fragment. As predicted, older ramets allocated more biomass to roots than younger ramets, and the chlorophyll content was greater in the younger than in the older ramet. These differences in RMR and chlorophyll content between ramets within the clone were detected mainly in the connected treatment: connection allows ramets to undertake different tasks within the clonal fragment. While older ramets specialize in acquisition of soil-based resources (determined by the increase in RMR), younger ramets specialize in aboveground expansion (determined by the reduction in RMR) and in harvesting aboveground resources (determined by a higher chlorophyll content). We detected a greater investment in

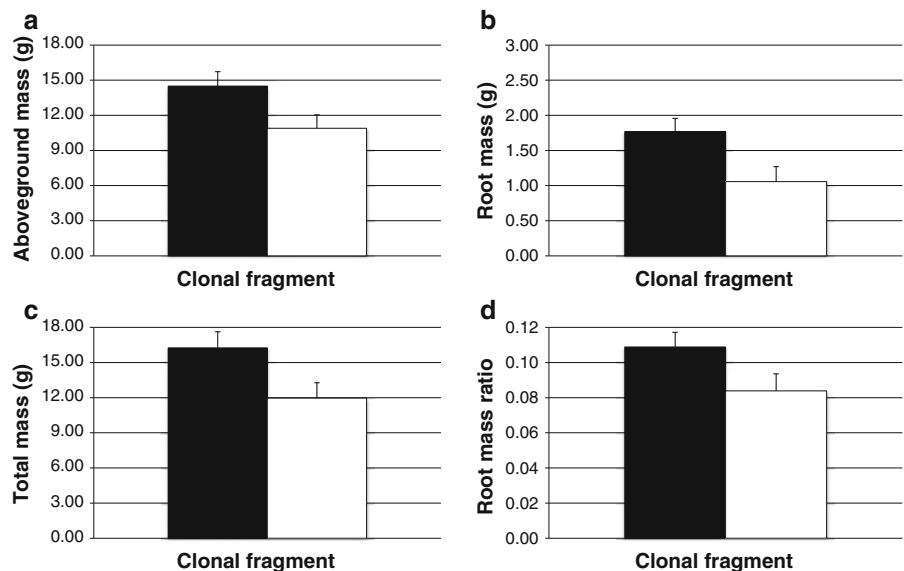
aboveground structures by connected younger ramets, and a high proportion of aboveground mass in *C. edulis* is invested in stolon growth. Therefore, the transfer of a greater proportion of mass to aboveground parts would be expected to lead to greater lateral expansion of stolons. In a recent experiment with *C. edulis* Roilola et al. (2010) have found an association between the increase in total biomass and the production of new ramets, supporting the idea that an increase of the biomass allocated to above-ground structures is directly associated with the production of new ramets and, as consequence, with lateral expansion. Similarly, in an experiment with the stoloniferous *Glechoma hederacea*, Roilola and Hutchings (2012) have proposed that clonal fragments could develop a form of division of labour in which ramets



**Fig. 4** Aboveground mass (**a**) root mass (**b**), total mass (**c**) and the proportional biomass allocated to roots estimated as the root mass ratio (**d**) of connected (*filled bars*) and severed (*empty bars*)

*bars*) individual ramets (mean  $\pm$  SE). See Table 2 for ANOVA and Scheirer–Ray–Hare test results

**Fig. 5** Aboveground mass (**a**) root mass (**b**), total mass (**c**) and the proportional biomass allocated to roots estimated as the root mass ratio (**d**) of clonal fragments (mean  $\pm$  SE). Connected treatment (*filled bars*) and severed treatment (*empty bars*) are shown. See text for ANOVA results



that were not allowed to take root specialized in lateral expansion, while ramets that were allowed to take root specialized in acquisition of soil-based resources. In our experiment, the increase in chlorophyll content detected in younger ramets could contribute to their aboveground growth, as the higher light energy harvest was mainly allocated to aboveground structures. Roiloa et al. (2007) also reported an increase in the chlorophyll content of younger ramets of *Fragaria*

*chiloensis* as a specialization to acquire light. This physiological specialization detected in our experiment supports that division of labour can be also detected in physiological traits (de Kroon et al. 1996; Roiloa et al. 2007; Wang et al. 2011).

Division of labour has been extensively studied in clonal plants, and it has been mainly associated with a heterogeneous distribution of resources. Most of the studies have used a design with two resources



negatively correlated in the space to induce division of labour. In this situation, ramets specialize to acquire the resource that is more abundant to them, and the subsequent reciprocal resource sharing increases the overall performance of the clone. This prediction is based on the logical fact that the acquisition of the abundant resource is likely to be more efficient than the acquisition of the scarce resource (Friedman and Alpert 1991; Birch and Hutchings 1994; Stuefer et al. 1996; Alpert and Stuefer 1997; Hutchings and Wijesinghe 1997; Stuefer 1998). Our study reports division of labour in a homogeneous environment, with all the ramets of the clone subjected to identical growing conditions and having access to identical amount of below and aboveground resources. Similarly, previous research has showed functional and structural specialization not environmentally induced in rhizomatous species (Schmid 1990; Stuefer 1998). As far as we know, we report here the first evidence that division of labour can be also developed under homogeneous conditions by a stoloniferous species. This finding is significant, because it indicates that the benefits of clonal integration are not only important under heterogeneous conditions, as it has been widely proposed (Price and Marshall 1999; Pennings and Callaway 2000; Saitoh et al. 2002; Roiloa and Retuerto 2006), but also relevant in homogeneous environments (Stuefer 1998). Ramet specialization reported in our experiment can be defined as a developmentally programmed division of labour (Stuefer 1998). This is an inherent division of labour, characterized by the presence of specialization implicit in the ontogeny of the ramets. Therefore, this type of division of labour is expressed in absence of induction due to environmental heterogeneity (Stuefer 1998).

We found an increase in photochemical efficiency and growth in connected fragments. This supports our second hypothesis that division of labour, mediated by physiological integration, increases the overall performance of the clonal fragment. As we expected, the photochemical efficiency and biomass production were higher in connected than in severed clonal fragments. The movement of resources between connected ramets of clonal plants has been widely described and generally confers net benefits to the whole clone (e.g. Hartnett and Bazzaz 1983; Alpert and Mooney 1986; Slade and Hutchings 1987; Birch and Hutchings 1994; Alpert 1999; Saitoh et al. 2002;

Roiloa and Retuerto 2006). Physiological traits can be considered as a sensible indicator of plant responses to the environment, and usually its effect on plants can be detected earlier at physiological than at morphological level. The current research contributes to prove that the benefits of clonal integration can be detected at physiological level (Hartnett and Bazzaz 1983; Roiloa and Retuerto 2006; Roiloa et al. 2007; Liu et al. 2008; Zhang et al. 2009). Roiloa and Retuerto (2006) have reported a similar benefit of integration in photochemical efficiency in a study with the clonal *Fragaria vesca*. In our study, the benefits of connection showed by clonal fragments in terms of photochemical efficiency were transferred into benefits in biomass production. As a result of these benefits at physiological and morphological level connected clonal fragments could have a higher growth rate, and therefore colonization capacity, than severed clonal fragments.

In short, connected clonal fragments of *C. edulis* showed developmentally programmed division of labour, and their photochemical efficiency and growth was enhanced by integration. This benefit of division of labour could be closely associated with the invasive expansion of *C. edulis*. The importance of clonal integration in plant invaders has been scarcely explored before (Amsberry et al. 2000; Peltzer 2002; Otfinowski and Kenkel 2008; Wang et al. 2008; Aguilera et al. 2010; Roiloa et al. 2010), and our study is the first exploring the idea that division of labour could contribute to the expansion of an invader, and therefore that clonal traits could increase the invasiveness of plant species (Pyšek 1997; Liu et al. 2006).

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