



## Original article

The effects of density on size-dependent gender plasticity in the monoecious species *Sagittaria potamogetifolia* (Alismataceae)Xiaowen Wang<sup>a</sup>, Lanjie Huang<sup>b</sup>, Andrew Wanyoike Gichira<sup>c,d</sup>, Xiaofan Wang<sup>e,\*</sup><sup>a</sup> Laboratory of Plant Mating Systems and Evolutionary Genetics, College of Life Sciences, Wuhan University, Wuhan, P.O. Box: 430072, Hubei Province, China<sup>b</sup> College of Life Sciences, Hubei University, Wuhan, P.O. Box: 430072, China<sup>c</sup> Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, P.O. Box: 430074, Hubei Province, China<sup>d</sup> Life Science College, University of Chinese Academy of Sciences, Beijing, P.O. Box: 100049, China<sup>e</sup> College of Life Sciences, Wuhan University, Wuhan, P.O. Box: 430072, Hubei Province, China

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## ABSTRACT

**Aim:** To test the fitness-gain curve model proposes that cosexual plants adjust their sex ratios and resource allocation depending on their size. In this study, the monoecious species *Sagittaria potamogetifolia* was used as a model to determine the effects of plant size and density on gender modification and reproductive allocation.

**Methods and materials:** Various traits, including flower number and plant biomass, were measured under four different artificially constructed population density treatments. More male flowers were produced than female flowers per individual at high densities, while the opposite trend was observed at low densities. This trend was particularly evident in the highest density treatment.

**Results:** A trade-off was discovered between male–female sex allocations in the highest density treatment (40 individuals m<sup>-2</sup>). The allometric growth of reproductive organs compared with plant size was detected, as evidenced by the reproductive structures' biomass and flower numbers. However, in the highest density treatment, size was weakly negatively correlated with femaleness.

**Conclusion:** Thus, *S. potamogetifolia* has a reproductive strategy that easily adjusts to different reproductive environmental densities.

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## 1. Introduction

Flowering plants possess highly diverse sexual systems, ranging from unisexuality to hermaphroditism (Lloyd and Bawa, 1984). This variability can be found among species, among populations or even among individuals within a population of a species (Lloyd and Bawa, 1984). Sex expression is also highly variable in response to environmental conditions (Freeman et al., 1980, 1981; Lloyd and Bawa, 1984; Sarkissian et al., 2001; Stehlik et al., 2008). These environmental factors include, for example, water or nutrient availability (Freeman et al., 1980; Litrico et al.,

2005; Dorken and Mitchard, 2008), exogenous applications of phyto-hormones, such as auxins, gibberellic acid and cytokinins (Chailakhyan and Khrianin, 1987), and affect population or individual density levels within species (Pannell, 1997; Delph, 2003; Dorken and Pannell, 2008; Litrico and Maurice, 2013).

Environmental conditions affect sex expression in cosexual plant species mainly through the alteration of resource allocation to female and male functions, which is usually dependent on plant size, known as size-dependent sex allocation (SDS; Gilbert and Bolker, 2003; Liu et al., 2009). Environmental differences in resource availability can generate different plant gender distributions (Gunton and Kunin, 2007). In general, smaller plants are expected to have a male-biased sex expression because female functions involve more resource costs (Weiner, 1988; Koelewijn and Hunscheid, 2000). The male allocation (pollen production) decreases with plant size in a population under a closed canopy in the monocarpic perennial herb *Cardiocrinum cordatum* (Thunb.). However, male production did not change with plant size under a sparse canopy (Cao and Kudo, 2008). Exceptions occur in some wind-pollinated plant species, in which larger plants show greater male-biased gender expression than smaller plants (Ackerly and

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Jasienski, 1990; Bickel and Freeman, 1993; Dajoz, 1997; Pannell, 1997). Additionally, exceptions occur in several animal-pollinated plant species (Han et al., 2011), which suggests that to maximize reproductive possibilities, more male flowers are produced (Eseyin et al., 2018; Gao and Wang, 2017; Ge et al., 2017). In *Daphne jezoensis*, an insect-pollinated summer deciduous shrub, which has two sex morphs, there is a similar reproductive cost in larger individuals for hermaphrodites and females (Akari and Gaku, 2016) because, in natural environments, female fruit production is strongly restricted by pollen limitations under a sparse canopy. Because different SDS patterns may exist in different plant species, diverse plant species should be studied to elucidate the mechanisms of complex environmental effects on patterns of size-dependent gender plasticity (Demir et al., 2017; Isik et al., 2017; Mukattash et al., 2018).

Different pollination, nutrient and density treatments can affect resource allocation between sexual reproduction and asexual reproduction, as well as the sex expression, in some *Sagittaria* species (Dorken and Barrett, 2004; Liu et al., 2008, 2009, 2010; Han et al., 2011). For example, Liu et al. (2008, 2010) found that individuals of *Sagittaria potamogetifolia* could produce more inflorescence under high nutrient level or low pollination treatments, and the sex ratio (female to male flower ratio) decreased with increased inflorescence numbers and plant size. In highly dense insect-pollinated herb *S. trifolia* populations, nearly half of the individuals were male and lacked female flowers, while in low-density populations all of the individuals were monoecious (Han et al., 2011). However, whether density affects gender variation in *S. potamogetifolia* remains unknown. Density, one of various environmental factors that can influence sex expression in plants, is also an important ecological factor because it influences fitness by affecting growth, survival and reproduction through competition for light, nutrients, water and pollinators (Kleunen and Fischer, 2003; Gunton and Kunin, 2009). It affects biomass allocation and sex variation among plant individuals as a result of resource competition (Ackerly and Jasieński, 1990; Pannell, 1997; Dorken and Pannell, 2008). For example, Ackerly and Jasieński (1990) found that in high density stands of the monoecious annual *Ambrosia artemisiifolia* (Asteraceae), the variability levels in biomass and gender were greater under a higher nutrient treatment, and there were significant positive correlations between maleness and both height and biomass. Moreover, Pannell (1997) found that the male frequency increased with density within an androdioecious population of *Mercurialis annua* from southern Spain, and Dorken and Pannell (2008) further found that the progeny sex ratio of *M. annua* was strongly dependent on density, with fewer male progeny growing under low density conditions. Because different plant species may express different SDS patterns, the manner in which individual density levels affect SDS patterns in various species requires further investigation.

The genus *Sagittaria* (Alismataceae) comprises approximately 30 species, most of which are monoecious (Bogin, 1955; Chen, 1989). Some species display remarkable variation in gender expression, making this genus a suitable model system for studying gender variation within and between species (Sarkissian et al., 2001; Huang et al., 2002; Dorken and Barrett, 2003; Liu et al., 2008, 2009; Han et al., 2011). For example, Dorken and Barrett (2003) discovered that individuals of *Sagittaria latifolia* sampled from two populations in East Anglia, UK, produced male-biased floral sex ratios, and SDS occurred in both populations. Liu et al. (2008) found that the ratio of female to male flowers of the monoecious species *Sagittaria potamogetifolia* decreased with increasing plant size, although the numbers of both female and male flowers increased. Liu et al. (2009) did not observe a correlation between increases in female flower production and size in *Sagittaria pygmaea*, but increased male flower production was observed. Contrary to the prediction of SDS, Han et al. (2011) found

that a decrease in male flower production was not correlated with a decrease in plant size in the Asian species *Sagittaria trifolia*.

Here, we investigated the effects of different densities on SDS patterns in the monoecious species *S. potamogetifolia*. To exclude the confounding effects of other environmental variables on the gender variation of this species, the experiments were conducted on individuals grown in a common garden at Wuhan University, China. We created different artificial individual densities and measured the variation in individual gender in each density treatment.

## 2. Materials and methods

### 2.1. Study species

*S. potamogetifolia* is a herbaceous aquatic plant. The species is endemic to China and grows in paddy fields and marshes in the south-eastern regions (Chen, 1989). It produces unisexual flowers. The plant flowers from May to October, with each individual developing 3–12 raceme inflorescence. Each inflorescence produces 1–3 female flowers that bloom for half a day in the basal whorl, followed by 1–24 male flowers in the apical whorls that bloom for 3–5 days per inflorescence. This species can reproduce sexually by selfing and outcrossing (producing viable seeds) or vegetatively through rhizomes or rhizome bulbils (Liu et al., 2010; pers. obs. of first author). This species is insect- and wind-pollinated, but pollen flows are mediated mainly by insect pollination (Wang and Chen, 2001).

### 2.2. Experimental design

Individuals of *S. potamogetifolia* were collected from a population in Wutun Cun, Nanping, Fujian Province (27° 51' N, 118° 08' E). The seeds were harvested at the end of the growing season in 2012 and stored in a cold room at 5 °C for 5 months. During the spring of 2013, the seeds were germinated in separate pots containing sediment (50 ml of a 1.25% solution of soluble 20:20:20:20 N: P: K: Na: Mg fertilizer) in the Wuhan University greenhouse. Once they had germinated, they were then randomly assigned to synthetic populations (plots) and placed into toughened plastic water tanks (60 cm × 50 cm × 40 cm cuboids) with drainage holes at the base and watered daily in a spontaneous pollination environment. These different plots were planted at low (13.33 individuals m<sup>-2</sup>), medium (26.67 individuals m<sup>-2</sup>), medium-high (33 individuals m<sup>-2</sup>) and high (40 individuals m<sup>-2</sup>) densities. Each treatment was replicated six times, resulting in a total of 204 plants in the 24 experimental populations. Sediment (clay) containing 50 ml of a 1.25% solution of soluble 20:20:20:20 N: P: K: Na: Mg fertilizer was applied to the experimental treatments once a week. Once they had flowered, individuals were grown right outside of the greenhouse in order for them to be naturally pollinated by insects during the flowering season, which lasted until late October. They were harvested randomly from each density in late October 2012. We monitored the water levels daily to maintain fully saturated sediments in each plot. Plots were maintained at 1 m distances from each other to avoid crowding.

To determine the SDS patterns, inflorescence sequences and the number of female and male flowers for each plant were recorded throughout the flowering period (4 June to 13 September 2013). We measured plant height, as well as leaf length, which was assessed as the midvein length of the basal leaf having the longest petiole, in the 24 randomly selected individuals from each treatment. For each plant, the dry biomass (dried at 60 °C for 48 h) of both vegetative (roots and leaves) and reproductive (inflorescence stalks and fruits) parts were calculated across the entire growing season by collecting the wilting parts every day.

A phenotypic gender index ( $G_i$ ) had previously been developed to measure femaleness within a population, and is calculated using the following formula (Lloyd and David, 1980; DeSoto and Quintanilla, 2008):

$$G_i = \frac{d_i}{d_i + l_i E}$$

where  $d_i$  and  $l_i$  represent the numbers of female and male flowers per plant, respectively, and  $E = \Sigma d_i / \Sigma l_i$  represents the ratio of the total female flower number to the total male flower number for each density treatment.  $G$  varies from 0 (complete maleness) to 1 (complete femaleness). We calculated the  $G_i$  of each plant to determine whether there was any relationship between female/male flower production and plant vegetative biomass.

All female and male flowers were counted on each individual every day throughout the flowering period to calculate the sex allocation.

### 2.3. Data analysis

All variables were tested (Méndez and Traveset, 2003). When homogeneity of variance for the data was not present, the data were log-transformed to achieve normality and homogeneity of variance. If the variances were still uneven, then Dunnett's T3 or LSD tests were used as the post hoc test.

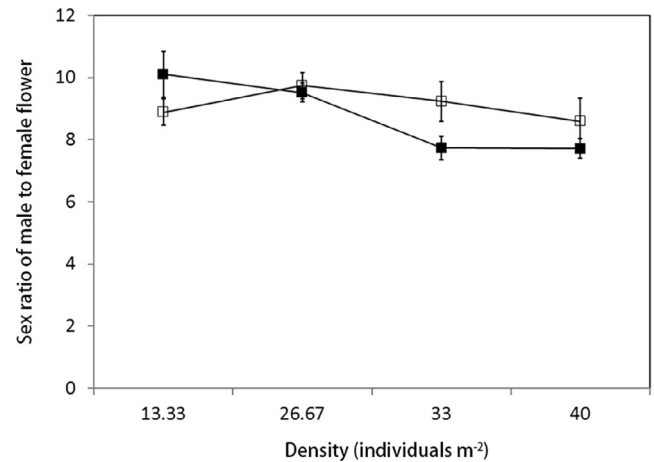
A one-way analysis of variance was used to determine the effects of population density and plant size (dry weight of individual) on plant traits (phenotypic gender, inflorescence number, plant height, vegetative biomass, reproductive biomass, male flower number, female flower number and display size). When the variance was homoscedastic, the LSD post-hoc test was used to determine the significance of multiple comparisons of means. When data were non-normally distributed, Dunnett's T3 post hoc test was used to determine the significance of multiple comparisons of means. The relationships between the number of flowers and plant size (dry weight), and phenotypic gender and plant size (dry weight), were investigated using regression analyses. To test for linear correlations, the reduced major axis II regression slopes (Buxton and Lord, 2016) were calculated based on the allometric model proposed by Klinkhamer and De Jong (1997).

To handle the temporal sex variation during flowering, the flowering period was divided into five flowering stages at 20-d intervals. We defined every 20 d as a node to divide growth conditions. To perform analyses of the size-dependent production of female and male flowers, some variables, such as the covariate representing plant size, were log-transformed (Méndez and Traveset, 2003). The daily sex ratio was determined as the ratio of the male flower number to the female flower number on every individual, while the total daily sex ratio was that of the male flower number to the female flower number per density level (Fig. 1). The homoscedasticity between the variable indices of different densities for flowering stage sex allocation variation and temporal changes in flowering were determined. An analysis of covariance was conducted on male and female flower numbers and plant size (total dry weight). We used Pearson correlation coefficient to test for a linear relationship between individual density levels and reproductive biomass. The least-square method was used to fit the curve of mean fruit production to those of the density levels. All statistical tests were conducted in SPSS version 20.0 (SPSS, Chicago, IL, USA).

## 2.4. Results

### 2.4.1. Temporal variation in sex expression

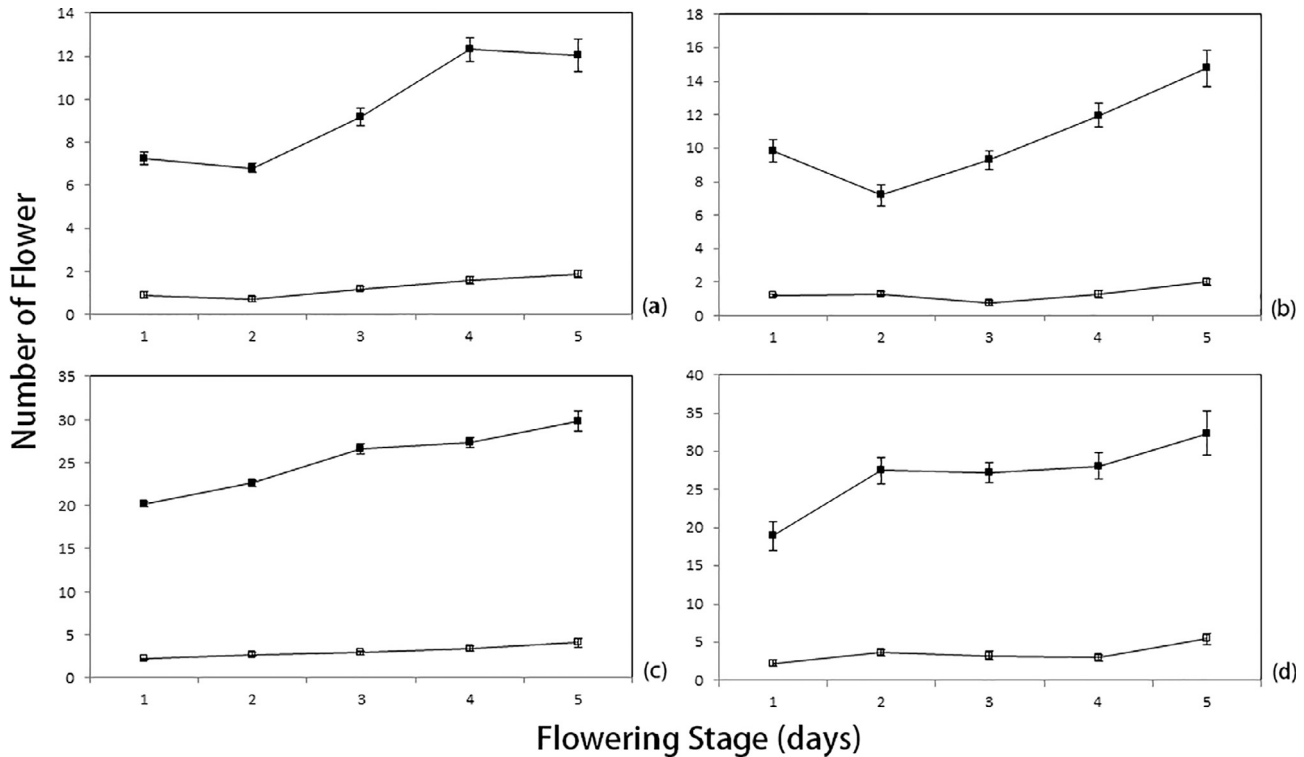
The variances of the daily sex ratio were not homoscedastic between individual densities. However, the variances of the total sex ratio were homoscedastic between all densities (Test of



**Fig. 1.** The mean individual daily floral display sex ratio and ratio of male to female flowers in individuals with different densities of *S. potamogetifolia*. (■) sex ratio of male to female in the individual per day. (□) total sex ratio of male to female in the individual.

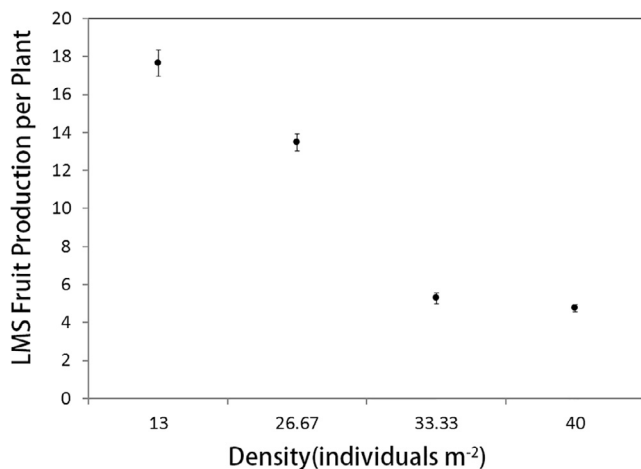
Homogeneity of Variances: Levene index = 21.488,  $P < 0.0001$ ). The Dunnett's T3 Post Hoc Tests showed that a significant decrease ( $P = 0.002$ ) in the daily sex ratio at the medium (26.67 individuals  $m^{-2}$ ) density compared with at the medium-high (33.33 individuals  $m^{-2}$ ) density (Fig. 1). The total sex ratio values between pairs of density levels were not significantly different from LSD Post Hoc Tests. There was no significant difference between the daily sex ratio at the low (13.33 individuals  $m^{-2}$ ) and the medium (26.67 individuals  $m^{-2}$ ) density levels. The mean sex ratio was affected by the individual density treatments and changed across the flowering stages. The daily sex ratio decreased as the density level increased.

In contrast to the sex phenotype variation based on the total flower numbers, during the flowering stages, the variances of male/female production were not homoscedastic between any of the experimental densities (Fig. 2). Male flower production at low density level: Levene index = 2.6,  $P = 0.041$ ; Female flower production at low density level: Levene index = 3.187,  $P = 0.017$ ; Male flower production at medium density level: Levene index = 16.378,  $P < 0.001$ ; Female flower production at medium density level: Levene index = 10.762,  $P < 0.001$ ; Male flower production at medium-high density level: Levene index = 2.913,  $P = 0.025$ ; Female flower production at medium-high density level: Levene index = 3.021,  $P = 0.021$ ; Male flower production at high density level: Levene index = 10.926,  $P < 0.000$ ; Female flower production at high density level: Levene index = 3.676,  $P = 0.008$ ). At the high density, there were significant differences between each pair of density levels at the 2nd, 3rd and 4th flowering stages. Male flower production increased from the 2nd to 4th flowering stages (Fig. 2a. Post Hoc Tests:  $P_{2-3} < 0.000$ ,  $P_{3-4} < 0.000$ ), while female production decreased significantly from the 2nd to 3rd flowering stages (Fig. 2a. Post Hoc Tests:  $P_{2-3} = 0.030$ ). At the medium-high density, male flower production increased significantly from the 1st to 2nd flowering stages (Fig. 2b. Post Hoc Tests:  $P_{1-2} = 0.056$ ). At the medium (26.67 individuals  $m^{-2}$ ) density, male flower production significantly decreased from the 1st to 2nd flowering stages (Fig. 2c. Post Hoc Tests:  $P_{1-2} < 0.000$ ), but increased significantly from the 2nd to 3rd flowering stages (Fig. 2c. Post Hoc Tests:  $P_{2-3} < 0.000$ ). At the low density, there was a significant increase in male flower production during the early flowering stages (Fig. 2d. Post Hoc Tests:  $P_{1-2} = 0.022$ ), but female flower production was not significantly changed (Fig. 2d). Overall, in both treatments, male flower production was more prevalent.



**Fig. 2.** The relationship between 5 flowering stages and flower number in *S. potamogetifolia* of different densities. (■) male flower number. (□) female flower number. (a) Individual density of  $40 \text{ m}^{-2}$  plants. (b) Individual density of  $33.33 \text{ m}^{-2}$  plants. (c) Individual density of  $26.67 \text{ m}^{-2}$  plants. (d) Individual density of  $13.33 \text{ m}^{-2}$  plants. The sample size ( $n = 20$ ) in all cases. Each stage separated by 20 days of *S. potamogetifolia*.

The fruit production of individuals, based on least-squares regressions, differed significantly among the density treatments. Variances of fruit production were not homoscedastic among the experimental densities (Test of Homogeneity of Variances: Levene index = 12.856,  $P < 0.0001$ ). Thus, Dunnett's T3 post hoc test was used. There was a significant decrease in fruit production at the low ( $13.33 \text{ individuals m}^{-2}$ ) compared with the medium ( $26.67 \text{ individuals m}^{-2}$ ) density level (Fig. 3. LSD Post Hoc Test:  $P = 0.003$ ) and in fruit production at the medium ( $26.67 \text{ individuals m}^{-2}$ ) compared with the medium-high ( $33.33 \text{ individuals m}^{-2}$ ) density level (Fig. 3. LSD Post Hoc Test:  $P < 0.000$ ).

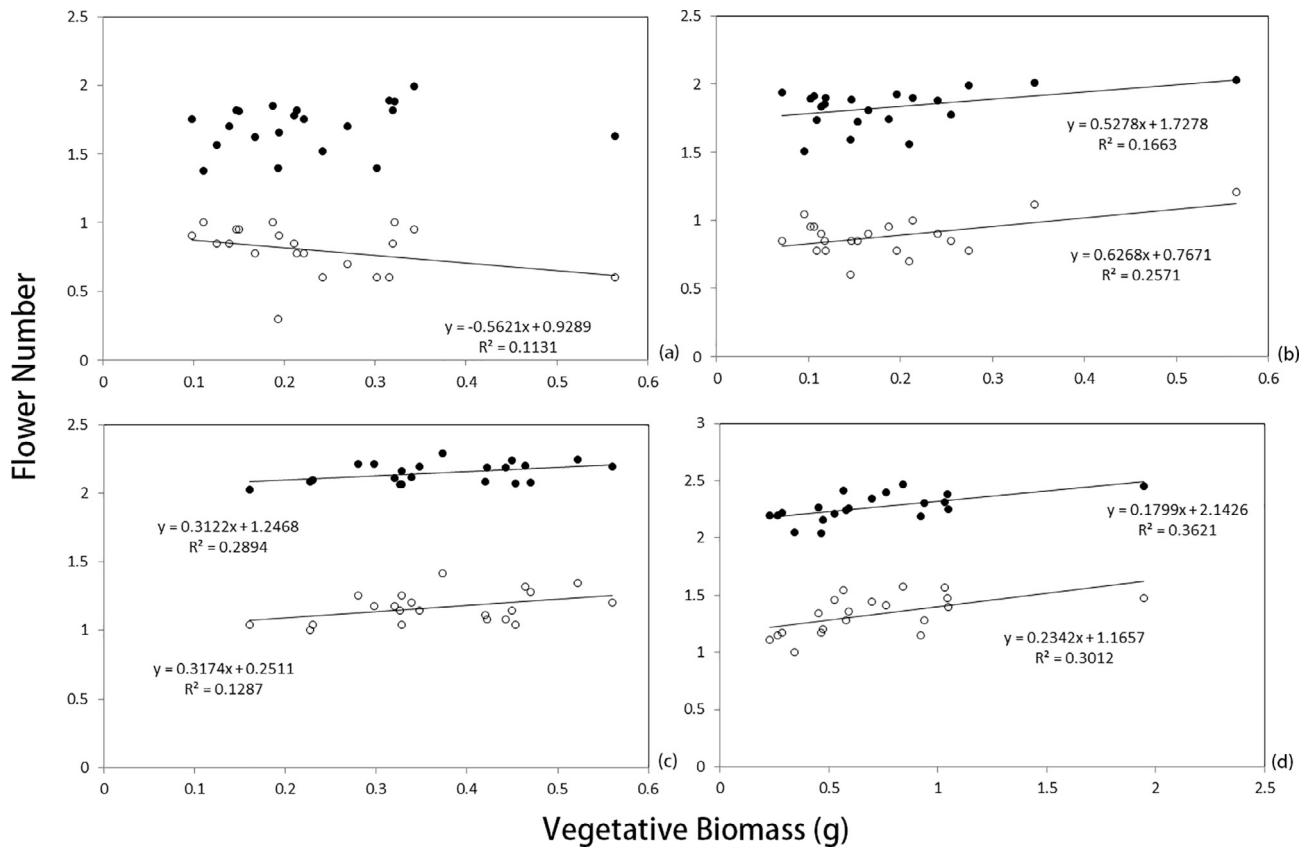


**Fig. 3.** Least-square means (LSM) total fruit biomass per plant of *S. potamogetifolia*.

#### 2.4.2. Sex allocation, biomass and flower numbers at different densities

We found significant differences in male flower production among the different density treatments. Because of missing variance, at medium ( $26.67 \text{ individuals m}^{-2}$ ) density level and medium-high ( $33.33 \text{ individuals m}^{-2}$ ) density level, we used  $t$ -test for double sample heteroscedasticity hypothesis to test significance (Relation between male flower production and vegetative biomass at low density level:  $P_{F\text{-test}} < 0.0001$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.6017$ ; Relation between female flower production and vegetative biomass at low density level:  $P_{F\text{-test}} = 0.003$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.5488$ ; Relation between male flower production and vegetative biomass at medium density level:  $P_{F\text{-test}} = 0.0839$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.538$ ; Relation between female flower production and vegetative biomass at medium density level:  $P_{F\text{-test}} = 0.3183$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.3587$ ; Relation between male flower production and vegetative biomass at medium-high density level:  $P_{F\text{-test}} = 0.1283$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.4078$ ; Relation between female flower production and vegetative biomass at medium-high density level:  $P_{F\text{-test}} = 0.1753$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.5071$ ; Relation between male flower production and vegetative biomass at high density level:  $P_{F\text{-test}} = 0.0188$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.1735$ ; Relation between female flower production and vegetative biomass at high density level:  $P_{F\text{-test}} = 0.0131$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.3363$ ;  $r$ : Pearson correlation coefficient). We use linear correlation analysis to test significance of regression equation significant inspection. At the medium-high, medium and low densities, the allocation to female flower.

Number increased with the increase in plant vegetative biomass (Fig. 4. At high density level:  $P_{\text{male}} = 0.452$ ,  $F_{\text{male}} = 0.589$ ,  $t_{\text{male}} = 0.767$ ;  $P_{\text{female}} = 0.136$ ,  $F_{\text{female}} = 2.422$ ,  $t_{\text{female}} = -1.556$ ; At medium-high density level:  $P_{\text{male}} = 0.067$ ,  $F_{\text{male}} = 3.789$ ,  $t_{\text{male}} = 1.946$ ;  $P_{\text{female}} = 0.019$ ,  $F_{\text{female}} = 6.574$ ,  $t_{\text{female}} = 2.564$ ; At medium density level:  $P_{\text{male}} = 0.055$ ,  $F_{\text{male}} = 4.199$ ,  $t_{\text{male}} = 2.049$ ;  $P_{\text{female}} = 0.066$ ,  $F_{\text{female}} = 3.82$ ,  $t_{\text{female}} = 1.954$ ; At low density level:



**Fig. 4.** Relationship between female flower production (open circles)/male flower (closed circles) production and vegetative biomass (g) in individuals with different densities of *S. potamogetifolia*. Slopes presented are based on reduced major axis (RMA) regression. (a) Individual density of 40 m<sup>-2</sup> plants. (b) Individual density of 33.33 m<sup>-2</sup> plants. (c) Individual density of 26.67 m<sup>-2</sup> plants. (d) Individual density of 13.33 m<sup>-2</sup> plants.

$P_{\text{male}} = 0.005$ ,  $F_{\text{male}} = 10.216$ ,  $t_{\text{male}} = 3.196$ ;  $P_{\text{female}} = 0.012$ ,  $F_{\text{female}} = 7.759$ ,  $t_{\text{female}} = 2.786$ ;  $P$ : the significance of regression equation significant inspection,  $F$ : significance of the regression equations,  $t$ : significance of regression parameter). In contrast, at the high density (40 individuals m<sup>-2</sup>), there was a trade-off between female and vegetative biomass (Fig. 4a). The allocation to male flower number had a weak negative correlation with the increase in plant vegetative biomass. There were isometric relationships between male and female flower numbers with increases in vegetative biomass at medium-high, medium and low densities (Fig. 4b, c, d).

#### 2.4.3. Phenotypic gender variation in *S. potamogetifolia*

Table 1 showed the individual differences between four different density levels. A positive correlation between plant size and phenotypic gender was observed. The  $G_i$  did not vary among the density treatments (Fig. 5). At low density level:  $P_{F\text{-test}} < 0.0001$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.419$ ; At medium density level:  $P_{F\text{-test}} < 0.0001$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.0032$ ; At medium-high density level:  $P_{F\text{-test}} = 0.0057$ ,  $P_{T\text{-test}} < 0.0001$ ,  $r = 0.1643$ ; At high density level:  $P_{F\text{-test}} = 0.0292$ ,  $P_{T\text{-test}} = 0.0145$ ,  $r = 0.33$ ;  $r$ : Pearson correlation coefficient). Thus, the investment in male function was not greater than in female function as predicted. At the high density, phenotypic gender values changed from femaleness to maleness as size increased. The phenotypic gender values varied at medium-high, medium and low density levels. However, positive correlations between plant size and phenotypic gender (i.e., femaleness and increasing size) were observed at the medium-high (Fig. 5b) and low (Fig. 5d) densities, while a negative correlation was observed at the high density (Fig. 5a). There was

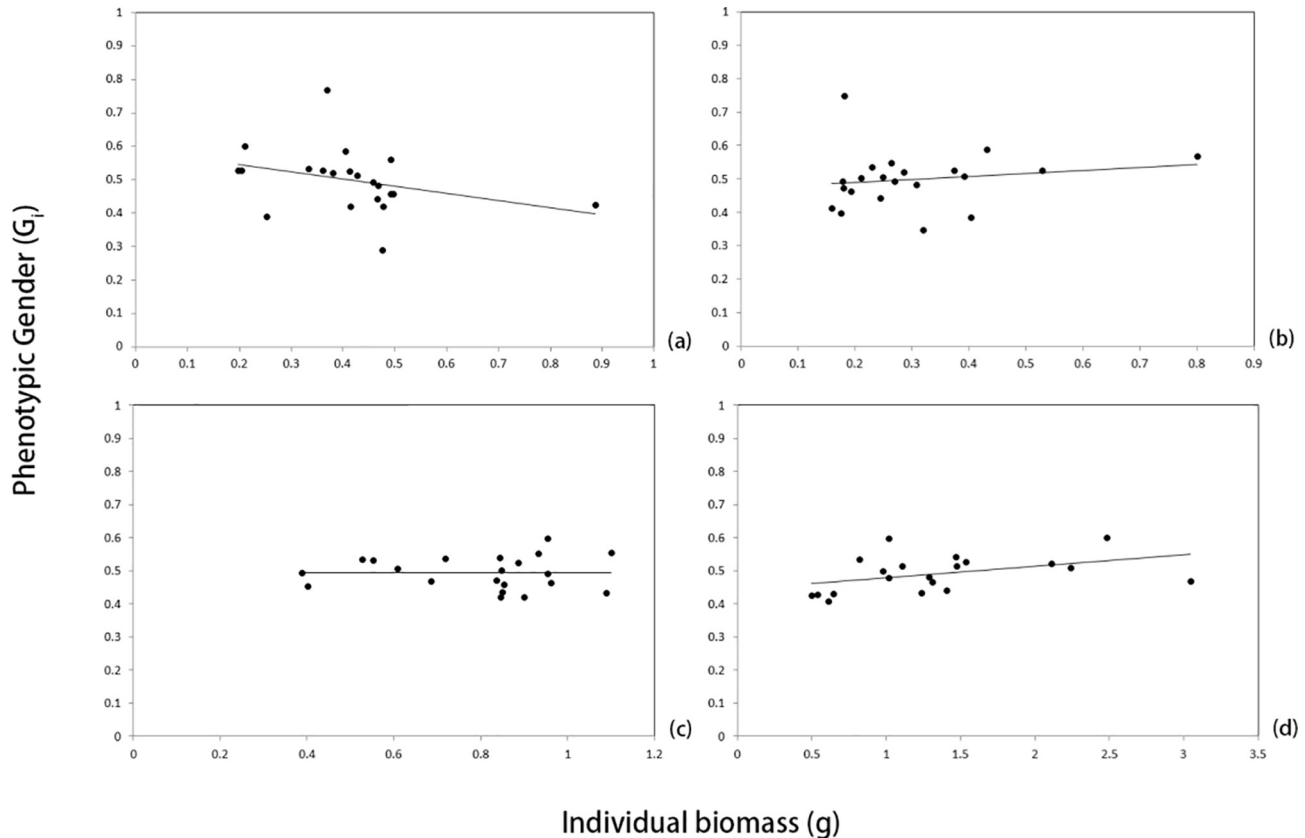
no correlation between plant size and phenotypic gender at the medium density (Fig. 5c). Thus, phenotypic gender appears to be influenced by density. Inflorescence production per individual was significantly positively correlated with plant size (At high density level:  $P = 0.553$ ,  $F = 0.364$ ,  $t = 0.603$ ; At medium-high density level:  $P = 0.068$ ,  $F = 3.751$ ,  $t = 1.937$ ; At medium density level:  $P = 0.021$ ,  $F = 6.339$ ,  $t = 2.578$ ; At low density level:  $P = 0.002$ ,  $F = 13.537$ ,  $t = 3.679$ .  $P$ : the significance of regression equation significant inspection,  $F$ : significance of the regression equations,  $t$ : significance of regression parameter). In the highest density treatment, inflorescence production per individual increased more slowly with plant size than in the other densities (Fig. 6). The mean female production per inflorescence did not vary among the different densities, and the mean male production per inflorescence at different densities fluctuated between 7 and 11 (Fig. 7). Additionally, at the highest density (40 individuals m<sup>-2</sup>) treatment, there was no linear correlation between inflorescence production and plant size. However, inflorescence production per individual increased with plant size at the medium (26.67 individuals m<sup>-2</sup>), medium-high (33.33 individuals m<sup>-2</sup>) and low (13.33 individuals m<sup>-2</sup>) density levels (Fig. 7).

### 3. Discussion

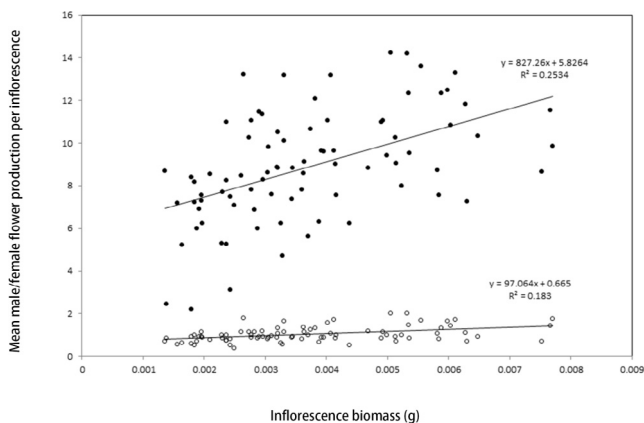
Here, strong correlations among sex expression, biomass and density were found. For example, larger plants produced more male flowers at the high density, while at the low density, phenotypic gender increased with plant size. At the medium (26.67 individuals m<sup>-2</sup>) density, femaleness did not vary with plant size. The observations differ from those of Han et al. (2011) who found

**Table 1**  
Mean allocation of resources to different plant structures in *S. potamogetifolia* in terms of different structure parameters ( $n = 24$ ). SD (%).

Population density (plant per $m^2$ )		Plant height (cm)	Number			Dry weight (g)			
			Inflorescence	Male	Female	Root	Leaf	Inflorescence	Fruit
40	Mean	16.66	7.76	54.05	6.76	0.02	0.09	0.02	0.16
	SD	1.63	2.39	19.58	2.32	0.01	0.03	0.01	0.07
33.33	Mean	19.98	9.33	70.33	8.05	0.03	0.07	0.02	0.09
	SD	1.41	2.71	20.62	2.75	0.02	0.06	0.01	0.06
26.67	Mean	25.53	14.9	142.43	15.19	0.03	0.14	0.07	0.35
	SD	2.61	1.95	24.6	4.21	0.01	0.04	0.02	0.16
13.33	Mean	28.26	17.8	192.2	22.9	0.11	0.3	0.08	0.58
	SD	3.4	4.07	52.08	8.52	0.06	0.19	0.04	0.34

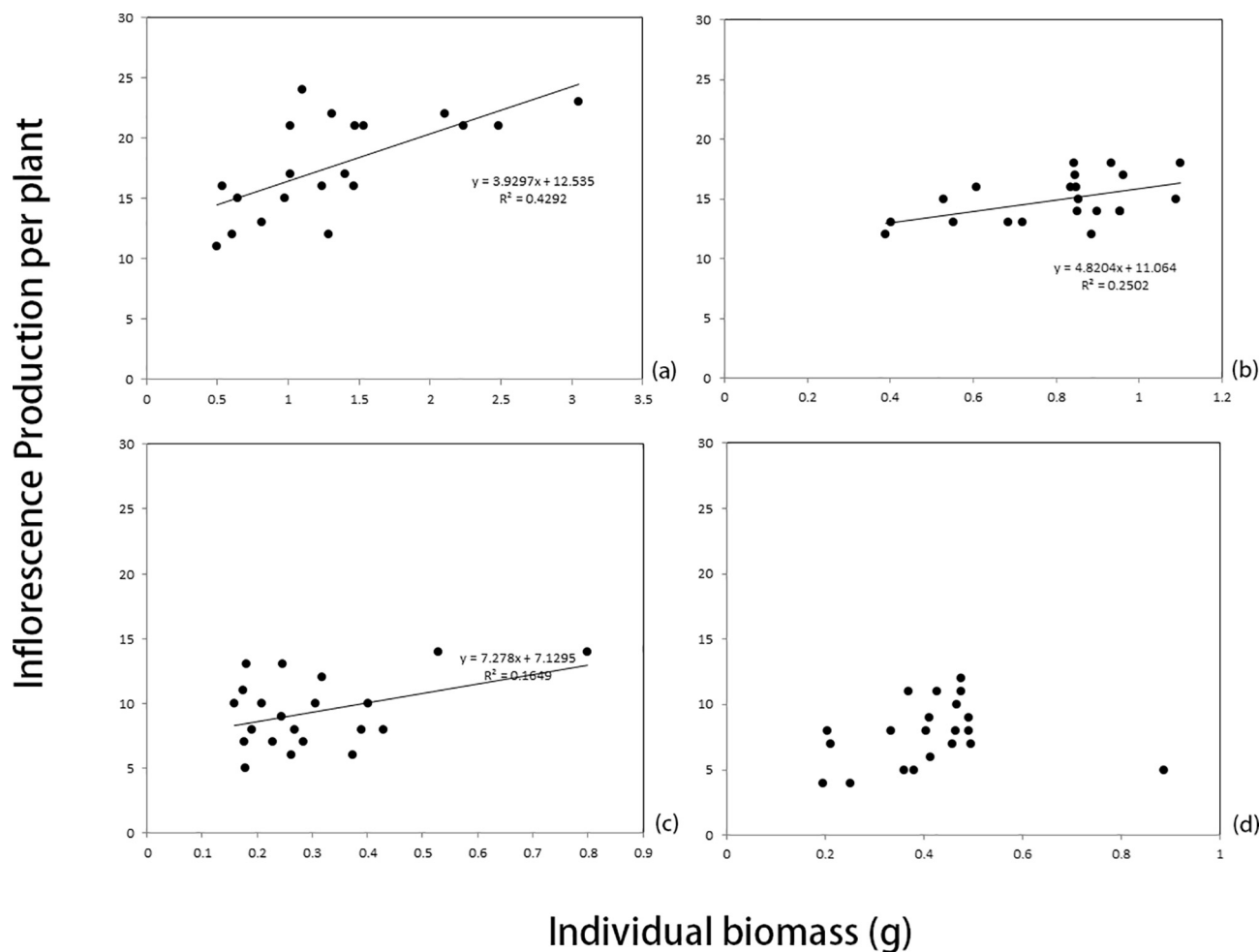


**Fig. 5.** Relationship between phenotypic gender and individual biomass (g) in individuals with different densities in *S. potamogetifolia*. (a) Individual density of  $40\ m^{-2}$  plants. (b) Individual density of  $33.33\ m^{-2}$  plants. (c) Individual density of  $26.67\ m^{-2}$  plants. (d) Individual density of  $13.33\ m^{-2}$  plants.



**Fig. 6.** The relationship between mean male or female flower number per inflorescence and mean inflorescence size biomass (g) in *S. potamogetifolia*. (●) male flower number. (○) female flower number.

isometric increases in both male and female flower production in two cultivated *S. trifolia* populations (a high-density and a low-density population), even though both male and female flower production levels in the low-density population increased faster than in the high-density population. The results corroborated previous results in which monoecious species mostly showed an allometric increase in male and female investments as plant size increased (Torices and Méndez, 2011). For example, Liu et al. (2009) concluded that in *S. pygmaea* male flower production increased with plant size (measured as midvein length), while female flower production was not correlated with plant size. The optimal resource configuration probably maximizes reproductive benefits. However, opposite trends have also been observed. For example, a field investigation of *Sagittaria sagittifolia* found that phenotypic gender increased with plant size (Sarkissian et al., 2001). However, no relationship was observed between plant size and floral sex ratio in *Arum italicum* (Méndez, 1998).



**Fig. 7.** The relationship between inflorescence number per individual and plant size individual biomass (g) in *S. potamogetifolia*.

Here, density affected the mean daily flower production and sex ratios, in that daily differences were observed in the number of flowers at the four individual densities. As in all other *Sagittaria* species (Huang et al., 2006), the female flowers of *S. potamogetifolia* opened first. In small populations, the effect of the first-stage opening of female flowers may be greater than in larger populations because the flowers (males and females) in small populations exhibit greater levels of synchronization (Wang et al., 2012). In our study, the smaller individuals at the high (40 individuals  $m^{-2}$ ) and medium-high (33.33 individuals  $m^{-2}$ ) densities from 1st to 2nd flowering stages exhibited no increased allocation of resources to sexual reproduction. However, at low and medium densities, during the 1st and 2nd flowering stages, individuals allocated more resources to sexual reproduction. All of the sex allocation levels at different densities showed a high degree of consistency. However, lower densities allocated more resource to male flower production than higher densities, resulting in a larger daily floral display and a greater gene flow. Thus, *S. potamogetifolia* individuals that are experiencing resource competition are inclined to produce enough females to gain reproductive assurance as opposed to focusing on pollen dispersal (Sakai and Sakai, 2003).

Previous studies indicated that larger inflorescences are female-biased (Torices and Méndez, 2011). For example, in *S. trifolia*, smaller inflorescences have no more than two whorls of female flowers, and some even produced no female flowers (Han et al., 2011). Similarly, in *S. brevirostra*, larger inflorescences have relatively more male than female flowers than the smaller inflorescences (Kaul, 1979). Here, the production of female, but not male, inflorescence

in *S. potamogetifolia* is dependent on inflorescence size. Thus, in *S. potamogetifolia*, individuals can adjust the sex ratio by producing more inflorescence. Individuals allocate resources to sexual reproduction based on the competition level for resources but not on plant size.

Individuals invest more resources to vegetative reproduction under resource (light, soil and space) competition. Clonal or vegetative reproduction in *S. potamogetifolia* passes more stable genetic information to the next generation than sexual reproduction, although the results of studies on the allocation of resources for asexual and sexual reproduction are quite inconsistent (Snow and Whigham, 1989; Saikkonen et al., 1998; Mendez, 1999). Here, at the high density level, *S. potamogetifolia* individuals allocated more resources to sexual production than asexual production. Thus, they consider this the optimal manner to achieve maximum fitness.

#### 4. Conclusion

Our findings are not adapted to the size-advantage model. We discovered that increasing female and male flower production patterns in *S. potamogetifolia* correlated with increasing plant size, indicating that gender dynamics stem from relative changes in individual density levels. There are several costs to individuals associated with investing in flower production, but the two costs relevant in this context are as follows: reduced spending on the production of male flowers and increased spending on the production of female flowers. Thus, when the individual is small and lacks

resources, the plant exclusively produces male flowers (Barrett, 1992; Bierzychudek, 1984; Ishii, 2004). If the blooming of female flowers is genetically controlled, then the development of female flowers will be aborted in the individual that lacks sufficient resources (Huang et al., 2002). Thus, in *S. potamogetifolia* at the highest density level, female flowers decreased with plant size only under severe conditions, such as under minimal environmental resource competition.

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