

# Allocation to clonal and sexual reproduction and its plasticity in *Vallisneria spinulosa* along a water-depth gradient

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**Abstract.** Variations in water level profoundly affect functional stability of freshwater ecosystems, as well as macrophyte growth and reproduction. Although the trade-off between allocation to clonal and sexual reproduction in clonal plants can be influenced by a variety of environmental factors, whether variations of reproductive allocation (RA) in response to different environments are driven only by a size-dependent effect (apparent plasticity) or whether RA can also change independently of plant size (true plasticity) is uncertain. We conducted an experiment in nine outdoor mesocosms (6400 L) to investigate the response of clonal and sexual reproduction and vegetative growth of a perennial submerged macrophyte *Vallisneria spinulosa* at water depths of 50, 100, or 150 cm. We evaluated size-dependent and size-independent effects of water depth on sexual and clonal RA. Deep water reduced vegetative size and sexual output (mass of fruits produced), but increased tuber production of *V. spinulosa*. There was an apparent trade-off between reproductive modes in terms of biomass investment; plants in deep water allocated more resources into clonal propagation and reduced investment in sexual reproduction compared to plants in shallow or intermediate water. Slopes of allometric relationships (sexual vs. vegetative biomass and clonal vs. vegetative biomass) were significantly affected by water depth. Shifts in sexual RA in response to varying water depths were largely size-dependent, but there were also size-independent effects. In contrast, size-independent effects were more important than effects of size changes in determining clonal RA. We concluded that *V. spinulosa* adapted to a water-depth gradient by plastic trade-offs between clonal propagation and sexual reproduction. Furthermore, a size-independent effect on RA suggests a flexible reproductive strategy that could be critical for plant performance in changing aquatic environments.

**Key words:** allometry; biomass partitioning; clonal reproduction; Poyang Lake; reproductive allocation; reproductive strategy; sexual reproduction; submerged macrophytes; trade-off; water level changes.

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## INTRODUCTION

Many perennial plants reproduce both vegetatively and sexually (Klimeš et al. 1997, Fischer and van Kleunen 2002, Vallejo-Marín et al. 2010, Barrett 2015). Vegetative propagules (stolons,

rhizome, tubers, etc.) facilitate local dynamics of plant species, namely maintenance and expansion of established populations (Grace 1993, Piquot et al. 1998, Lenssen et al. 2004). Seeds, however, enable long-distance dispersal and colonization of empty habitats (Eriksson 1992,

Bacles et al. 2006). Trade-off between life-history traits is a principal hypothesis of life-history evolution theory. For example, investment of resources in seed substantially decreased clonal propagule production of *Butomus umbellatus* (Thompson and Eckert 2004) and *Sagittaria latifolia* (Van Drunen and Dorken 2012). Additionally, expression of resource allocation between sexual and clonal reproduction likely further depends on environmental conditions (Sun et al. 2001, van Kleunen 2007). For instance, *Carex brevicuspis* invested more resources to sexual vs. asexual reproduction in disturbed habitats with fertile soils (Chen et al. 2015). Thus, perhaps trade-off of resource allocation between reproductive modes is an adaptive strategy for clonal plants in various environments. Alterations in sexual and clonal allocation may be influenced by plant size (Schmid et al. 1995), density (Rautiainen et al. 2004), and environmental factors (Liu et al. 2009).

Because plant allocation is allometric in the broad sense, that is, changes with plant size (Weiner 2004), factors that influence plant size will also affect reproductive allocation (RA). A size-dependent effect on plant RA has been reported frequently, particularly in ephemeral and annual plants (Schmid and Weiner 1993, Sugiyama and Bazzaz 1998, Cheplick 2005), establishing a relationship between seed output and plant size. In some studies, reproductive allometry for a genotype was predicted to be not particularly plastic; rather, variation of RA was attributed to differences in the position of individuals on a fixed developmental trajectory (apparent plasticity; Weiner et al. 2009). Conversely, it is argued that the relationship between plant size and reproductive output may change in response to environmental factors (Weiner 2004, Cheplick 2005), that is, the trajectory itself is plastic. These shifts in RA are not determined solely by size changes, but may also represent accommodation in biomass partitioning that could be conducive to maintain plant fitness in a changing environment (size-independent effect or true plasticity; Weiner 2004, Niu et al. 2009). Unfortunately, except for a few cases (Hartnett 1990, Schmid et al. 1995, Xie and Yu 2011), little is known about size-dependent vs. size-independent effects on clonal propagule production in clonal perennials, especially in aquatic habitats where clonal

propagation occurs frequently. Elucidating size-dependent vs. size-independent effects is critical for understanding causes of variation in plant RA modes and predicting responses to environmental changes.

Water depth is a primary influence on species distribution and community structure of submerged macrophytes (Dong et al. 2014, Fu et al. 2014), as well as plant growth and production (Søndergaard et al. 2013). As water depth increases, submerged macrophytes usually show adaptations through a variety of adjustments of plant growth, biomass reallocation, and root traits (Maberly 1993, Strand and Weisner 2001, Hussner and Busch 2009). However, very few studies have focused on the RA modes and allometry of RA in response to varying environmental conditions for submerged macrophytes. Water level changes not only affect plant biomass, but can also affect allocation to plant reproduction. Clonal propagules confer survival potential and clonal populations often persist in environments in which ecological conditions are unfavorable for recruitment of sexually produced progeny (Grace 1993, Barrett 2015). Investment in sexual reproduction ensures that seed set is successful under favorable conditions, even if they only occur infrequently. Thus, a balance between clonal and sexual reproduction is crucial for long-time survival of plant populations and maintaining genetic diversity, and this balance may be affected by water depth. We hypothesize that increasing water depth contributes to a change in reproductive strategies in perennial aquatics, investing resources preferentially to clonal propagation, at the expense of sexual reproduction.

Constructions of dams, sand mining, and changes in local rainfall have strongly altered water levels of the Poyang Lake, China's largest freshwater lake (Feng et al. 2012, Lai et al. 2014). To test our hypothesis, *Vallisneria spirulosa*, a perennial submerged macrophyte with sexual and clonal reproduction, as well as a dominant species in the Poyang Lake, was studied. This species frequently distributed at depths of 20–180 cm in shallow lakes along the middle-lower reaches of the Yangtze River. Plants were grown at three water depths to address the following questions: (1) What are the effects of water depth on clonal and sexual output and vegetative size?

(2) Does increasing water depth affect sexual reproduction negatively and affect clonal reproduction positively? and (3) Are changes in RA among treatments caused exclusively by plant size, or is there a size-independent effect? Finally, is there a trade-off between these two modes of reproduction?

## MATERIALS AND METHODS

### Study species

Reproductive allometry was determined in *Vallisneria spinulosa* S. Z. Yan (Hydrocharitaceae). This plant species is a vital component of Poyang Lake ecosystem, as it purifies the water and provides food (tubers) for rare and endangered waterfowls, for example, Siberian crane (*Grus leucogeranus*) and white-naped crane (*Grus vipio*). Like most aquatic angiosperms, it is capable of both sexual and clonal reproduction (via seeds and tubers, respectively). Tubers are the sole perennating structure for this plant, representing the only asexual biomass at the end of the growing season. Clonal growth results from production of rosette-like ramet with ribbon-like leaves (0.4–1 cm wide and up to 2 m long) along elongating stolons during the growing season. At the end of the growing season, each ramet may produce several tubers which overwinter (buried in sediment) and sprout the following spring. Flowering and fruiting occur from June to October. Male inflorescences are located among bases of leaves, containing hundreds of minute flowers enclosed by a short-peduncled spathe. At maturity, male flowers are released to the water surface. A single female flower develops underwater and is brought to the surface by elongation of its slender peduncle. Pollination occurs on the surface of the water. After pollination, the peduncle of the female flower tightly coils and retracts the pollinated flower underwater, where fleshy fruits further develop.

### Experimental material

Mature fruits of *V. spinulosa* were collected from female plants growing naturally in Meixi Lake (116°03' E, 29°13' N) in the Poyang Lake area (115°49'–116°46' E, 28°24'–29°46' N). The water table in Meixi Lake usually fluctuated, resulting in plants being at a depth range of

20–180 cm. Seeds were immediately removed from their capsules and placed in ambient-temperature water to allow the mucilage to naturally degrade (5–7 d). Prior to experimentation, seeds were stored in a plastic container filled with water, in darkness at 4°C for 5 months.

### Experimental setup

A manipulative experiment was designed to explore differences in RA between sexual and clonal reproduction (fruit and seed production as an indicator of sexual production and tubers as indicator of clonal production) at various water depths. The experiment was conducted in an outdoor mesocosm facility located at the Poyang Lake Laboratory for Wetland Ecosystem Research, Chinese Academy of Sciences (116°03' E, 29°26' N), in the northwestern part of Poyang Lake Basin. During April 2015, over 300 seeds of *V. spinulosa* were germinated in each of three bins (56 × 38 × 28.5 cm) containing ~10 cm of sterilized Poyang Lake sediment and 10 cm of water. Approximately 8 weeks later, when seedlings had four or five leaves, 270 plants of uniform size (5–6 cm tall) were selected and individually transplanted into 270 plastic pots (18 cm diameter and 12 cm deep) containing 10 cm deep Poyang Lake sediment (TN: 2.41 mg/g, TP: 0.75 mg/g, organic matter content: 5.82%;  $n = 3$ ). The experimental design was a randomized block design with three water depths (50, 100, and 150 cm), defined as shallow-, intermediate-, and deep-water treatments, respectively, representative of common water depths where *V. spinulosa* grows. After transplantation, the experiment was conducted in nine outdoor mesocosms (2 × 2 × 1.6 m deep) arranged in a randomized block design with three repetitions per water-depth treatment. That is, one of three water-depth treatments was randomly assigned in each of the three replicate blocks. Thirty pots of planted *V. spinulosa* were submerged in one of the nine mesocosms full of lake water (TN: 1.49 mg/L, TP: 0.06 mg/L;  $n = 9$ ) at 50 cm depth for 10-d acclimation. To exclude aquatic animals, water was continuously filtered using a plankton net (pore size: 0.505 mm). Ten days later, in three mesocosms the water depth was adjusted to 100 cm above the sediment surface in pots, in three it was adjusted to 150 cm, and in the other three it remained at 50 cm. Pots were suspended

at the three depths using ropes tied to galvanized metal tubes. Additional water was added to each mesocosm two or three times every week to maintain a consistent water level and promote water circulation. Throughout the study, phytoplankton visible on the water surface were removed using a filter net (pore size: 0.03 mm), whereas epiphyton on plants were removed using a soft brush.

Light intensity in the water column (50, 100, and 150 cm deep) was recorded in each mesocosm using a Hydrolab DS5X Multi-parameter sonde with PAR sensor (Hach Company, Loveland, Colorado, USA). Light intensities at 50, 100, and 150 cm under water were 485.7, 251.7, and 115.3  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (mean  $\pm$  standard error,  $n = 9$ ), respectively, at noon on 25 September 2015.

### Sampling procedures

Treatments were maintained over 18 weeks (June–October 2015). We did not use male individuals, due to the problem of quantifying reproductive biomass for males that release minute flowers to the water surface at maturation. Plant height, leaf number, and fruit numbers were recorded biweekly. Height was measured as the distance from the soil surface to the apex of the longest leaf (to the nearest 1 mm). Size at first flowering was recorded as the number of leaves at initiation of reproduction (Fazlioglu et al. 2016). Some pots were damaged by unidentified aquatic insects during the experiment and were excluded from analyses. When the fruits were fully mature, all pots with healthy and ungrazed female plants were removed from the mesocosms. Ultimately, there were 21 female plants (pots) for each water depth. We began by counting the number of reproductive and vegetative (mature but no flower) ramet number in each pot (each ramet was defined as a single shoot with roots). All fruits per genet were harvested and counted. Then, all pots were emptied, and tubers were carefully harvested from each genet and counted. Roots and tubers were hand-washed. Total seed production (seed number) per female plant was measured based on all fruits from every female plant. Finally, every genet was partitioned into vegetative (including leaves, stolons, and roots), sexual (including peduncles and fruits), and clonal (tubers) structures, separately

dried at 70°C in paper bags for 72 h, and weighed immediately after drying. Vegetative biomass was the sum of leaf, stolon, and root biomasses. The percentage of biomass allocated to each part was calculated by the ratio of each part's biomass to the total biomass of a plant.

### Data analyses

A general linear model (GLM) was used, with water depth as a fixed factor and block as a random factor, to analyze variations in plant traits (height, vegetative biomass, fruit mass, tuber mass, number of reproductive ramets and vegetative ramets, number of fruits, seeds, and tubers, and number of leaves at first flowering) and allocation to various parts. Comparisons of means were done with a LSD test, followed by Bonferroni's correction ( $\alpha' = \alpha/3$ ,  $\alpha = 0.05$ ). Pearson's correlation analysis was used to determine relationship between sexual and clonal allocations, and the significance level was 0.05. All data were analyzed with SPSS statistical software (version 19.0; SPSS, Chicago, Illinois, USA).

Relationships between biomass of sexual structures (S) and vegetative (i.e., non-reproductive) biomass (V), and between biomass of clonal structures (C) and V of individuals at each depth of water were analyzed using the log-transformed version of the classical allometric model:  $\log Y = a \log X + \log b$ .  $X$  is vegetative biomass, and  $Y$  is biomass of sexual or clonal structures in the current study. Parameters  $a$  and  $b$  are usually referred to as the allometric exponent and the allometric coefficient, respectively. An exponent significantly different from 1 indicates an allometric (non-isometric) relationship. Data were log-transformed to analyze allometric scaling in biomass patterns and homogenize variances. Standardized major axis regression was used to fit allometric data and estimation of the parameters under the three treatments, using the SMATR package in R, version 3.11 (Warton et al. 2006). Whether the slope among individuals of each treatment differed from 1 was determined. Significant differences in slopes among water-depth treatments were detected with multiple post hoc comparisons. For the full analysis, a general linear model was used, with  $\log S$  and  $\log C$  as response variable, water depth as fixed variable, and  $\log V$  as covariate. An ANCOVA was



used to test the effects of water depth, log  $V$ , and their interaction on reproduction.

## RESULTS

### Size variation and size-dependent reproductive biomass

Water depth had significant effects on plant size (expressed as vegetative mass; Fig. 1a) and clonal and sexual reproduction of *Vallisneria spirulosa* (Fig. 1a). The vegetative biomass of *V. spirulosa* was greatest in intermediate water (100 cm) and least in deep water (150 cm; Fig. 1a). Sexual biomass was greatest in intermediate water and lowest in deep water (Fig. 1a). In contrast, plants accumulated the least clonal structure (tuber)

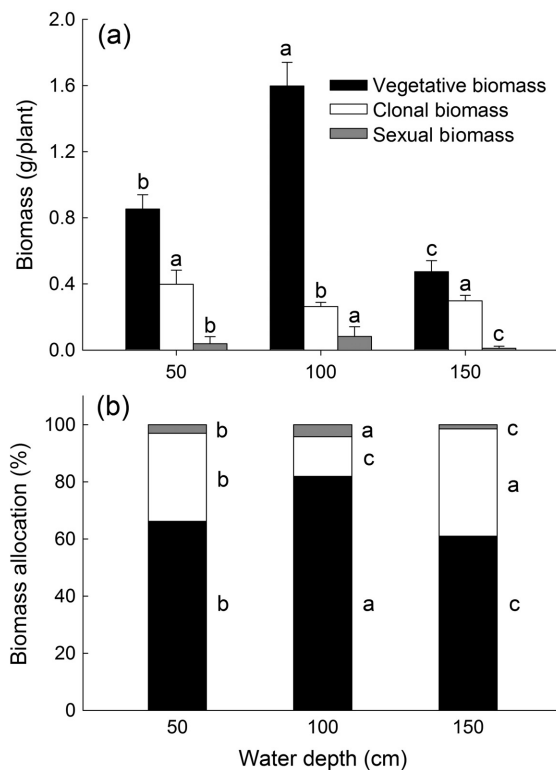


Fig. 1. Effects of water depth on vegetative biomass, tuber biomass, and sexual structure biomass (a) and allocation to various fractions (b) of *Vallisneria spirulosa* biomass (vegetative parts, black; clonal structures, white; sexual structures, gray) growing in shallow, intermediate, and deep water (50, 100, and 150 cm, respectively). Values are mean  $\pm$  standard error. Means without a common letter differed, a–c ( $P < 0.05$ ).

Table 1. General linear model analysis of effects of water depth and log (vegetative biomass) on log (sexual biomass).

Source	SS	df	F	P
Water depth	0.248	2	68.8	<0.001
Log (vegetative biomass)	0.400	1	222.5	<0.001
Water depth $\times$ log (vegetative biomass)	0.052	2	14.5	<0.001
Error	0.103	57	0.002	

Notes: Adjusted  $R^2$  for the model is 0.99. SS, sum of squares.

biomass in intermediate water, with no detectable differences between shallow and deep water (Fig. 1a).

Besides water depth, the GLM revealed that the reproductive out of this species was significantly affected by plant size and the interaction between water depth and plant size (Tables 1 and 2). Half of the variations (50%) in log  $S$  were due to log  $V$ , whereas water depth accounted for 31% of the variations in log  $S$  (Table 1). By contrast, half of the variations (50%) in log (tuber biomass) were explained by the effects of water depth when compared to variation in log  $V$  (37%; Table 2). In addition, there was also a significant water depth  $\times$  log  $V$  interaction; therefore, populations at different water depths differed in their allometric relationships (log  $S$  vs. log  $V$  or log  $C$  vs. log  $V$ ; Tables 1 and 2).

### Quantitative traits of sexual and clonal propagation

Traits of sexual and clonal propagation had distinct responses to water depths. Size at first flowering was significantly greater in deep vs. shallow and intermediate water (Table 3); therefore, threshold size for sexual reproduction was

Table 2. General linear model analysis of effects of water depth and log (vegetative biomass) on log (clonal biomass).

Source	SS	df	F	P
Water depth	0.567	2	186.6	<0.001
Log (vegetative biomass)	0.424	1	278.9	<0.001
Water depth $\times$ log (vegetative biomass)	0.054	2	17.8	<0.001
Error	0.087	57	0.002	

Notes: Adjusted  $R^2$  for the model is 0.90. SS, Sum of squares.

Table 3. Effects of water depth on sexual and clonal reproductive traits of *Vallisneria spinulosa* plants.

Sources	No. of leaves at first flowering	No. of fruits	No. of seed	No. of tubers
Shallow water	60.2 ± 7.8 <sup>b</sup>	2.2 ± 0.4 <sup>b</sup>	283.2 ± 30.2 <sup>b</sup>	10.6 ± 1.5 <sup>a</sup>
Intermediate water	49.1 ± 5.3 <sup>c</sup>	4.0 ± 0.5 <sup>a</sup>	633.7 ± 56.6 <sup>a</sup>	5.3 ± 0.8 <sup>b</sup>
Deep water	84.6 ± 11.0 <sup>a</sup>	1.1 ± 0.2 <sup>c</sup>	111.4 ± 12.9 <sup>c</sup>	8.3 ± 1.2 <sup>a</sup>

Note: Within a column, means without a common letter differed, a–c ( $P < 0.05$ ).

highly variable among treatments. On the contrary, deep-water plants reached a given allocation to clonal propagation at smaller vegetative sizes (Fig. 2b). In addition, numbers of fruits and seeds were highest in intermediate water and lowest in deep water (Table 3). Furthermore, there were significant differences in clonal production (e.g., number of tubers) among water depths, with deep- and shallow-water plants producing the most tubers (Table 3).

#### Biomass allocation to reproductive modes

The percent allocation to sexual, clonal, and vegetative biomass of the species varied significantly among treatments (Fig. 1b). Plants in deep water had greatest allocation to clonal propagation and least allocation to sexual reproduction. In contrast, plants in intermediate water were constrained in their allocation to clonal propagation, but had a significantly higher allocation to sexual reproduction than shallow- or deep-water plants (Fig. 1b). On analyzing the correlation in allocation between sexual and clonal reproduction, plants showed a negative trade-off relationship across water depths ( $r = -0.91$ ,  $P < 0.001$ ).

#### Reproductive allometric relationships among individuals

There was a highly significant positive log–log relationship between S and V for all treatments (Fig. 2a, Table 4). The log S–log V relationship was affected by water depth, as indicated by different slopes (Fig. 2a). The log S–log V slope was significantly  $<1$  in deep water, significantly  $>1$  in intermediate water, but not significantly different from 1 in shallow water (Table 4). There was also a strong positive log–log relationship between C and V within all populations (Fig. 2b, Table 4), and there were significant effects of water depth on the allometric slope. The regression slope was significantly  $>1$  in deep water, significantly  $<1$  in

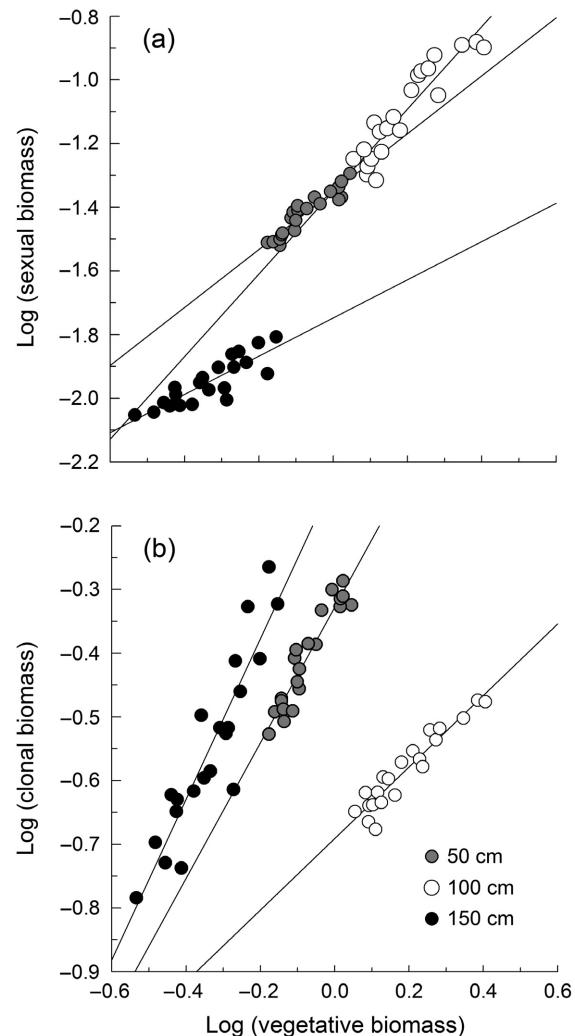


Fig. 2. Relationships between log (sexual biomass) vs. log (vegetative biomass) and between log (clonal biomass) vs. log (vegetative biomass) among individuals of *Vallisneria spinulosa* grown in shallow, intermediate, and deep water (50, 100, and 150 cm, respectively), with standardized major axis lines for each treatment.

intermediate water, but not significantly different from 1 in shallow water (Table 4).

## DISCUSSION

Water depth had prominent effects on plant growth and reproduction. There was an alteration in reproductive strategies of *Vallisneria spirulosa* along the water-depth gradient. Environmental selection pressures on *V. spirulosa* growing in deep or shallow water drove the population to increase its capacity to clonal propagation. Clonality in aquatic habitats is likely an adaptive response to restriction on pollination, or a strategy to exploit stable environments (Reusch 2006, Zhang et al. 2013).

### Plant size and RA in response to water-depth change

In the present study, plant size of *V. spirulosa*, in terms of vegetative biomass, was obtained at a moderate depth (100 cm). Light availability is severely attenuated by water depth, which may have been the main factor restricting the growth of *V. spirulosa*, as photosynthesis of submerged macrophytes can be constrained in deep water. Moreover, considering that plants grown in shallow water (50 cm) had a height of 25.2 cm (Appendix S1: Fig. S1), it is likely that very high photosynthetically active radiation arrived at their leaves, resulting in photo-inhibition. Although optimal water depths can promote growth of submerged macrophytes, for example, *Myriophyllum spicatum* (Strand and Weisner 2001), *Potamogeton obtusifolius* (Maberly 1993), excessively high or low water depths greatly inhibit growth of submerged macrophytes if they exceed the plants' physiological limits (Søndergaard et al. 2013).

As emphasized in life-history theory, shifts in RA can be instrumental for maintaining plant fitness in a changing environment. The present results explicitly demonstrated that individual modes of RA varied across water depths. At a moderate water depth, plants invested more resources in production of fruits and seeds; consequently, resources invested in clonal reproduction were relatively reduced. However, in deep water, sexual reproduction is likely to be negatively influenced by water depth, so that plants invest more resources in production of more

Table 4. Estimated parameters in allometric regression between log (sexual biomass) vs. log (vegetative biomass) and log (clonal biomass) vs. log (vegetative biomass) of *Vallisneria spirulosa* grown at three levels of water depth (using standardized major axis).

Parameters	Water depth (cm)		
	50	100	150
Log (sexual biomass) vs. log (vegetative biomass)			
Slope	0.97 <sup>NS,b</sup>	1.41 <sup>**a</sup>	0.70 <sup>**c</sup>
95% CI	0.82–1.15	1.17–1.69	0.54–0.89
Intercept	–1.35	–1.37	–1.72
R <sup>2</sup>	0.88	0.85	0.73
Log (clonal biomass) vs. log (vegetative biomass)			
Slope	1.13 <sup>NS,a</sup>	0.59 <sup>***b</sup>	1.38 <sup>**a</sup>
95% CI	0.96–1.31	0.51–0.69	1.14–1.67
Intercept	–0.32	–0.70	–0.09
R <sup>2</sup>	0.90	0.90	0.84

Notes: NS, not significant. Within a row, treatments without a common letter differed, a–c ( $P < 0.05$ ).  
<sup>\*\*</sup> $P < 0.01$ , <sup>\*\*\*</sup> $P < 0.001$ .

tubers. In that regard, flowering and seed production of *V. spirulosa* were very limited in deep water, and clonal reproduction might compensate for decreased sexual reproduction.

The shift to much more clonal propagation may be attributed to difficulties in production of successful female flower in deep water (maintenance of long peduncles to bear flowers on the water surface is highly costly). Additionally, *V. spirulosa* responded to high water level by increasing its reproductive threshold (Table 3), that is, by delayed flowering and fruiting. Reduced light availability in deep water might inhibit or restrict the developmental shift of meristems to reproductive organs. Consistent with the present results, spatial variations in water depth and current velocity have been shown to affect the mode of reproduction within populations of several plant species, by limiting the plants' ability to produce emergent flower-bearing stems (Van Wijk 1988, Boeger and Poulson 2003).

Shallow-water plants also produced fewer fruits than intermediate-water plants, though they must invest much less in reproductive support structures. This may have been a response to physiological stresses imposed on growth and

survival of submerged macrophytes by a low water level, namely enhanced plant respiration and photo-inhibition as a result of excessive light intensity (Masini et al. 1995, Peralta et al. 2002). These changes will cause a decrease in production of reproductive ramets that can potentially produce sexual organs (Appendix S1: Fig. S2), thereby reducing the number of flowers and fruits observed.

#### **Evidence of trade-offs between sexual and clonal reproduction**

Our study provided clear evidence for a negative correlation between sexual reproduction and clonal propagation. Thus, *V. spinulosa* shifted the balance between the two reproductive modes to adapt to different environments. Trade-offs in resource allocation between sexual and clonal reproduction have been demonstrated for aquatic species (Thompson and Eckert 2004, Reusch 2006, Van Drunen and Dorken 2012), whereas other studies failed to detect clear trade-off relationships (Eckert et al. 2000, Han et al. 2011). Based on the variability and trade-off of resource allocation between these two reproductive modes, we inferred that different populations of this species living in contrasting habitats are able to use different reproductive strategies. As a dominant species in Poyang Lake, trade-off between allocation to sexual reproduction and clonal propagation along a water-depth gradient could be particularly important for *V. spinulosa* to succeed in new environments.

Because both modes of propagation should be closely linked to fitness, the relative investment in these two modes of reproduction should reflect fitness gains through clonal vs. sexual investment (Van Drunen and Dorken 2012). Clonal reproduction in perennial species may be particularly advantageous as physical and physiological stresses linked with high water levels may lower seed set, seed germination, or seedling establishment. Clonal propagules, due to their relatively large size and their ability to store resources and colonize space, may have a higher potential than seeds for successful colonization (Grace 1993, Johansson and Nilsson 1993). In previous investigations, the amount of tubers produced by *Vallisneria* spp. was a key factor in determining population density of the following growing season in the field where seedlings were rare (Li 2004). However, compared

to seeds, clonal propagules are generally more vulnerable to desiccation and therefore, more short-lived. Therefore, seeds surviving in seed banks have critical roles in the pioneer stages of aquatic vegetation, allowing (re)establishment of genetically diverse population (Pollux et al. 2007, Boedeltje et al. 2008). If so, at a moderate water depth, increased allocation to seeds might reflect enhanced allocation to traits that promote re-establishment after disappearance and contribute to genetic diversity. In deep-water habitats, clonal propagation of *V. spinulosa* may assure population maintenance; by contrast, sexual reproduction might be a luxury investment, and enabling population restoration from extreme events.

#### **Size-dependent vs. size-independent variations of RA**

Because RA within a population changes with plant size, allometry is a useful tool to disentangle the size-dependent and size-independent effects on plant RA variation (Bonser and Aarssen 2009). Herein, we confirmed that biomass of both sexual structures and clonal structures was positively related to plant size. Most of the variation in individual sexual biomass was due to plant size (Table 1), meaning that a size-dependent effect was a dominant mechanism underlying the changes in sexual RA of *V. spinulosa*. Within a population, plant size is usually the best predictor of the amount of reproduction (e.g., number of flowers and seeds produced; Hartnett 1990, Schmid and Weiner 1993). Our results were also in agreement with previous conclusion that clonal propagule production was size-dependent in clonal plants (Schmid et al. 1995, Brown and Eckert 2005, Xie and Yu 2011). Size-dependent RA has been described as a low-risk, bet-hedging strategy, which ensures that the plant species commit resources into reproduction in changing environments to increase plant fitness (Cheplick 2005). Perennial submerged macrophytes in Poyang Lake usually expose to large spatial and temporal water-depth changes. Under these circumstances, a bet-hedging strategy in RA could be adaptive for perennial species to balance the functions between survival and reproduction.

However, theory predicts that plants could respond to environmental changes not only by a size-dependent effect, but also by a size-independent effect (also called true plasticity;



Weiner 2004). Water depth affected allometric slopes of S–V and C–V relationships, as well as plant size, which clearly indicated that a size-independent effect was also a mechanism for regulation of RA in *V. spinulosa*. Moreover, water depth accounted for most of the variation in tuber biomass (Table 2). Thus, a size-independent effect was more important in regulating clonal RA when compared to variation in plant size. Results in *V. spinulosa* were consistent with empirical evidence in terrestrial plants that changes in RA were explainable by environmental effects on biomass partitioning (Sugiyama and Bazzaz 1998, Niu et al. 2009). It has been suggested that size-independent changes in biomass partitioning may buffer plant populations from environmental changes (Ghalambor et al. 2007), and hence may be of evolutionary significance. Thus, the ability to modify biomass investment among sexual and clonal reproduction and vegetative growth along a water-depth gradient may be an important strategy of *V. spinulosa* to adapt to water level changes. Further studies are needed to understand how this variability in biomass partitioning affects population and community dynamics of submerged macrophytes in the context of hydrological changes induced by climate and human activities.

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