

# Sex-specific plasticity of reproductive allocation in response to water depth in a clonal, dioecious macrophyte

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**PREMISE OF THE STUDY:** Sex-specific differences in reproductive investment contribute to sexual dimorphism in dioecious plants. Along environmental gradients, males and females may plastically adjust reproductive allocation differently because of contrasting reproductive costs. In dioecious macrophytes, variation in water depth is likely to influence reproductive allocation but has not been investigated in detail.

**METHODS:** *Vallisneria spinulosa* was grown in aquatic mesocosms at water depths of 50, 100 and 150 cm for 14 weeks. Plasticity in allocation was measured to investigate whether sexual dimorphism in reproductive allocation and vegetative growth changed in response to varying water depths.

**KEY RESULTS:** Females invested a higher fraction of resources to sexual reproduction than males across all water depths and decreased proportional allocation to sexual structures in shallow and deep water compared to intermediate water depth. In contrast, males maintained similar sexual allocation across all water depths. Females displayed larger vegetative size than males, despite greater sexual investment, but decreased vegetative biomass more than males in shallow or deep water. The sexes invested similarly in clonal propagation by tubers at all water depths, but a trade-off with sexual reproduction was only evident in females.

**CONCLUSIONS:** Our results suggest that females of *V. spinulosa* have mechanisms to compensate for the costs of sexual reproduction in heterogeneous environments. Compared to males, females expressed greater plasticity in biomass allocated to sexual reproduction and vegetative growth in response to water depth variation. Environmental variation in underwater light availability probably caused the sex-specific allocation strategies found in *V. spinulosa*.

**KEY WORDS** cost of reproduction; dioecy; Hydrocharitaceae; Poyang Lake; reproductive effort; sexual dimorphism; submerged aquatic; *Vallisneria spinulosa*; water depth variation.

Plants partition their finite resources among reproduction, growth, and maintenance, and allocating more resources to one function decreases resource availability to other functions (Stearns, 1989). Because of this trade-off, differences in reproductive allocation result in relative differences in life-history traits (e.g., vegetative growth and size, mortality, frequency of flowering; Obeso, 2002; Harris and Pannell, 2008; Matsuyama and Sakimoto, 2008) among species and populations. Patterns of resource allocation may differ between the sexes in dioecious plants as a consequence of their different requirements for male and female reproduction (Delph, 1999). Typically, females invest a greater proportion of their resources to reproductive structures compared to their male counterparts, largely due to

investment in fruits and seeds (Barrett and Hough, 2013). If females of dioecious species invest significantly more resources to reproduction than males do, then vegetative growth, survival, and flowering may be lower.

Sexual dimorphism in reproductive investment, vegetative growth, and size has been documented in a variety of terrestrial plant species, including trees, shrubs, and herbaceous plants (Lloyd and Webb, 1977; Delph, 1999; Obeso, 2002; Barrett and Hough, 2013). In long-lived species, males often exceed females in size and capacity for clonal propagation, whereas females of short-lived species can be significantly larger than males (e.g., Hesse and Pannell, 2011; Teitel et al., 2016). In some species, vegetative growth does

not differ between males and females (Delph et al., 1993; Costich, 1995). Importantly, the degree of sexual dimorphism not only varies among plant and animal species (Laiolo et al., 2013; Tonnabel et al., 2014), but can also vary among populations of the same species (Stillwell et al., 2007; Tonnabel et al., 2017). Determining the relative contribution of genetic and environmental factors to variation in allocation patterns in dioecious species is a major challenge for understanding the evolution of sexual dimorphism.

According to the differential plasticity hypothesis, males and females differ in their plastic responses to environmental factors, which can result in variation in the degree of sexual dimorphism along environmental gradients (Delph and Bell, 2008). The differing requirements for reproduction between the sexes in plant populations can cause differential responses to environmental conditions (Leigh and Nicotra, 2003). For example, the generally higher costs of reproduction for females compared to males for a given reproductive episode may cause a more pronounced reduction in reproductive allocation under environments with low resource availability (Dawson and Geber, 1999; Pickering and Hill, 2002). Because of higher reproductive requirements, females may be more sensitive than males to environmental stress in terms of vegetative growth, leading to enhanced sexual differences in growth under stressful conditions (Dawson and Ehleringer, 1993; Eckhart and Chapin, 1997). Alternatively, females in suboptimal growth conditions may suppress reproductive allocation but conserve vegetative allocation to ensure survivorship (e.g., Jönsson and Tuomi, 1994), thus leading to decreased sexual dimorphism in vegetative investment. For clarifying these predictions, experimental studies on the manner in which sexual dimorphism in reproductive and vegetative investment may change plastically across different environments is necessary.

Aquatic macrophytes provide opportunities to investigate the adaptive significance of plasticity of reproductive investment in growth and reproduction along environmental gradients. Many macrophytes have high levels of phenotypic plasticity and diverse mechanisms for clonal propagation, e.g., via corms, rhizomes, tubers, and turions (Sculthorpe, 1967; Barrett et al., 1993; Grace, 1993; Eckert et al., 2016). Clonal plants allocate resources to sexual reproduction and clonal propagation from similar resource pools during reproductive episodes. Thus, in addition to the growth–reproduction trade-off, a trade-off between sexual and clonal reproduction can be expected and, indeed, has been reported in several aquatic plants (Thompson and Eckert, 2004; Van Drunen and Dorken, 2012). Patterns of differential resource allocation to sexual reproduction between the sexes of dioecious aquatics may therefore play a significant role in influencing resource allocation to clonal propagation.

Because many aquatic plants occur in habitats with strong environmental gradients, they are especially suitable for investigating plasticity in sexual dimorphism. Water depth is one of the most important determinants of the distribution, growth, and reproduction of submersed macrophytes, especially in relatively shallow lakes (Strand and Weinsner, 2001; Fu et al., 2014). Different areas of a lake can have variable water depths because of the topography of the lake bottom, and human activities can greatly affect changes in water levels in lakes and rivers, including flooding (Lai et al., 2014; Munoz et al., 2018). Increased water depth reduces light penetration to the lake bottom (photic zone, reviewed by Sculthorpe, 1967), especially in eutrophic water bodies. In lakes with a wide range of depths, deep water can represent an energy-limited and stressful

environment for many aquatics owing to the low availability of light for photosynthesis and growth.

Aquatics generally modify their patterns of reproductive allocation in different environmental conditions, but relatively little is known about sex-specific responses, the major focus of this study. Here, we investigate the plasticity of sexual dimorphism in allocation patterns in the dioecious, clonal, submersed aquatic *Vallisneria spinulosa* S.Z.Yan using an experimental mesocosm approach. We compared vegetative growth and resource allocation to sexual and clonal reproduction of male and female individuals along a manipulated water-depth gradient. Our study addressed the following questions: (1) Overall, do females invest more resources in reproduction and fewer resources in vegetative growth than males across varying water depths? (2) In response to decreased light availability in deep water conditions, are there sexual differences in relative allocation to reproduction and vegetative growth? (3) Is there evidence for a trade-off between sexual and clonal reproduction in male and female plants? Our results demonstrate striking differences in sex-specific plasticity across water depth treatments with important implications for the reproductive ecology of dioecious aquatics.

## MATERIALS AND METHODS

### Study species

*Vallisneria spinulosa* S.Z.Yan (Hydrocharitaceae) is a clonal, dioecious, submersed aquatic with a hydrophilous pollination system. It is found in ponds and lakes in Jiangsu, Zhejiang, Hubei, Hunan, and Jiangxi provinces of China (Chen et al., 2012) and is endemic to the mid-lower reaches of the Yangtze River (Wang et al., 2010). *Vallisneria spinulosa* plays an important role in freshwater ecosystems by providing food for waterfowl, nursery habitat for fishes, substrate for invertebrates, and it also serves to purify water. In common with other *Vallisneria* species (reviewed by Sculthorpe, 1967; Cox, 1988), female flowers develop underwater, are brought to the surface singly on elongated slender peduncles and, at anthesis, float on the water surface. Male inflorescences are produced underwater, and at maturity, the male spathes containing numerous male flowers detach and float to the water surface. Male flowers are dispersed across the water surface and aggregate around female flowers, resulting in pollination. After pollination, the peduncles of female flowers coil tightly and retract pollinated flowers underwater, where fleshy fruit develop. During the growing season, clonal growth results from ramet production along elongating stolons, and in the autumn, ramets produce several tubers that overwinter in the sediment and germinate the following spring.

### Experimental material

On January 2016, we collected numerous tubers of *V. spinulosa* from a large dioecious population at Bang Lake (115°55′–116°06′E, 29°11′–29°18′N), a part of Poyang Lake (Jiangxi Province, China, 115°49′–116°46′E, 28°24′–29°46′N). The population covered an area of approximately 0.5 ha. The water table in Bang Lake usually fluctuates, resulting in plants at depths from 20 to 180 cm. The tubers were randomly collected at 20-m intervals throughout the population to maximize the number of genets sampled. The sexes were unknown when the tubers were collected. We then returned

the tubers to the laboratory where they were thoroughly washed by hand. Tubers were then stored in a water-filled plastic container in the dark at 4°C for approximately 4 months until used for the experiment.

### Experimental design

To investigate the influence of water depth on reproductive allocation and vegetative growth, we started an experiment on 1 June 2016 in nine outdoor mesocosms (2 × 2 × 1.6 m deep) at the Poyang Lake Laboratory for Wetland Ecosystem Research, Chinese Academy of Sciences (116°03'E, 29°26'N). We selected 225 mature tubers of *V. spinulosa* of uniform size (mean fresh mass ± SE = 0.895 ± 0.017 g; range of tuber mass = 0.751–1.023 g), and these were individually transplanted into plastic pots (23 cm diameter and 17 cm deep) containing deep sediment from Poyang Lake (mud to sand = 4:1, TN: 2.30 mg g<sup>-1</sup>, TP: 0.62 mg g<sup>-1</sup>, organic matter content: 5.32%; *n* = 3 pots). The tubers were randomly distributed among the three water depth treatments so that there was no significant difference in mean tuber mass among treatments ( $F_{2, 222} = 0.120$ ;  $P = 0.887$ ). The experiment was set up as a randomized block design with three water depths: 50 (shallow), 100 (intermediate), and 150 cm (deep), representative of water depths in which *V. spinulosa* commonly grows. We randomly assigned the pots to the three water depth treatments in each of the three replicate blocks, and we adjusted water depths so that they were 50, 100 and 150 cm above the sediment surface in pots. A total of 75 pots were assigned to each treatment. We used ropes tied to galvanized metal tubes to suspend pots at the three depths, and additional water was added to each mesocosm two or three times every week to maintain a consistent water level and promote water circulation. To exclude aquatic animals, we continuously filtered water using a plankton net (pore size: 0.505 mm) and removed phytoplankton visible on the water surface using a filter net (pore size: 0.03 mm). We also removed epiphyton from plants throughout the study using a soft brush.

We recorded light intensity at 50, 100, and 150 cm depths at noon under clear skies on 8 August 2016 in each mesocosm using a Hydrolab DS5X Multi-parameter sonde with PAR sensor (Hach Co., Loveland, CO, USA). The light intensities at 50, 100 and 150 cm depth were 905.5 ± 20.5, 469.2 ± 12.9 and 214.8 ± 9.8 μmol m<sup>-2</sup> s<sup>-1</sup> (mean ± SE, *n* = 9), respectively.

### Harvesting of plant parts

From 6 to 9 September (at fruit maturation), we harvested the above- and belowground portions of well-developed, intact plants of both sexes in each mesocosm. The final numbers of harvested male plants were 25, 23, and 19 for the three water depths, and those of harvested female plants were 22, 25, and 20, respectively. Because some stunted plants grew very poorly and did not flower and others were grazed by aquatic insects, our final harvest only involved plants that grew vigorously and flowered. Each plant was hand-washed free of sediment and epiphytic aquatic organisms. For each harvested plant, we counted the number of tubers and for males the number of spathes and for females the number of fruits. Every plant was partitioned into vegetative (leaves, roots, and stolons), sexual (spathes or peduncles and fruits) and clonal (tubers) components. We dried plant parts in an oven to constant mass at 70°C and weighed. Because dispersal of pollen from some male spathes occurred when plants were harvested, we randomly sampled 10

nondehiscent, mature male spathes from five other male plants within each depth that were not harvested for biomass measurements and weighted the biomass per spathe, respectively. We then multiplied the mean biomass of 10 male spathes obtained at each water depth by the total number of spathes per male to give an estimate of the biomass of the sexual reproductive component of male plants for each water depth. In this study, the dry mass of leaves, roots, and stolons (i.e., nonreproductive parts) was combined as vegetative investment (i.e., vegetative growth). Biomass allocation to sexual and clonal reproduction was determined by dividing the dry mass of each by the total biomass of the plant.

### Data analyses

Weiner (2004) proposed a linear model of reproductive allometry as an appropriate null hypothesis for understanding variability in reproductive allocation, and we used this approach. For both sexes, we analyzed relations between sexual reproductive biomass (SR) and vegetative biomass (V), and between clonal reproductive biomass (CR) and V among individuals of each water depth treatment using the log-linear model:  $\log y = a \log x + \log b$ , where parameter *a* is the allometric exponent and *b* the allometric coefficient, *x* is vegetative biomass, and *y* is the biomass of the sexual or clonal components. A value of *a* different from 1 indicates an allometric (i.e., non-isometric) relation between reproductive and vegetative biomass. This model allowed us to test whether allocation to reproduction increased ( $a > 1$ ) or decreased ( $a < 1$ ) more than linearly (allometrically) with size. We used a standardized major axis (SMA) regression to fit log-transformed data and for estimating the parameters under different water depth treatments and for different sexes. We tested whether the slope among individuals within each treatment was different from 1 and whether there were significant differences in slopes among water depth treatments and between sexes. All standardized major axis (SMA) analyses used the package SMATR 3 (Warton et al., 2012) in R version 3.3.1 (R Core Team, 2016).

We used linear mixed-effect models to test for differences between the sexes in their response to water depth, with biomass allocation to sexual and clonal reproduction, and vegetative biomass as response variables, and block as a random effect. We conducted post hoc comparisons between means using a Tukey's honestly significant difference (HSD) test for multiple comparisons, with  $P < 0.05$ . We used Pearson's correlation analysis to determine the relation between allocation to sexual and clonal reproduction for the two sexes, respectively, with a significance level of 0.05. We performed these data analyses using SPSS statistical software (version 19.0; SPSS, Inc., Chicago, IL, USA).

## RESULTS

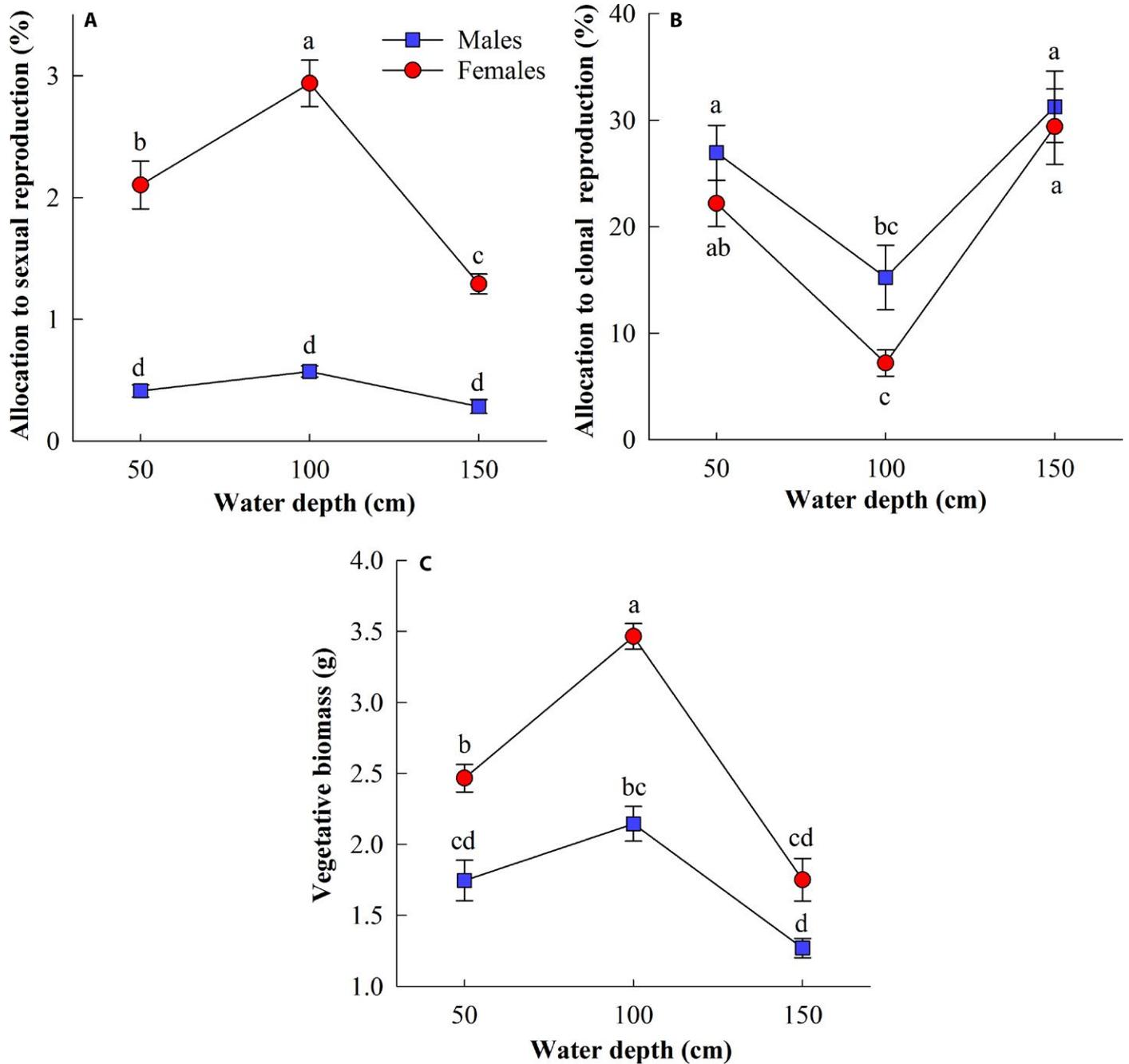
### Resource allocation to sexual and clonal reproduction

At all water depths, female plants allocated a greater proportion of biomass to sexual reproduction than male plants ( $P < 0.001$ ; Table 1, Fig. 1A). Post hoc comparisons indicated that although water depth did not significantly affect sexual allocation in males (male:  $F_{2, 64} = 7.164$ ,  $P = 0.200$ ), in females, there were significant differences among water depths (female:  $F_{2, 64} = 28.72$ ,  $P < 0.001$ ). Biomass allocated to sexual structures in females was lowest in deep water (150

**TABLE 1.** Summary of linear mixed-effects models of allocation to sexual and clonal reproduction and vegetative biomass of *Vallisneria spinulosa* males and females (sex) grown at different water depths.

Source	Allocation to sexual reproduction		Allocation to clonal reproduction		Vegetative biomass	
	F	P	F	P	F	P
Depth	28.403 <sub>2,128</sub>	<0.001	26.644 <sub>2,128</sub>	<0.001	62.476 <sub>2,128</sub>	<0.001
Sex	265.017 <sub>1,128</sub>	<0.001	4.942 <sub>1,128</sub>	0.058	80.399 <sub>1,128</sub>	<0.001
Depth × sex	13.737 <sub>2,128</sub>	<0.001	0.559 <sub>2,128</sub>	0.573	7.674 <sub>2,128</sub>	0.001

Note: Subscripts after F values are the respective numerator and denominator degrees of freedom.



**FIGURE 1.** Allocation to (A) sexual and (B) clonal reproduction and (C) vegetative biomass for *Vallisneria spinulosa* males and females as a function of water depth (50, 100, and 150 cm). Values are means ± SE; values with different letters differed significantly among water depths in a Tukey HSD test at  $P < 0.05$ .

cm), intermediate in shallow water (50 cm), and highest at an intermediate water level (100 cm) (Fig. 1A). Thus, sex-differential allocation to sexual structures depended on water depth (sex  $\times$  depth interaction:  $P < 0.001$ ; Table 1, Fig. 1A).

The proportion of biomass invested in clonal propagation tended to be lower in females than males across all water depths, but this trend was not significant ( $P = 0.058$ ; Table 1, Fig. 1B). In both sexes, resource allocation to clonal propagation was significantly lower in intermediate than in shallow or deep water (male:  $F_{2,64} = 7.563$ ,  $P < 0.001$ ; female:  $F_{2,64} = 17.73$ ,  $P < 0.001$ ; Fig. 1B), but water depth had no significant effect on the degree of sex-differential allocation to clonal structures (sex  $\times$  depth interaction:  $P = 0.573$ ; Table 1, Fig. 1B). Females showed a moderate negative correlation between resource allocation to sexual and clonal reproduction across water depths ( $r = -0.47$ ,  $P < 0.001$ ), whereas there was no evidence for this effect in males ( $r = -0.15$ ,  $P = 0.236$ ).

### Vegetative growth

At both shallow and intermediate water depths, females displayed greater vegetative biomass (size) than males ( $P < 0.001$ ; Table 1, Fig. 1C). Post hoc comparisons indicated that water depth significantly affected female vegetative biomass; female plants grown at an intermediate depth had the greatest vegetative biomass, whereas those grown in deep water had the lowest ( $F_{2,64} = 35.12$ ,  $P < 0.001$ ; Fig. 1C). In males, vegetative biomass was significantly reduced in deep water compared with shallow or intermediate water depths ( $F_{2,64} = 11.89$ ,  $P < 0.001$ ; Fig. 1C). The relative size of male versus female plants was greatly affected by water depth (sex  $\times$  depth interaction:  $P < 0.001$ ; Table 1) with vegetative biomass decreasing more in females than males in deep water (Fig. 1C).

### Reproductive allometry

In both males and females, the amount of biomass allocated to sexual reproduction increased disproportionately with increases in vegetative biomass (males:  $a = 2.18$ ,  $P < 0.001$  for  $a > 1$ ; females:  $a = 1.86$ ,  $P < 0.001$ ; Table 2). The slope of log SR–log  $V$  among females was significantly affected by water depth and was lower in deep water depths than in shallow or intermediate water depths (Table 2 and Fig. 2B). In contrast, the log SR–log  $V$  slope among males remained unchanged across water depths (Table 2, Fig. 2A). Clonal investment increased with increasing biomass in males ( $a = 1.34$ ,  $P = 0.019$ ; Table 3), but not in females ( $a = -1.51$ ,  $P < 0.001$ ; Table 3). In both sexes, the log CR–log  $V$  slope varied significantly among water depth treatments (Table 3, Fig. 3).

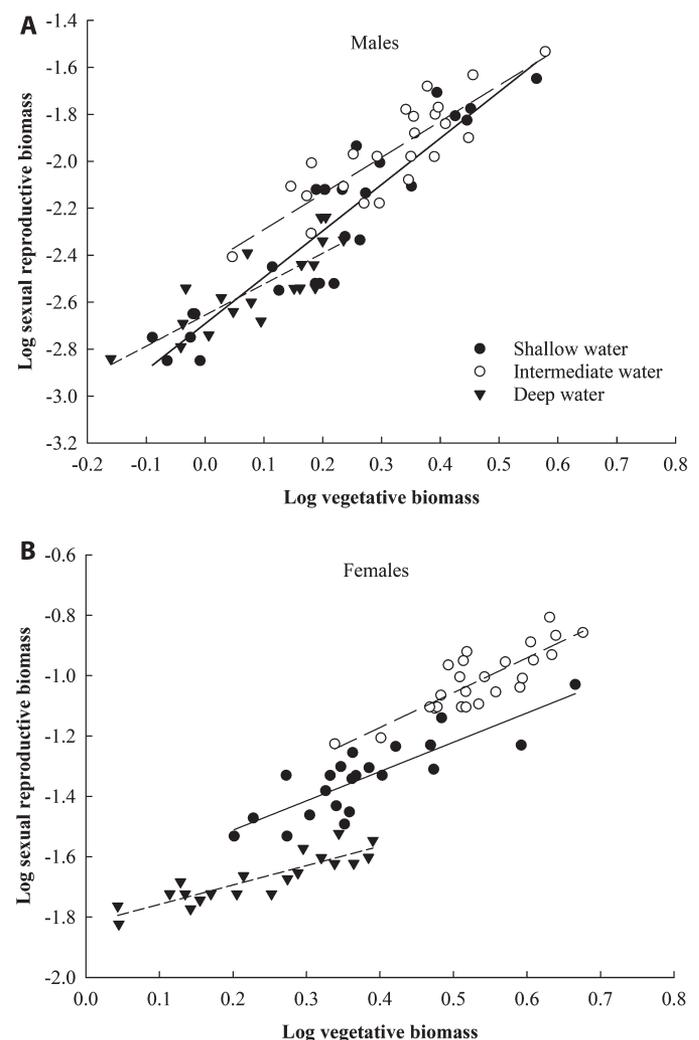
### DISCUSSION

The effect of water depth on growth and reproduction in submerged macrophytes has been widely studied (e.g., Strand and Weisner, 2001; Zhou et al., 2017; reviewed by Sculthorpe, 1967), including in several *Vallisneria* species (Lovett Doust and Laporte, 1991; Li et al., 2017, 2018). However, earlier studies of *Vallisneria* did not consider whether variation in water depth may lead to plasticity in sexual dimorphism. In our experiment, vegetative growth and sexual investment in females and males of *V. spinulosa* did not show straightforward linear relations with increasing water depth. Rather, most of the variables that we

**TABLE 2.** Estimated slopes and intercepts in allometric regression between log sexual biomass ( $y$ ) versus log vegetative biomass ( $x$ ) of males and females of *Vallisneria spinulosa* in different water depth treatments (using standardized major axis regression).

Sex	Depth	Slope = $a$	95% CIs of slope	Intercept	$R^2$
Males <sup>a</sup>	SW <sup>a</sup>	2.13***	1.82–2.50	–2.75	0.86
	MW <sup>ab</sup>	1.81***	1.43–2.29	–2.53	0.73
	DW <sup>abc</sup>	1.63**	1.23–2.17	–2.69	0.68
	Overall	2.18***	1.97–2.40	–2.70	0.85
Females <sup>b</sup>	SW <sup>c</sup>	1.16 <sup>NS</sup>	0.90–1.49	–1.78	0.70
	MW <sup>bc</sup>	1.37**	1.09–1.73	–1.75	0.71
	DW <sup>d</sup>	0.73*	0.58–0.92	–1.84	0.78
	Overall	1.83***	1.66–2.01	–2.04	0.85

SW: shallow water; MW: intermediate water; and DW: deep water. Asterisks represent slopes that differed from 1: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , NS, not significant. The superscript letters in the depth column are the result of post hoc comparisons; depths with different letters are significantly different ( $P < 0.05$ ).



**FIGURE 2.** The allometric relationship between log sexual reproductive biomass and log vegetative biomass for (A) males and (B) females of *Vallisneria spinulosa* in shallow water (●, solid line), intermediate water (○, long dashed line), and deep water (▼, short dashed line), with standardized major axis regression lines for each treatment.

**TABLE 3.** Estimated slopes and intercepts in allometric regression between log clonal biomass (y) versus log vegetative biomass (x) of males and females of *Vallisneria spinulosa* in different water depth treatments (using standardized major axis regression).

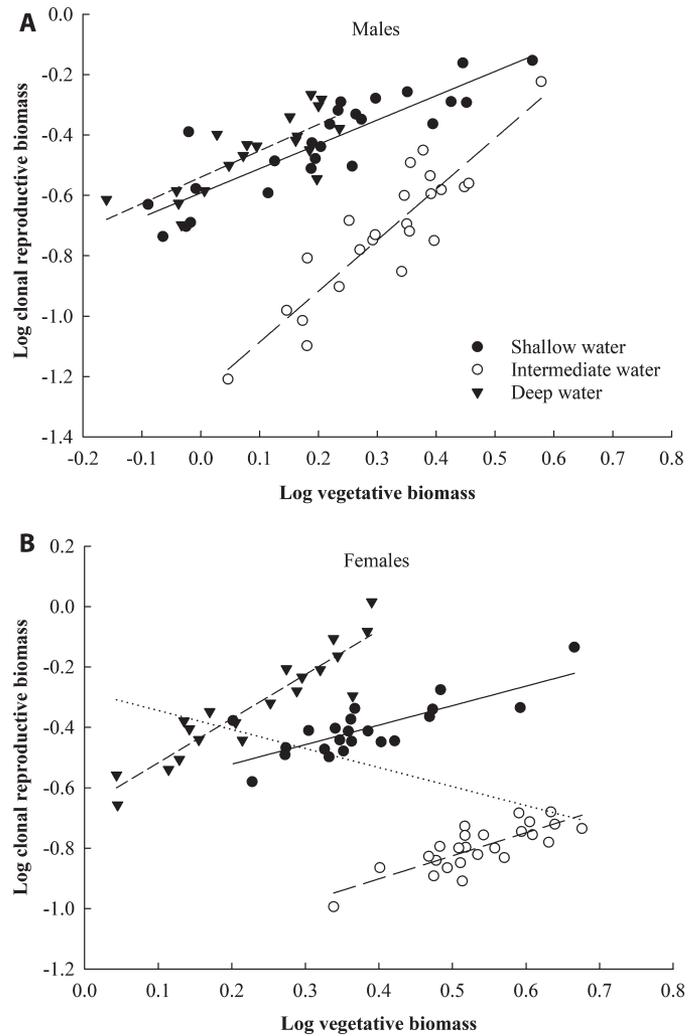
Sex	Depth	Slope = <i>a</i>	95% CIs of Slope	Intercept	R <sup>2</sup>
Males <sup>a</sup>	SW <sup>c</sup>	0.93 <sup>NS</sup>	0.75–1.14	–0.62	0.76
	MW <sup>a</sup>	1.87 <sup>***</sup>	1.54–2.28	–1.31	0.81
	DW <sup>bc</sup>	1.13 <sup>NS</sup>	0.82–1.55	–0.56	0.60
	Overall	1.34 <sup>**</sup>	1.06–1.69	–0.82	0.08
Females <sup>a</sup>	SW <sup>c</sup>	0.84 <sup>NS</sup>	0.62–1.12	–0.72	0.60
	MW <sup>c</sup>	0.96 <sup>NS</sup>	0.74–1.25	–1.31	0.63
	DW <sup>ab</sup>	1.58 <sup>***</sup>	1.31–1.90	–0.69	0.86
	Overall	–1.51 <sup>***</sup>	–1.89 to –1.21	0.07	0.17

SW: shallow water; MW: intermediate water; and DW: deep water. Asterisks represent slopes different from 1: <sup>\*\*</sup>*P* < 0.01, <sup>\*\*\*</sup>*P* < 0.001, NS, not significant. The superscript letters in the depth column are the result of post hoc comparisons; depths with different letters are significantly different (*P* < 0.05).

measured exhibited a peaked response with intermediate water depth different from the other two water depth treatments. This general pattern probably occurred because shallow water subjects these submersed aquatic plants to high light conditions that may interfere with photosynthesis, whereas our deep-water treatment decreased light availability, photosynthesis, and energy-use efficiency. Similar nonlinear responses to these three water depth treatments in growth have also been reported in semelparous *V. natans* (Li et al., 2017). Our range of water depth treatments therefore probably encompassed both stressful and less stressful conditions for growth. We next consider the specific sex-specific responses to patterns of reproductive allocation that were revealed by our study and suggest why they may be adaptive for submersed aquatics occupying heterogeneous environments.

**Sexual differences in resource allocation to reproduction**

Our results revealed that females invested more in sexual reproduction than males across all experimental water depth treatments. Female-biased reproductive allocation is well documented in animal-pollinated, dioecious taxa (Delph, 1999; Obeso, 2002; Barrett and Hough, 2013), but has also been reported in the clonal dioecious macrophyte *V. americana* (Lovett Doust and Laporte, 1991). Female-biased allocation is usually explained by the fact that reproductive investment in males is limited to flowering, whereas females not only produce flowers but also costly fruits and seeds. Also, water-pollinated plants such as *Vallisneria* may also have considerable energetic investment in accessory structures (e.g., peduncles) in females to position flowers at or just above the water surface to facilitate pollination (Cox, 1988). Male plants do not bear this cost; male structures float to the surface and have much reduced peduncles. It is important to emphasize that our analysis does not consider any differences in gamete production between the sexes. It is possible that allocation to pollen in *Vallisneria* may involve a substantial investment of specific resources. For example, in wind-pollinated *Mercurialis annua*, the high nitrogen demands of pollen production may exert a considerable cost, leading to males experiencing a greater reproductive burden than females (Harris and Pannell, 2008; Tonnabel et al., 2017). Different resource currencies (e.g., carbon versus nitrogen) between the sexes of dioecious plants may be an important factor driving sex-specific patterns of sexual dimorphism.



**FIGURE 3.** The allometric relationship between log clonal reproductive biomass and log vegetative biomass for (A) males and (B) females of *Vallisneria spinulosa* in shallow water (●, solid line), intermediate water (○, long dashed line), and deep water (▼, short dashed line), with standardized major axis regression lines for each treatment. The dotted line in (B) represents the orthogonal (SMA) line where data for all water depths for females were analyzed together, with overall negative slope (Table 3).

Our study detected a trade-off in females between sexual reproduction and clonal propagation via tubers, whereas reproductive costs for males had no apparent effect on tuber production. Several other studies of dioecious species have also detected a trade-off between sexual investment and clonal propagation in female plants. For example, in the dioecious emergent aquatic *Sagittaria latifolia*, a 1:1 trade-off between biomass investment in female function and clonal reproduction (ramet and corm production) occurs in females, but there was no evidence that male sexual investment had any influence on the production of ramets and corms (Van Drunen and Dorken, 2012). Similarly, in *Rubus chamaemorus*, a similar trade-off was evident between clonal propagation (rhizome production) and female function in females that were allowed to produce fruit (Ågren, 1988). On the basis of an analysis of fitness gain curves between the sexes of clonal dioecious species, Dorken and Van Drunen (2010) proposed that clonal expansion may benefit

males more strongly than females owing to fertility selection, which may explain the absence of a trade-off in males between sexual investment and clonal propagation.

### Sexual differences in vegetative growth

Males of *V. spinulosa* were smaller in terms of overall vegetative biomass than females in contrast to expectations of the cost of reproduction hypothesis (Obeso, 2002). This pattern is also evident in *V. americana*, and Lovett Doust and Laporte (1991) proposed that females had to grow larger than males before they could reproduce sexually to bear their increased reproductive costs. The fitness benefits of this size-advantage hypothesis have been explored theoretically in plants (Charnov, 1982; Andersson and Iwasa, 1996), and the earlier reproductive maturity of males compared to females has been reported in several dioecious species (Nicotra, 1999; Harris and Pannell, 2008; Barrett et al., 2010; Teitel et al., 2016). Males of *V. spinulosa* flowered earlier than females in our experiment, and as a consequence, male growth may have been partly compromised early in the growing season when plants flower and thus need to produce nitrogen-rich pollen that influences size later in life. In contrast, females generally allocate more to leaf tissue early in the season, enabling them to acquire more photosynthate for later allocation to costly fruits and seeds (Delph et al., 1993; Harris and Pannell, 2008). Photosynthesis of reproductive structures may provide a compensatory mechanism for reproduction that may conceal growth–reproduction trade-offs (Blanke and Lenz, 1989; Obeso, 2002). In our experiment, flowers and fruits of females were green and may therefore also contribute to reproductive allocation through photosynthesis.

### Plasticity in sexual dimorphism in response to water depths

Our previous investigation on the influence of variation in water depth on reproductive allocation in *V. spinulosa* reported considerable phenotypic plasticity in allocation patterns across the same water depths that were used in this study (Li et al., 2018). However, the sex of individuals was not considered, and in contrast to the present work, seeds rather than tubers were used in the experiment. Several studies of terrestrial dioecious species have demonstrated sex-specific plasticity in response to various environmental factors including nitrogen (Hesse and Pannell, 2011), moisture availability (Delph and Bell, 2008), and plant density (Tonnabel et al., 2017). However, to our knowledge, our study provides the first evidence for plasticity in sexual dimorphism in response to variation in water depth. This finding has broader implications because of the common occurrence of dioecy in aquatic macrophyte families, especially those that are submersed (Sculthorpe, 1967; Les, 1988). Additional investigations on other species would be useful to determine the extent to which sex-specific plasticity in sex dimorphism is widespread.

During the 14-week experiment, reproductively mature males and females differed in how they adjusted their resource allocation to sexual reproduction, probably as a response to different levels of underwater light availability. Whereas males maintained the same allocation of resources to sexual reproductive structures across the three water depth treatments, females decreased their allocation to sexual reproduction in shallow or deep water. These results indicate sex-differential plasticity to various water depths in females, consistent with the general prediction that females are likely to

reduce reproductive investment more than males in stressful environments. Carbon acquisition is likely to be more critical in females than males owing to the requirement for seed production. However, another contributing factor is that female reproductive structures produced in deep water are likely to also be more expensive than the equivalent structure of males in terms of biomass allocation because they are required to grow to the water surface. *Vallisneria spinulosa* is relatively shade tolerant and able to grow clonally in deep water conditions. It is therefore not surprising that, in our shallow water treatment, investment in sexual reproduction was also reduced, although not to the extent found in deep water conditions. The reduced investment may be associated with suboptimal conditions for photosynthesis including excessive light and possibly higher water temperatures.

We found that whole-plant investment in clonal tissue of males and females in *V. spinulosa* was responsive to changes in water depth and probably to underwater light conditions. Females of *V. spinulosa* allocated more biomass to sexual reproduction in the intermediate water depth than in shallow or deep water, but allocated more biomass to clonal propagation in both shallow and deep water compared to intermediate water depth. However, males of *V. spinulosa* were able to increase tuber production in shallow or deep water without a decrease in flowering. These results reflect the occurrence of sex-specific differences in trade-offs between investment in sexual reproduction and clonal propagation. Trade-offs between these two reproductive modes often occur when resources are limited (Zhang and Zhang, 2006; Liu et al., 2009). Thus, the allocation trade-off found in females but not males seems likely to be driven by different resource currencies for female and male function, perhaps reflecting the direct impact of light limitation on the production of carbon-rich reproductive structures in females.

Trade-offs in components of life history are a way to maximize lifetime reproductive success and safeguard the future capacity for population establishment and genet persistence, especially under environmental conditions that restrict opportunities for sexual reproduction (Eckert et al., 2016). Clonal plants may allocate more resources to vegetative propagation as a persistence mechanism under conditions of light limitation. Vegetative propagules produced in deep water may have a greater potential than seeds for successful colonization and establishment due to their relatively large size and ability to photosynthesize and take up nutrients (Grace, 1993; Johansson and Nilsson, 1993). Clonal propagation may also be more successful in environments experiencing recovery after disturbance (Eriksson, 1997; Barrat-Segretain, 2001; Zhang et al., 2013). For example, plants of *V. spinulosa* occurring around the periphery of lakes in shallow water are at risk of desiccation owing to water level fluctuations during dry summers. Significantly, plants in the shallow water treatment allocated significantly more resources to the production of tubers than plants at the intermediate water depth, which is likely to help populations persist in low water levels.

In our experiment, size disparities in vegetative biomass were significantly affected by water depth. However, the degree of size dimorphism in vegetative growth decreased significantly in deep water, probably as a result of light limitation. Asymmetrical competition in plants can compromise fitness, with the largest plants removing a disproportionate amount of resources (Weiner, 1990). In *V. spinulosa*, the observed responses in deep water should result in a relatively low size hierarchy between the sexes, which may avoid intersexual competition and light deprivation. Another possible explanation for the effect of water depth on size dimorphism

is that plants may have undergone plastic changes to their relative allocation to shoots in response to light limitation. Tonnabel and colleagues (2017) found that the sexes of dioecious *M. annua* adjusted their relative allocation to root and shoot growth in response to light limitation in competitive circumstances. Further analysis of sex-specific allocation to growth of resource-harvesting organs (roots and shoots) in the context of reproductive investment by plants with different genders would be beneficial.

In conclusion, our results revealed a trade-off between sexual reproduction and clonal propagation in females but not males of *V. spinulosa*. This plasticity-mediated response to a pervasive feature of heterogeneous aquatic environments—water depth—represents an important sex-specific life-history strategy. Females responded more sensitively than males to water depths suboptimal for growth and sexual reproduction. Although we have not shown that these plastic responses in sexual dimorphism are adaptive, they seem likely to contribute to the widespread occurrence of *V. spinulosa* across the vast floodplain area of Poyang Lake and in other regions of China. Flooding and water level fluctuations in freshwater habitats play a key role in the ecology and evolution of aquatic macrophytes (reviewed by Eckert et al., 2016). Our findings on sex-specific differences in plasticity in *V. spinulosa* in response to water level variation therefore provide novel insights into the life-history strategies of submerged dioecious macrophytes in freshwater habitats.

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## AUTHOR CONTRIBUTIONS

L.L., Z.P.S., and J.K.C. conceived and designed the experiments; L.L. executed the experiment, analyzed the data statistically, prepared the figures, and wrote the first draft of the manuscript; S.C.H.B. provided scientific advice; S.C.H.B. and L.L. revised the paper.

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