

# Phenotypic responses of a submerged macrophyte (*Vallisneria natans*) to low light combined with water depth

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## ABSTRACT

Adaptations to low light and water depth stresses are crucial for the survival of submerged macrophytes. To determine the phenotypic responses of *Vallisneria natans* to such stresses, we combined a field investigation, a light control experiment and an *in situ* response experiment to evaluate adaptive variations in fresh weight and morphological and physiological characteristics. In the field investigation and the light control experiment, water depth and light intensity were the main environmental factors affecting the fresh weight and morphological characteristics of *V. natans*. Fresh weight and leaf length were the most significant responding variables, and they were positively correlated with water depth and negatively correlated with underwater light intensity, although the root length and leaf number exhibited no variation. The leaf length elongated more rapidly at sites with intermediate water depth and low light intensity, and the allometric slopes were steeper. Furthermore, the *in situ* response experiment results showed a unimodal distribution of the chlorophyll-a concentrations of *V. natans* along with increasing water depths (from 0.5 m to 8.5 m). The turning point of the chlorophyll-a concentration occurred at 5.5 m, which is almost the maximum water depth at which *V. natans* occurs in Lake Erhai. Overall, our results evidenced that light availability is an important driving factor that controls the status of *V. natans* by affecting not only its morphology and physiology but also its biomass allocation and ramet production. An alternative resource allocation pattern of *V. natans* could be a shift between light acquisition and clonal reproduction.

## 1. Introduction

Phenotypic plasticity is an important ecological strategy (Sultan, 1995; Mal and Lovett-Doust, 2005; Bradshaw, 2006), which is ubiquitous in plants (Sultan, 1987, 1995; Tollrian, 2002). It can increase the resistance and adaptability of plants and provide them with a wider ecological niche (Sultan and Bazzaz, 1993; Orr, 1999; Burns and Winn, 2006; Cao et al., 2016). As emphasised in life history theory, phenotypic plasticity may occur at different life stages of the plants, and it is

essential for maintaining plant fitness in heterogeneous habitats (Huey et al., 2000; Debat and David, 2001; Tollrian, 2002; Li et al., 2018).

Submerged macrophytes are important for the maintenance of the clear-water phase and biodiversity of aquatic ecosystems (Phillips et al., 1978; Blindow et al., 1993; Scheffer and Jeppesen, 1998). Water depth and underwater light intensity are crucial for the survival and growth of submerged plants (Riis et al., 2012; Zhu et al., 2012; Wei et al., 2018). Several studies have indicated that submerged macrophytes exhibit high phenotypic plasticity in response to changes in water depth and light

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intensity (Yang et al., 2004; Zhu et al., 2012; Malheiro et al., 2013; Reckendorfer et al., 2013; Atapaththu and Asaeda, 2015; Baastrup-Spohr et al., 2016; Wei et al., 2018). The response reflects different strategies such as morphology adjustment, biomass reallocation and changes in the content of photosynthesis pigments and root traits (Maberly, 1993; Strand, 2001; Hussner et al., 2009). Yang et al. (2004), found that increased water depth made *Myriophyllum spicatum* allocate less biomass to roots and stems and more to leaves, leading, overall, to increased plant height. Contrary to this, *Potamogeton maackianus* allocated more biomass to stems and less to roots and leaves, and shoot length showed a non-linear response to water depth. In an analysis of the response of five submerged macrophyte species to water depth, Zhu et al. (2012) found that, for all five species, biomass was negatively correlated with water depth. Cao et al. (2016) and Chou et al. (2019) showed that changes in the chlorophyll-a content of leaves are the main physiological adaptive mechanism employed by submerged macrophyte species in response to light attenuation. These studies thus collectively show that phenotypic plasticity is an adaptive strategy used by multiple submerged macrophyte species in response to the changing environment.

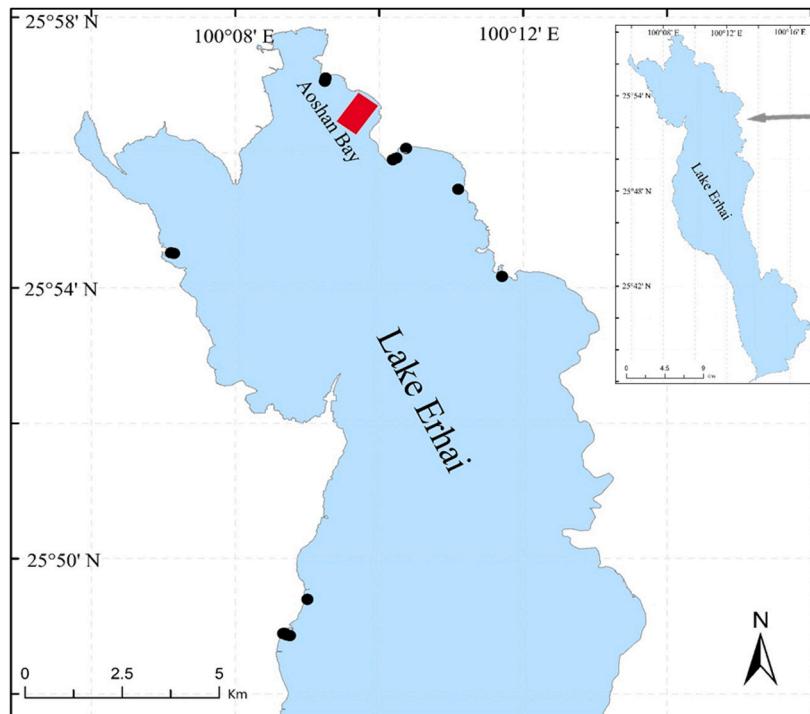
*Vallisneria natans* L. (Hydrocharitaceae) is a perennial submerged clonal freshwater macrophyte (Lowden, 1982; Mcconchie and Kadereit, 1987). It is widely distributed in tropical and subtropical areas and it usually occupies an extensive range of water depths (Lowden, 1982; Mcconchie and Kadereit, 1987). Recently, the species has also been widely used in the ecological restoration of freshwater ecosystems (Ke and Li, 2006; Li et al., 2018; Liu et al., 2020). However, studies investigating the adaptation characteristics of this species to water depth are

rare except for a few controlled experiments and field investigations (Fu et al., 2012; Chen et al., 2016; He et al., 2019; Li et al., 2020). Water depth is an integrated environmental factor that affects many variables, including light intensity, dissolved oxygen, pH and nutrient concentrations, making it difficult to separate the effect of light on plant growth from that of other variables. Therefore, we combined a field investigation with a light control experiment to elucidate the adaptive strategy of this species by phenotypic plasticity. In the field investigation, we aimed to determine the adaptive strategies of *V. natans* at different water depths and light conditions, and the objective of the light control experiment was to verify the results of the field investigation using an established relationship between light intensity and water depth. Finally, we conducted an *in situ* response experiment to explore the effects of water depth and light intensity on physiological factors. We hypothesised that: (1) in deeper and lower light intensity habitats, *V. natans* would allocate more biomass to leaves for rapid elongation to optimise light harvesting and thus avoid the adverse effects of low light; (2) in addition to morphological plasticity, *V. natans* would also develop physiological adaptions, including changes in the chlorophyll-a concentrations in the leaves, to compensate for light attenuation in deeper water.

## 2. Materials and methods

### 2.1. Field investigation

Lake Erhai (25°36'–25°58'N, 100°05'–100°18'E) is a plateau freshwater lake (Fig. 1) located in Yunnan Province, southwest China. Lake



**Fig. 1.** Map showing the field investigation sampling sites and the location of the light control and physiological response experiments. Black solid pentagram = Lake Erhai, Dali, China; black hollow pentagram = light control experiment, Donghu Experimental Station, Wuhan, China; black solid dots = field investigation sampling sites; red area = *in situ* response experiment in Aoshan Bay.

Erhai has a surface area of 252 km<sup>2</sup>, a catchment area of 2565 km<sup>2</sup> and a volume of approx.  $2.88 \times 10^9$  m<sup>3</sup>. Its mean water depth is 10.17 m, with a maximum depth of 20.9 m (Wan et al., 1988). Before the 1960s Lake Erhai was oligotrophic, between the mid-1970s and 1980s the lake was oligo-mesotrophic and remained mesotrophic during the 1990s, and since the 2000s the lake has been eutrophic (Yan et al., 2005). The lake has become increasingly eutrophic due to enhanced external point and non-point source nutrient inputs. The point source pollution is mainly domestic sewage from nearby scattered dwellings, while the non-point source pollution is due to farming activities in the lake basin (Wang et al., 2012). Combined with the changing state of eutrophication, the aquatic vegetation in the lake has varied dramatically. Thus, since the 1980s, the distribution area, community structure and maximum distribution depth of aquatic macrophytes have changed significantly (Fu et al., 2014; He et al., 2015, 2019; Yuan et al., 2018, 2019).

We chose Lake Erhai for field investigation mainly because its water depth gradient fully meets our experimental design. We conducted a simple field investigation before the light control experiment, but as the data recorded at that time was not comprehensive, we conducted a systematic field investigation following the experiment. The field investigation was conducted in September 2016. All the samples were collected on the same day and, therefore, the influence of differences in investigation time on the biological characteristics can be ignored. We collected the *V. natans* using a rotary scythe (covering 0.2 m<sup>2</sup>) at five water depths (WD): 0.5, 1.5, 2.5, 3.5 and 4.5 m (the distribution boundary of *V. natans* in Lake Erhai is 5.07 m, unpublished data). In total, 786 individuals of *V. natans* were collected from the 16 sampling sites (Fig. 1, Table S1), ranging between 41 and 54 individuals per site. The intact individuals were first washed carefully to remove mud, attached algae, fish eggs, zoobenthos and the moisture adsorbed on the leaf surface before determining their fresh weight (FW). Then, the morphological characteristics were determined: (1) length of the longest leaf (LL), (2) length of the longest root (RL) and (3) leaf number (LN).

During the investigation, the major physical and chemical variables in the water and sediment of each sampling site were measured using the standard methods described by APHA (2012). Water temperature (Temp) was measured with a YSI Professional Plus Water Quality Monitor (YSI Inc., Yellow Springs, Ohio, USA), water total nitrogen (TN) and total phosphorus (TP) were measured with UV spectrophotometry (UV-2550, Shimadzu Suzhou Instruments, China), and chlorophyll-a (Chl-a) was measured using ethanol extraction colorimetry (UV-2550, Shimadzu Suzhou Instruments, China). Surface sediments were collected from the top 10 cm layer with a Peterson sediment collector. Sediment nitrogen (SN) concentrations were determined by an elemental analyzer (Flash EA 1112 series, CE Instruments, Italy) and sediment phosphorus (SP) concentrations following the method described by Bao (2000). The sediment samples were first digested using sulphuric acid/hydrogen peroxide and acid/perchloric. Then, total P concentrations were determined using the ammonium molybdate ascorbic acid method. Underwater photosynthetically active radiation (PAR) was measured at noon during the day of sample collection by a handheld Li-COR sensor coupled with a data logger (Li-1400; Li-Cor Company, Lincoln, NE, U.S.A.) to estimate the vertical light profile in the lake. The light extinction coefficient (K) was calculated based on the equation:  $K = (lnI_1 - lnI_2)/(d_2 - d_1)$ , where  $d_1$  and  $d_2$  stand for water depth, and  $I_1$  and  $I_2$  are light intensity at water depth  $d_1$  and  $d_2$ , respectively (Chen et al., 2016). The K value was used to calculate the corresponding water depth in the light control experiment.

## 2.2. Light control experiment

The light control experiment was performed outdoors at the Donghu Experimental Station of Lake Ecosystem, Institute of Hydrobiology, Chinese Academy of Sciences, from 20 April 2015 to 20 April 2016. We chose the Donghu Experimental Station of Lake Ecosystem for our light control experiment mainly because all equipment necessary for our

experiment was available. Newly produced seedlings of *V. natans* (height  $31.7 \pm 8.7$  cm, mean  $\pm$  SD) and sediment for the experiment were collected from Lake Donghu. Seedlings of similar height were chosen and kept in a nursery pond with water and sediment from the lake. Three randomly selected seedlings from the nursery pond were planted in each outdoor glass aquarium (length: 50 cm; width: 50 cm; height: 80 cm) with a 10 cm sediment layer and 70 cm water. The glass aquarium was placed on a platform on the lake surface (Fig. S2). When the water in the aquarium evaporated more than 5 cm, we refilled the tank to the initial water level. As Lake Donghu is eutrophic, the water used in the experiment was a mixture of lake water and tap water (with a volume ratio of 3:7) to avoid algae blooming (Chen et al., 2016). The experiment involved four different light shading treatments, obtained by one-layer black nylon mesh (L1), two-layer black nylon mesh (L2), three-layer black nylon mesh (L3) and four-layer black nylon mesh (L4), which did not alter the spectrum of the incident light. The actual light intensity of each treatment was 39.5% (L1), 17.1% (L2), 7.1% (L3) and 2.8% (L4) of full daylight ( $2251.5 \pm 2.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ , at noon on sunny days). We calculated the water depth corresponding to the specific shading light intensity (based on survey data from the field investigation), which were 0.66, 1.52, 2.52 and 3.5 m for L1, L2, L3 and L4, respectively. Each treatment had three replicates (aquarium). The plants were harvested after 12 months, counting the number of ramets per aquarium to analyse the clonal reproduction of the species. For each plant individual, FW and the three morphological characteristics (LL, RL, LN) were measured.

During the light control experiment, PAR, Temp, TN, TP and Chl-a in the water column were monitored every two weeks using the same methods and equipment as in the field investigation.

## 2.3. In situ response experiment

The *in situ* response experiment was conducted in Aoshan Bay in the northern part of Lake Erhai (Fig. 1) from 18 September to 18 November 2016. We chose Aoshan Bay for the *in-situ* response experiment mainly because its water depth gradient fully meets our experimental design. The bay holds a high abundance of submerged macrophytes, and the bottom is relatively flat (He et al., 2015). A total of 450 *V. natans* seedlings having similar height and biomass ( $13.5 \pm 1.6$  cm height,  $1.1 \pm 0.3$  g fresh weight, mean  $\pm$  SD) were collected from the submerged macrophyte nursery raised on sediment and in water from Lake Erhai at the Erhai Lake Research Station. The sediment for the experiment was collected in Aoshan Bay. Seedlings were planted in plastic buckets (diameter: 35 cm, height: 26 cm, 10 seedlings per bucket), filled with 20 cm sediment and then suspended in the experimental area (Fig. 1) at nine different water depths: 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 m (Fig. S1, maximum water depth  $> 9$  m). There were five replicates (buckets) of each depth. The plants were harvested after 60 days and washed carefully to remove mud and attached algae, after which they were weighed to determine FW. The relative growth rate (RGR) of the *V. natans* plants was calculated as:  $RGR = \ln(FW_2 / FW_1) / \Delta t$ , where  $FW_1$  and  $FW_2$  are the fresh weight at the beginning and end of the experiment, respectively, and  $\Delta t$  is the duration of the experiment. Leaf chlorophyll-a concentration was determined with a spectrophotometer following liquid nitrogen extraction (Murata et al., 1973). Both the RGR and the leaf chlorophyll a concentration of *V. natans* were used to analyse the physiological response of the species.

During the experiment, PAR, Temp, TN, TP and Chl-a in the experimental water column were measured every two weeks applying the same methods and equipment as in the field investigation.

## 2.4. Statistical analyses

As for the field investigation data, differences in environmental factors and biological parameters between sampling sites were detected using principal component analysis (PCA) within Canoco 5.0. The effects

of water depth gradients (grouping all the samples from the same site) on FW, LL, RL and LN of *V. natans* L. were analysed using one-way ANOVA ( $p < 0.05$ ), after which we ran post-hoc tests using the Tukey's method, and the relationship between FW, LL, RL and LN at the different water depths was analysed with linear regression in OriginPro 9.0 ( $p < 0.05$ ). Spearman correlation analyses of WD, PAR, FW, LL, RL and LN ( $p < 0.05$ ) were run using the psych package of R. As for the light control experiment data, we used the same analysis as for the field investigation data. In the *in situ* response experiment, the differences in fresh weight at the start and at the end of the experiment, the change in RGR and leaf chlorophyll-a concentrations at different water depths were determined using one-way ANOVA ( $p < 0.05$ ) followed by Tukey's post-hoc test. The nonlinear fitting function (Gaussian peak function) was used to predict the water depth at which the leaf chlorophyll-a concentrations peak appears.

### 3. Results

#### 3.1. Field investigation

##### 3.1.1. Biomass and morphological variations of *V. natans* with increasing water depth

Water depth had prominent effects on plant size (Table S1). ANOVA analysis showed that there were significant differences ( $p < 0.05$ ) in the fresh weight and morphological characteristics (LL, RL, LN) of *V. natans* between the different water depths. Based on the PCA results (Fig. 2a) and the linear fitting of biological indicators with WD (Fig. 4a), we found that FW and LL exhibited the most significant changes with increasing water depth. The PCA eigenvalues of Axis 1 and Axis 2 were 92.0% and 4.9% (Fig. 2a), respectively, of which FW, LL, RL and LN contributed 61.4%, 35.8%, 2.6% and 0.2%, respectively, to the eigenvalues of Axis 1. FW ( $r^2 = 0.42, p < 0.001$ ), LL ( $r^2 = 0.64, p < 0.001$ ), RL ( $r^2 = 0.23, p < 0.001$ ) and LN ( $r^2 = 0.21, p < 0.001$ ) all correlated significantly with water depth (Table 1). Through linear regression, we found that the FW and LL of each ramet were significantly ( $p < 0.05$ ) higher and longer in deeper water, whereas root length and leaf number showed no significant variation ( $p > 0.05$ ) (Fig. 4a).

##### 3.1.2. Variations of main environmental factors with increasing water depth

Detailed information about Temp, TN, TP, Chl-a, SN and SP at the sampling sites is given in Appendix A (Table S2). The PCA analysis showed distinct differences between sampling sites in WD and PAR, the

eigenvalues of Axis 1 and Axis 2 being, respectively, 81.6% and 9.5% (Fig. 3a), implying that 91.1% of the total variance could be explained by the two axes. Here, WD and PAR contributed 5.5% and 81.5%, respectively, to the eigenvalues of Axis 1. Nonlinear fitting indicated that PAR, as expected, decreased exponentially with increasing water depth (Fig. 4a). These results suggest that WD and PAR were the main environmental factors related to the fresh weight and the morphological (LL, RL, LN) variation of *V. natans*.

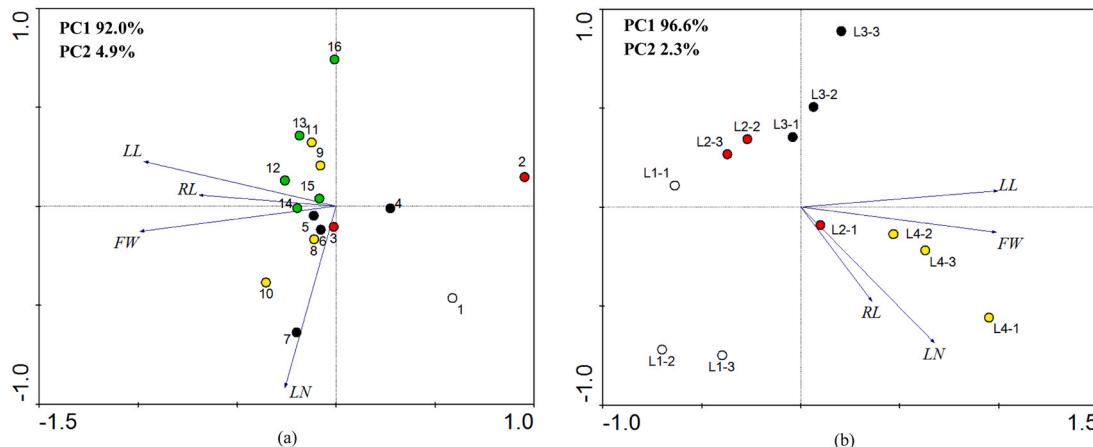
#### 3.1.3. Correlations between various biological indicators

Significant positive correlations ( $p < 0.001$ ) were found between fresh weight and all the morphological indicators included in the field investigation (Table 1). The LL-FW, RL-FW and LN-FW allometric relationships were significantly related to water depth but had different slopes (Fig. 5). Thus, the LL-FW slopes first increased and then gradually decreased with increasing water depth, while the RL-FW and LN-FW slopes decreased, indicating a biomass allocation favouring leaf growth rather than root and leaf number growth. Leaf length and fresh weight were closely correlated ( $p < 0.001$ , Fig. 5); thus, with increasing fresh weight, the leaf length increased as well, and the slope (allometric growth rate) rose with increasing water depth. Root length also varied relative to fresh weight ( $p < 0.001$ , Fig. 5) and increased with increasing fresh weight; however, the allometric growth rate decreased with increasing water depth. Also leaf numbers increased with increasing fresh weight ( $p < 0.001$ , Fig. 5), and the allometric growth rate declined with rising water depth (Fig. 5). Accordingly, it was the allometric relationships of *V. natans* that differed at the different water depths.

### 3.2. Light control experiment

#### 3.2.1. Biomass and morphological variations of *V. natans* along the light gradient

Light intensity also had prominent effects on plant size (Appendix A Table S3). ANOVA analysis showed that there were significant differences ( $p < 0.05$ ) in the fresh weight and morphological characteristics (LL, RL, LN) of *V. natans* under different light intensities. Based on the PCA results (Fig. 2b) and linear fitting of biological indicators with the calculated water depths (Fig. 4b), we found that both FW and LL showed the most significant changes with diminished light intensity. The eigenvalues of Axis 1 and Axis 2 were 96.6% and 2.3% (Fig. 2b), respectively, to which FW, LL, RL and LN contributed 32.5%, 66.2%, 0.1% and 1.1%, respectively, to the eigenvalues of Axis 1. FW ( $r^2 = 0.36, p < 0.001$ ) and LL ( $r^2 = 0.36, p < 0.001$ ) were significantly correlated

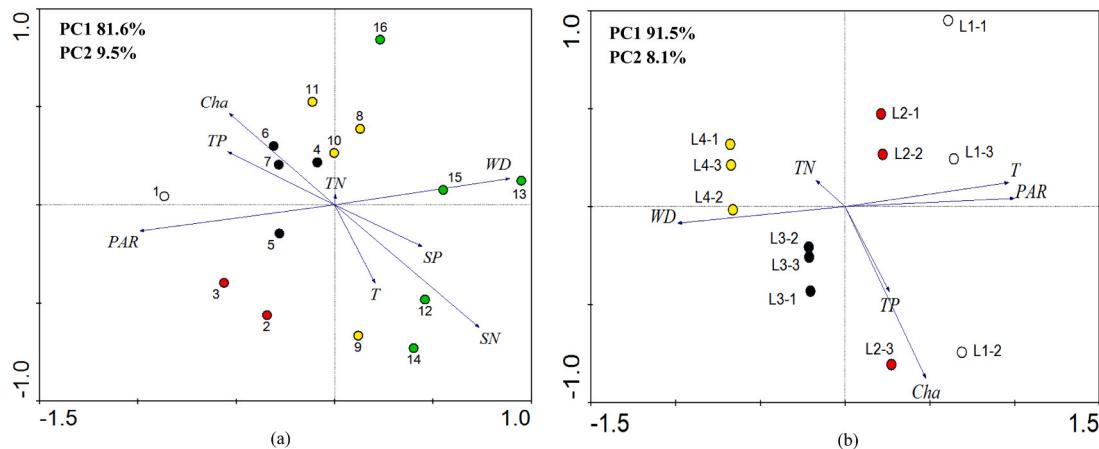


**Fig. 2.** Biomass and morphological indicators in the PCA analysis conducted for the field investigation (a) and the light control experiments (b). Same colours in the field investigation and the light control experiment mean that water depth and light treatment gradients were identical. FW = fresh weight, LL = leaf length, RL = root length, LN = leaf number. In the field investigation (a): 1 = 0.5 m; 2 and 3 = 1.5 m; 4, 5, 6 and 7 = 2.5 m; 8, 9, 10 and 11 = 3.5 m; 12, 13, 14, 15 and 16 = 4.5 m. In the light control experiments (b): L1-1, L1-2 and L1-3 = 39.5% of full daylight; L2-1, L2-2 and L2-3 = 17.1% of full daylight; L3-1, L3-2 and L3-3 = 7.1% of full daylight; L4-1, L4-2 and L4-3 = 2.8% of full daylight.

**Table 1**

Spearman correlation analysis between WD, PAR, FW, LL, RL and LN in the field investigation and light control experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and without asterisk  $P > 0.05$ . WD = water depth; PAR = photosynthetically active radiation; FW = fresh weight; LL = leaf length; RL = root length; LN = leaf number.

Indices	Field investigation					Light control experiment				
	PAR	FW	LL	RL	LN	PAR	FW	LL	RL	LN
WD	-1.00***	0.42***	0.64***	0.23***	-0.21***	-1.00***	0.36***	0.58***	-0.03	-0.01
PAR		-0.42***	-0.64***	-0.23***	0.21***		-0.36***	-0.58***	0.03	0.01
FW			0.76***	0.50***	0.53***			0.82***	0.62***	0.72***
LL				0.37***	0.14***				0.44***	0.63***
RL					0.16***					0.56***



**Fig. 3.** Environmental indicators in the PCA analysis of the results of the field investigation (a) and the light control experiment (b). Same colours in the field investigation and the light control experiment mean that water depth and light treatment gradients were identical. WD = water depth, PAR = photosynthetically active radiation, T = temperature, TN = total nitrogen in water, TP = total phosphorus in water, Chl- $\alpha$  = chlorophyll- $\alpha$  in water, SN = total nitrogen in sediment, SP = total phosphorus in sediment. In the field investigation (a): 1 = 0.5 m; 2 and 3 = 1.5 m; 4, 5, 6 and 7 = 2.5 m; 8, 9, 10 and 11 = 3.5 m; 12, 13, 14, 15 and 16 = 4.5 m. In the light control experiments (b): L1-1, L1-2 and L1-3 = 39.5% of full daylight; L2-1, L2-2 and L2-3 = 17.1% of full daylight; L3-1, L3-2 and L3-3 = 7.1% of full daylight; L4-1, L4-2 and L4-3 = 2.8% of full daylight.

with light intensity, while RL ( $r^2 = 0.03, p > 0.05$ ) and LN ( $r^2 = 0.01, p > 0.05$ ) did not exhibit any significant correlation with these two variables (Table 1). Finally, we found that the relationship between FW, LL, RL and LN and light intensity agreed with the relationship with water depth (Fig. 4b).

### 3.2.2. Variations of main environmental factors along the light gradient

One-way ANOVA revealed no significant differences ( $p > 0.05$ ) in Temp, TN, TP and Chl- $\alpha$  but a significant difference ( $p < 0.001$ ) in PAR results between the experimental treatments (Appendix A Table S4). As in the field experiment, PCA identified WD and PAR as the most important parameters, the eigenvalues of Axis 1 and Axis 2 being 91.5% and 8.1% (Fig. 3b), respectively. Thus, 99.6% of the total variance could be explained by the two axes. Among these, WD and PAR contributed 12.0% and 85.5%, respectively, to the eigenvalues of Axis 1. The results of nonlinear fitting showed that the changing trend of the light response in the light control experiment was consistent with that observed in the field investigation (Fig. 4b). Therefore, based on the PCA and nonlinear fitting results, we found that PAR was the main environmental factor related to the morphological variation of *V. natans*.

### 3.2.3. Correlations between various biological indicators

Significant positive correlations ( $p < 0.001$ ) were found between fresh weight and all morphological indicators studied in the light control experiment (Table 1). We used the same method as in the field investigation to analyse the correlations between various biological indicators at different light intensity gradients. The LL-FW, RL-FW and LN-FW allometric relationships were similar to those observed in the field investigation (Fig. 6). The LL-FW slopes first increased and then gradually decreased with declining light intensity, while the RL-FW and LN-

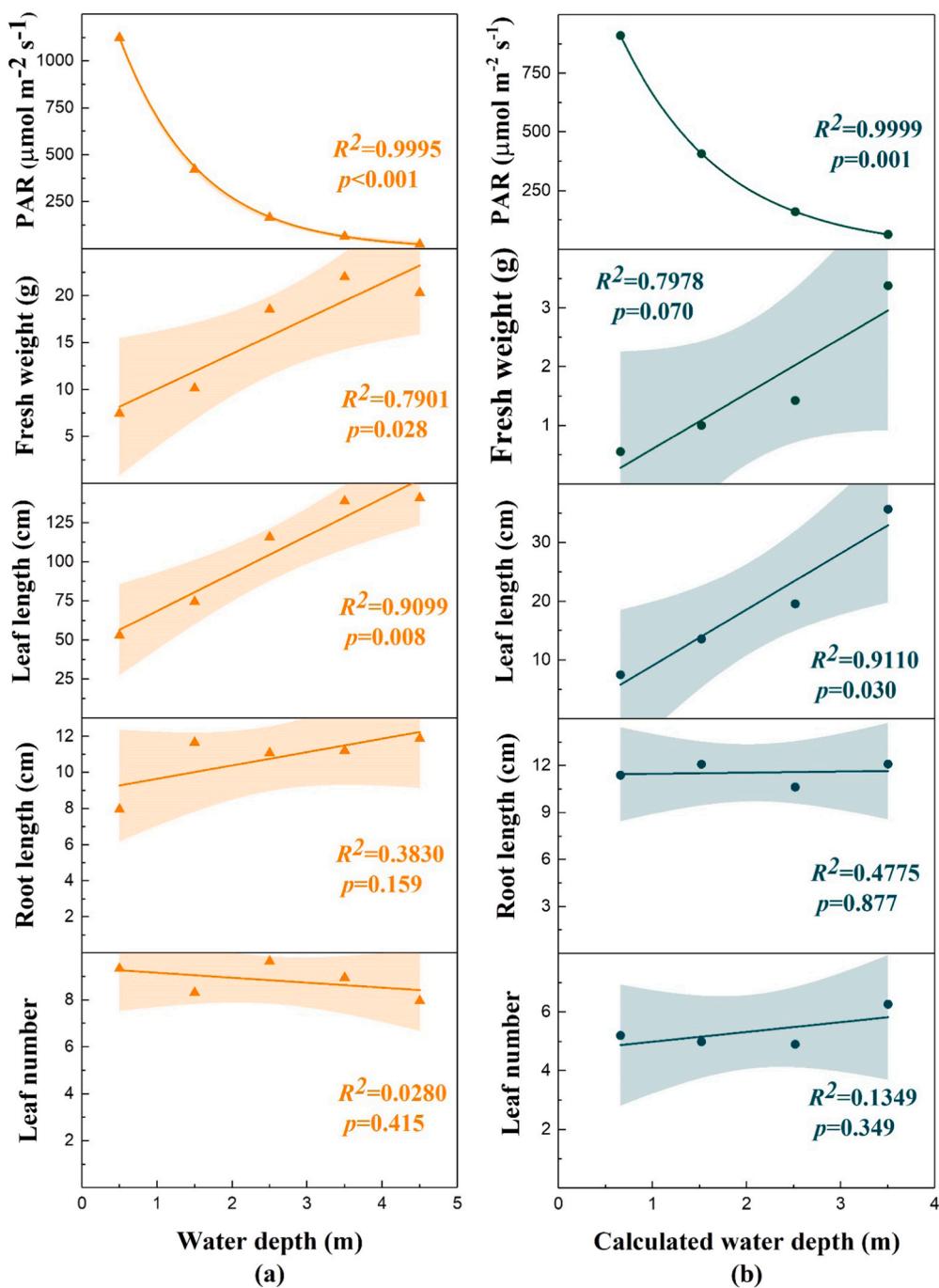
FW slopes decreased, indicating a biomass allocation favouring leaf growth rather than root and leaf number growth. Leaf length exhibited a significant positive correlation with fresh weight ( $p < 0.001$ , Fig. 6), and the slope (allometric growth rate) increased with the continuous weakening of the light intensity. Root length also demonstrated a significant positive correlation with fresh weight ( $p < 0.001$ , Fig. 6), the allometric growth rate declining with diminishing light intensity, and fresh weight exhibited a significant positive relation with leaf number ( $p < 0.001$ , Fig. 6) whose allometric growth rate decreased with decreasing light intensity (Fig. 6). Thus, the allometric relationships of *V. natans* differed at the different light intensities.

### 3.2.4. Ramet changes of *V. natans* at different light intensities

*Vallisneria natans* population growth was high at high light conditions and vice versa. The light intensity had significant effects on the ramet numbers of *V. natans* that, at the end of the experiment, were (means  $\pm$  SD)  $105 \pm 40^a$ ,  $92 \pm 3^b$ ,  $55 \pm 14^c$  and  $11 \pm 2^d$  for L1, L2, L3 and L4, respectively (Appendix A Table S3). Most ramets appeared in the L1 treatment where the leaves of *V. natans* grew slowly and more energy was allocated to expansion rather than to leaf growth.

### 3.3. In situ experiment: physiological responses of *V. natans*

At the end of the *in situ* response experiment, the mean fresh weight of the plants at 0.5, 1.5 and 2.5 m was significantly ( $p < 0.001$ ) higher than the initial weights, while there was no significant ( $p > 0.05$ ) difference in the mean fresh weight of the plants growing at 3.5, 4.5 and 5.5 m. In the 6.5, 7.5, and 8.5 m ramets, the fresh weight at the end of the experiment was significantly ( $p < 0.001$ ) lower than the initial weights (Fig. 7a). When water depth was greater than 5.5 m, RGR



**Fig. 4.** The relationships between PAR, fresh weight, leaf length, root length and leaf number of *V. natans* L. along a water depth gradient in the field investigation (a) and the light control experiment (b). Note: The data used are the mean of the samples.

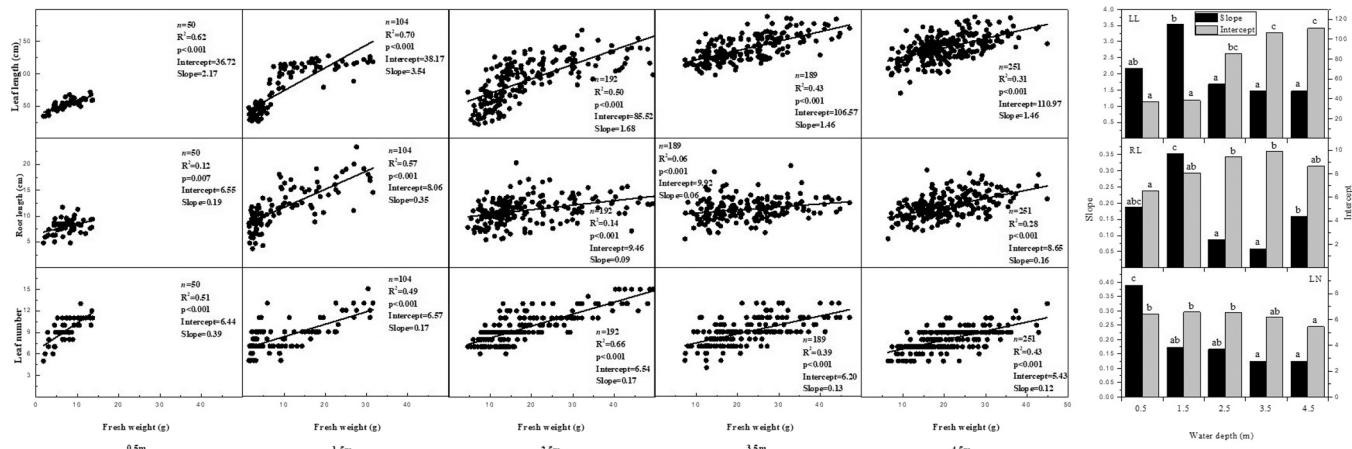
became negative (Fig. 7b), implying that the experimental *V. natans* seedlings did not survive for long at water depths  $> 5.5$  m. Typically, the chlorophyll-a concentration of *V. natans* first increased and then gradually decreased with the increase in water depth, and non-linear fitting showed that the turning point occurred at 5.5 m where RGR became negative (Fig. 7c).

In the *in situ* experiment, one-way ANOVA showed no significant differences ( $p > 0.05$ ) in Temp, TN, TP and Chl-a but a significant difference ( $p < 0.05$ ) in PAR with increasing water depth (Appendix A Table S5), and water depth also influenced the RGR and chlorophyll-a concentration of *V. natans* ( $r^2 = 0.99$ ,  $p < 0.001$ ) (Fig. 7).

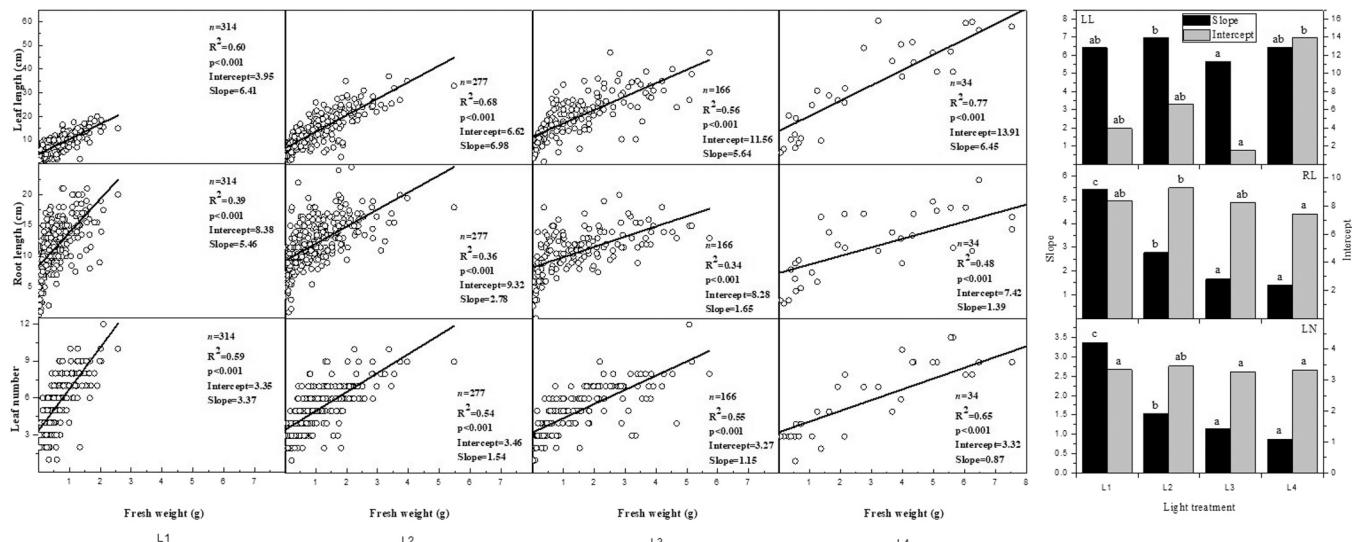
#### 4. Discussion

Our results clearly showed that phenotypic plasticity is an important adaptation mechanism/strategy for *V. natans* to the changes in water depth and light intensity. Aquatic plants typically adjust their biomass allocation and morphology to improve their ability to exploit essential resources (Westoby et al., 2002; Xiao et al., 2007; Xie et al., 2007; Poorter et al., 2012).

When we compared the results of the field investigation and the light control experiment, we found that fresh weight and the main morphological characteristics (leaf number, leaf length and root length) exhibited the same changing pattern, indicating that the underwater light intensity would be the key factor for the depth response. *Vallisneria*



**Fig. 5.** Relationships between fresh weight (FW) and leaf length (LL), root length (RL) and leaf number (LN) of *Vallisneria natans* L. at different water depth gradients and variations in slope and intercept of morphological parameters of *V. natans* L. with the water depth gradient in the field investigation. Different letters indicate significant differences ( $p < 0.001$ ).



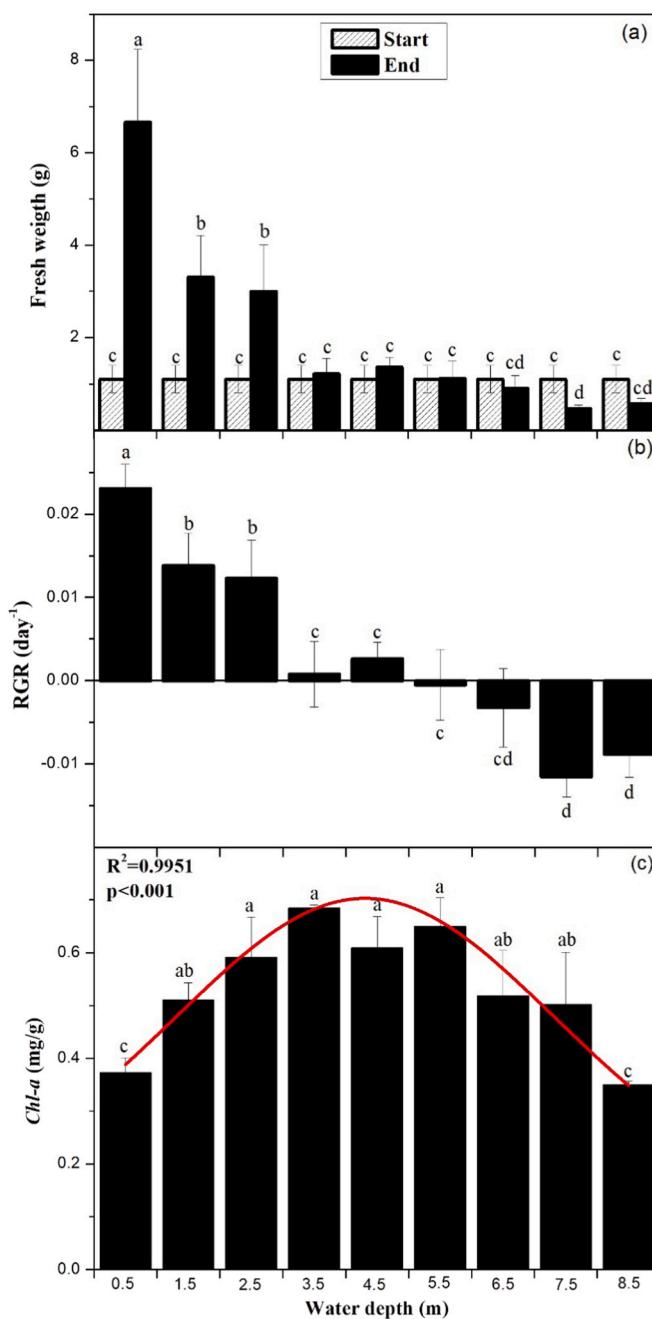
**Fig. 6.** Relationships between fresh weight (FW) and morphological parameters of *Vallisneria natans* L. at different light intensity gradients and variations in slope and intercept of morphological parameters of *V. natans* L. with the light intensity gradient in the light control experiment. Different letters indicate significant differences ( $p < 0.001$ ).

*natans* had the lowest fresh weight and the shortest leaf length in the shallow waters and in the high light treatment. Fresh weight and leaf length increased progressively with increasing water depth and decreasing light intensity. These results are consistent with our first hypothesis. Shallow water and high light intensity may inhibit the development of *V. natans* and reduce plant growth (Simpson et al., 1980; Bonser and Geber, 2005; Weischedé et al., 2006; Lu et al., 2013; Cao et al., 2016). According to our results, this inhibitory effect gradually disappeared as the water depth increased and the light intensity decreased, leading to higher plant height and allocation of more biomass to elongation of leaves while reducing root growth. In this way, leaves may more rapidly reach the water surface to benefit from the improved light conditions. This light-harvesting strategy, called 'light foraging', allows plants to obtain light at minimum metabolic cost (Hutchings and Dekroon, 1994). Other studies have also found that *V. natans* adopts plastic strategies and increases its leaf biomass and leaf length to reach a given plant size in response to increasing water depth and decreasing light intensity at the expense of branch number and belowground biomass (Lieffers and Shay, 1981; Nohara and Kimura, 1997; Vretare et al., 2001). However, Fu et al. (2012), contrary to our observations,

found small-sized *V. natans* individuals in deep and low light intensity waters. Their experiments lasted only 52 days, and the ability of the plants to fully adapt to the new habitat might therefore have been underestimated.

In agreement with our second hypothesis, we found that water depth had a significant effect on the chlorophyll-a concentration of *V. natans*, which increased with increasing depth until 5.5 m and then gradually decreased at deeper sites. *Vallisneria natans* may shift the light compensation point to cope with reduced light (Bai et al., 2015; Wei et al., 2018). Blanch et al. (1998) found that *V. natans* had a low photosynthetic light intensity compensation point of  $9.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which is close to the light intensity value ( $9.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 5.5 m water depth in our *in situ* response experiment. Accordingly, compared with the field investigation data on light intensity at 4.5 m, as well as the change of RGR in the *in-situ* experiment, we infer that the maximum survival boundary for *V. natans* in Lake Erhai is 5.5 m.

We found that *V. natans* faced a trade-off between biomass increase and clonal expansion. The vegetative reproduction strategy of *V. natans* differed with water depth and light conditions. In the light control experiment, population growth increased with increasing light, which



**Fig. 7.** (a) Average ramet biomass at the start and at the end of the *in-situ* experiment, (b) average relative growth rate (RGR) and (c) changes in chlorophyll-a levels along a water depth gradient in the *in situ* response experiment. Different letters above the bars indicate significant differences ( $p < 0.001$ ).

agrees with the trade-off theory (Thompson and Eckert, 2004; Reusch, 2006; Van Drunen and Dorken, 2012; Li et al., 2018). *Vallisneria natans* clonal growth and regeneration depend strongly on water depth and light availability (Ferreiro et al., 2013; Søndergaard et al., 2013; Dong et al., 2014; Fu et al., 2014; Wei et al., 2018). When light resources are sufficient, *V. natans* allocates more resources to producing new ramets as a more abundant population can share or prevent the likelihood of extinction, while under low light conditions, *V. natans* allocates more resources to light acquisition organs and less to reproduction (Li et al., 2018).

The plants with high plasticity have a distinct competitive advantage over species with low plasticity (Sultan, 1995; Orr, 1999; Bradshaw, 2006). They are able to occupy a wider geographical area and more

diverse habitats (Burns and Winn, 2006; Riis et al., 2010; Hyldgaard and Brix, 2012.). Species with high plasticity adapt quickly to a changing environment, and this competitive advantage may gradually ensure their dominance of the local species community. Therefore, the selection of species with high plasticity, such as *V. natans*, is recommended for the ecological restoration of aquatic environments including plant transplantations.

## 5. Conclusion

Our results reveal that phenotypic plasticity allows *V. natans* to adapt morphologically, physiologically and reproductively to different environments. At low light, the plants used more energy to increase the leaf length than at high light, while the growth of root length and leaf number declined; in contrast, when light resources were sufficient, more energy was directed towards clonal reproduction. Such phenotypic plasticity shaped the population characteristics of *V. natans* and may further influence the local community assembly. Light availability is the most important driving factor for the maintenance and expansion of *V. natans* populations, and the depth boundary for *V. natans* survival in Lake Erhai was estimated to 5.5 m, which is similar to the light compensation point depth. This is valuable information as *V. natans* is often considered for ecological restoration of lost aquatic plants in subtropical and tropical freshwater ecosystems.

## Author contributions

WZ, TC and LN planned and designed the research, QC, JC and WR performed experiments, QC and CY conducted fieldwork, QC analysed data and wrote the manuscript. QC, XZ and EJ revised the manuscript and gave important and critical input.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquabot.2021.103462.

## References

- APHA, AWWA, WEF, 2012. Standard methods for the examination of water and wastewater, 22nd ed. Washington DC. <https://doi.org/10.1080/23267224.1919.10651076>.
- Atapathithu, K.S.S., Asaeda, T., 2015. Growth and stress responses of Nuttall's waterweed *Elodea nuttallii* (Planch) St. John to water movements. Hydrobiologia 747 (1), 217–233. <https://doi.org/10.1007/s10750-014-2141-9>.
- Bastrup-Spohr, L., Møller, C.L., Sand-Jensen, K., 2016. Water-level fluctuations affect sediment properties, carbon flux and growth of the isoetid *Littorella uniflora* in oligotrophic lakes. Freshw. Biol. 61 (3), 301–315. <https://doi.org/10.1111/fwb.12704>.

- Bai, X., Chen, K.N., Zhao, H.G., Chen, X.M., 2015. Impact of water depth and sediment type on root morphology of the submerged plant *Vallisneria natans*. J. Freshw. Ecol. 30 (1), 75–84. <https://doi.org/10.1080/02705060.2014.970672>.
- Bao, S.D., 2000. Analysis of Soil Agrochemical, third ed. China Agriculture Press, Beijing (in Chinese).
- Blanch, S.J., Ganf, G.G., Walker, K.F., 1998. Growth and recruitment in *Vallisneria americana* as related to average irradiance in the water column. Aquat. Bot. 61 (3), 181–205. [https://doi.org/10.1016/S0304-3770\(98\)00065-5](https://doi.org/10.1016/S0304-3770(98)00065-5).
- Blindow, I., Andersson, G., Hargeby, A., Johansson, S., 1993. Long-term pattern of alternative stable states in 2 shallow eutrophic lakes. Freshw. Biol. 30 (1), 159–167. <https://doi.org/10.1111/j.1365-2427.1993.tb00796.x>.
- Bonser, S.P., Geber, M.A., 2005. Growth form evolution and shifting habitat specialization in annual plants. J. Evol. Biol. 18 (4), 1009–1018. <https://doi.org/10.1111/j.1420-9101.2005.00904.x>.
- Bradshaw, A.D., 2006. Unravelling phenotypic plasticity: why should we bother? New Phytol. 170 (4), 644–648. <https://doi.org/10.1111/j.1469-8137.2006.01761.x>.
- Burns, J.H., Winn, A.A., 2006. A comparison of plastic responses to competition by invasive and non-invasive congeners in the commelinaceae. Biol. Invasions 8 (4), 797–807. <https://doi.org/10.1007/s10530-005-3838-5>.
- Cao, Y., Wu, H., Zhang, S.J., Guo, Z.C., Wang, G., 2016. Effects of water depth on the seedling morphology and chlorophyll fluorescence of *Vallisneria natans*. J. Freshw. Ecol. 31 (3), 463–475. <https://doi.org/10.1080/02705060.2016.1167132>.
- Chen, J.F., Cao, T., Zhang, X.L., Xi, Y.L., Ni, L.Y., Jeppesen, E., 2016. Differential photosynthetic and morphological adaptations to low light affect depth distribution of two submerged macrophytes in lakes. Sci. Rep. 6, 1–9. <https://doi.org/10.1038/srep34028>.
- Chou, Q.C., Cao, T., Ni, L.Y., Xie, P., Jeppesen, E., 2019. Leaf soluble carbohydrates, free amino acids, starch, total phenolics, carbon and nitrogen stoichiometry of 24 aquatic macrophyte species along climate gradients in China. Front. Plant Sci. 10, 1–11. <https://doi.org/10.3389/fpls.2019.00442>.
- Debat, V., David, P., 2001. Mapping phenotypes: canalization, plasticity and developmental stability. Trends Ecol. Evol. 16 (10), 555–561. [https://doi.org/10.1016/S0169-5347\(01\)02266-2](https://doi.org/10.1016/S0169-5347(01)02266-2).
- Dong, B.L., Qin, B.Q., Gao, G., Cai, X.L., 2014. Submerged macrophyte communities and the controlling factors in large, shallow lake Taihu (China): sediment distribution and water depth. J. Gt. Lakes Res. 40 (3), 646–655. <https://doi.org/10.1016/j.jglr.2014.04.007>.
- Ferreiro, N., Giorgi, A., Feijoó, C., 2013. Effects of macrophyte architecture and leaf shape complexity on structural parameters of the epiphytic algal community in a Pampean stream. Aquat. Ecol. 47 (4), 389–401. <https://doi.org/10.1007/s10452-013-9452-1>.
- Fu, H., Yuan, G.X., Cao, T., Ni, L.Y., Zhang, M., Wang, S.R., 2012. An alternative mechanism for shade adaptation: implication of allometric responses of three submerged macrophytes to water depth. Ecol. Res. 27 (6), 1087–1094. <https://doi.org/10.1007/s11284-012-0991-z>.
- Fu, H., Zhong, J., Yuan, G., Xie, P., Guo, L., Zhang, X., Xu, J., Li, Z., Li, W., Zhang, M., Cao, T., Ni, L., 2014. Trait-based community assembly of aquatic macrophytes along a water depth gradient in a freshwater lake. Freshw. Biol. 59 (12), 2462–2471. <https://doi.org/10.1111/fwb.12443>.
- He, L., Zhu, T., Cao, T., Li, W., Zhang, M., Zhang, X., Ni, L., Xie, P., 2015. Characteristics of early eutrophication encoded in submerged vegetation beyond water quality: a case study in Lake Erhai, China. Environ. Earth Sci. 74 (5), 3701–3708. <https://doi.org/10.1007/s12665-015-4202-4>.
- He, L., Zhu, T., Wu, Y., Li, W., Zhang, H., Zhang, X., Cao, T., Ni, L., Hilt, S., 2019. Littoral slope, water depth and alternative response strategies to light attenuation shape the distribution of submerged macrophytes in a mesotrophic lake. Front. Plant Sci. 10, 1–9. <https://doi.org/10.3389/fpls.2019.00169>.
- Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D., Serra, L., 2000. Rapid evolution of a geographic cline in size in an introduced fly. Science 287 (5451), 308–309. <https://doi.org/10.1126/science.287.5451.308>.
- Hussner, A., Meyer, C., Busch, J., 2009. The influence of water level and nutrient availability on growth and root system development of *Myriophyllum aquanticum*. Weed Res. 49 (1), 73–80. <https://doi.org/10.1111/j.1365-3180.2008.00667.x>.
- Hutchings, M.J., Dekroon, H., 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Adv. Ecol. Res. 25, 159–238. [https://doi.org/10.1016/S0065-2504\(08\)60215-9](https://doi.org/10.1016/S0065-2504(08)60215-9).
- Hyldgaard, B., Brix, H., 2012. Intraspecific differences in phenotypic plasticity: invasive versus non-invasive populations of *Ceratophyllum demersum*. Aquat. Bot. 97 (1), 49–56. <https://doi.org/10.1016/j.aquabot.2011.11.004>.
- Ke, X.S., Li, W., 2006. Germination requirement of *Vallisneria natans* seeds: implications for restoration in Chinese lakes. Hydrobiologia 559, 357–362. <https://doi.org/10.1007/s10750-005-1276-0>.
- Li, H., Li, Q., Luo, X., Fu, J., Zhang, J., 2020. Responses of the submerged macrophyte *Vallisneria natans* to a water depth gradient. Sci. Total Environ. 701, 1–10.
- Li, L., Lan, Z.C., Chen, J.K., Song, Z.P., 2018. Allocation to clonal and sexual reproduction and its plasticity in *Vallisneria spinulosa* along a water-depth gradient. Ecosphere 9 (1), 1–11. <https://doi.org/10.1002/ecs2.2070>.
- Lieffers, V.J., Shay, J.M., 1981. The effects of water level on the growth and reproduction of *Scirpus maritimus* var. *paludosus*. Can. J. Bot. Rev. Can. Bot. 59 (2), 118–121. <https://doi.org/10.1139/b81-019>.
- Liu, H., Zhou, W., Li, X.W., Chu, Q.S., Tang, N., Shu, B.Z., Liu, G.H., Xing, W., 2020. How many submerged macrophyte species are needed to improve water clarity and quality in Yangtze floodplain lakes? Sci. Total Environ. 724, 10–23. <https://doi.org/10.1016/j.scitotenv.2020.138267>.
- Lowden, R.M., 1982. An approach to the taxonomy of *Vallisneria* L. (*Hydrocharitaceae*). Aquat. Bot. 13 (3), 269–298. [https://doi.org/10.1016/0304-3770\(82\)90064-X](https://doi.org/10.1016/0304-3770(82)90064-X).
- Lu, J., Wang, Z.X., Xing, W., Liu, G.H., 2013. Effects of substrate and shading on the growth of two submerged macrophytes. Hydrobiologia 700 (1), 157–167. <https://doi.org/10.1007/s10750-012-1227-5>.
- Maberly, S.C., 1993. Morphological and photosynthetic characteristics of *Potamogeton obtusifolius* from different depths. J. Aquat. Plant Manag. 31, 34–39.
- Mal, T.K., Lovett-Doust, J., 2005. Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, *Lythrum salicaria* (Lythraceae), in response to soil moisture. Am. J. Bot. 92 (5), 819–825. <https://doi.org/10.3732/ajb.92.5.819>.
- Malheiro, A.C.E., Jahns, P., Hussner, A., 2013. CO<sub>2</sub> availability rather than light and temperature determines growth and phenotypical responses in submerged *Myriophyllum aquanticum*. Aquat. Bot. 110, 31–37. <https://doi.org/10.1016/j.aquabot.2013.05.001>.
- McConchie, C.A., Kadereit, J.W., 1987. Floral structure of *Vallisneria caulescens* Bailey & F. Mueller. Aquat. Bot. 29 (2), 101–110. [https://doi.org/10.1016/0304-3770\(87\)90089-1](https://doi.org/10.1016/0304-3770(87)90089-1).
- Murata, N., Itoh, S., Okada, M., 1973. Induction of chlorophyll a fluorescence in isolated spinach chloroplasts at liquid nitrogen temperature. Biochim. Biophys. Acta 325 (3), 463–471. [https://doi.org/10.1016/0005-2728\(73\)90207-7](https://doi.org/10.1016/0005-2728(73)90207-7).
- Nohara, S., Kimura, M., 1997. Growth characteristics of *Nelumbo nucifera* Gaertn in response to water depth and flooding. Ecol. Res. 12 (1), 11–20. <https://doi.org/10.1007/BF02523605>.
- Orr, H.A., 1999. Phenotypic evolution: a reaction norm perspective. Science 285 (5426), 343–344. <https://doi.org/10.1126/science.285.5426.343>.
- Phillips, G.L., Eminson, D., Moss, B., 1978. Mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4 (2), 103–126. [https://doi.org/10.1016/0304-3770\(78\)90012-8](https://doi.org/10.1016/0304-3770(78)90012-8).
- Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193 (1), 30–50. <https://doi.org/10.1111/j.1469-8137.2011.03952.x>.
- Reckendorfer, W., Funk, A., Gschöpf, C., Hein, T., Schiemer, F., 2013. Aquatic ecosystem functions of an isolated floodplain and their implications for flood retention and management. J. Appl. Ecol. 50 (1), 119–128. <https://doi.org/10.1111/1365-2664.12029>.
- Reusch, T.B.H., 2006. Does disturbance enhance genotypic diversity in clonal organisms? A field test in the marine angiosperm *Zostera marina*. Mol. Ecol. 15 (1), 277–286. <https://doi.org/10.1111/j.1365-294X.2005.02779.x>.
- Riis, T., Lambertini, C., Olesen, B., Clayton, J.S., Brix, H., Sorrell, B.K., 2010. Invasion strategies in clonal aquatic plants: are phenotypic differences caused by phenotypic plasticity or local adaptation? Ann. Bot. 106 (5), 813–822. <https://doi.org/10.1093/aob/mcq176>.
- Riis, T., Olesen, B., Clayton, J.S., Lambertini, C., Brix, H., Sorrell, B.K., 2012. Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. Aquat. Bot. 102, 56–64. <https://doi.org/10.1016/j.aquabot.2012.05.002>.
- Scheffer, M., Jeppesen, E., 1998. Alternative stable states. In: Jeppesen, E., Sondergaard, M., Sondergaard, M., Christoffersen, K. (Eds.), The Structuring Role of Submerged Macrophytes in Lakes. Springer, New York, U.S.A, pp. 397–406. <https://doi.org/10.1007/978-1-4612-0695-8>.
- Simpson, P.S., Eaton, J.W., Hardwick, K., 1980. The influence of environmental factors on apparent photosynthesis and respiration of the submersed macrophyte *Eloea canadensis*. Plant Cell Environ. 3 (6), 415–423. <https://doi.org/10.1111/1365-3040.ep1586879>.
- Søndergaard, M., Phillips, G., Hellsten, S., Kolada, A., Ecke, F., Mäemets, H., Mjelde, M., Azzella, M.M., Oggioni, A., 2013. Maximum growing depth of submerged macrophytes in European lakes. Hydrobiologia 704 (1), 165–177. <https://doi.org/10.1007/s10750-012-1389-1>.
- Strand, J.A., Weisner, S.E.B., 2001. Morphological plastic responses to water depth and wave exposure in an aquatic plant (*Myriophyllum spicatum*). J. Ecol. 89 (2), 166–175. <https://doi.org/10.1046/j.1365-2745.2001.00530.x>.
- Sultan, S.E., 1987. Evolutionary implications of phenotypic plasticity in plants. Evol. Biol. 21, 127–178. [https://doi.org/10.1007/978-1-4615-6986-2\\_7](https://doi.org/10.1007/978-1-4615-6986-2_7).
- Sultan, S.E., 1995. Phenotypic plasticity and plant adaptation. Acta Bot. Neerl. 44 (4), 363–383. <https://doi.org/10.1111/j.1438-8677.1995.tb00793.x>.
- Sultan, S.E., Bazzaz, F.A., 1993. Phenotypic plasticity in *Polygonum persicaria* 3: the evolution of ecological breadth for nutrient environment. Evolution 47 (4), 1050–1071. <https://doi.org/10.2307/2409974>.
- Thompson, F.L., Eckert, C.G., 2004. Trade-offs between sexual and clonal reproduction in an aquatic plant: experimental manipulations vs. phenotypic correlations. J. Evol. Biol. 17 (3), 581–592. <https://doi.org/10.1111/j.1420-9101.2004.00701.x>.
- Tollrian, R., 2002. Phenotypic plasticity: beyond nature and nurture. Nature 416 (6881), 584–585. <https://doi.org/10.1038/416584b>.
- Van Drunen, W.E., Dorken, M.E., 2012. Trade-offs between clonal and sexual reproduction in *Sagittaria latifolia* (Alismataceae) scale up to affect the fitness of entire clones. New Phytol. 196 (2), 606–616. <https://doi.org/10.1111/j.1469-8137.2012.04260.x>.
- Vretare, V., Weisner, S.E.B., Strand, J.A., Granéli, W., 2001. Phenotypic plasticity in *Phragmites australis* as a functional response to water depth. Aquat. Bot. 69 (2–4), 127–145. [https://doi.org/10.1016/S0304-3770\(01\)00134-6](https://doi.org/10.1016/S0304-3770(01)00134-6).
- Wan, G., Xu, Y., Li, S., Chen, Z., 1988. Hydrochemical compositions in several lakes and reservoirs in Yunnan-Guizhou plateau. J. Environ. Sci. 9, 37–51 (in Chinese with English abstract).
- Wang, R., Dearing, J.A., Langdon, P.G., Zhang, E., Yang, X., Dakos, V., Scheffer, M., 2012. Flickering gives early warning signals of a critical transition to a eutrophic lake state. Nature 492 (7429), 419–422. <https://doi.org/10.1038/nature11655>.

- Wei, H., He, F., Xu, D., Zhou, Q., Xiao, E., Zhang, L., Wu, Z., 2018. A comparison of the growth and photosynthetic response of *Vallisneria natans* (Lour.) Hara to a long-term water depth gradient under flowing and static water. *J. Freshw. Ecol.* 33, 223–237. <https://doi.org/10.1080/02705060.2018.1432509>.
- Weischedé, J., Martíková, J., de Kroon, H., Huber, H., 2006. Shade avoidance in *Trifolium repens*: costs and benefits of plasticity in petiole length and leaf size. *New Phytol.* 172 (4), 655–666. <https://doi.org/10.1111/j.1469-8137.2006.01885.x>.
- Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A., Wright, I.J., 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annu. Rev. Ecol. Syst.* 33, 125–159. <https://doi.org/10.1146/annurev.ecolsys.33.010802.150452>.
- Xiao, K.Y., Yu, D., Wu, Z.H., 2007. Differential effects of water depth and sediment type on clonal growth of the submersed macrophyte *Vallisneria natans*. *Hydrobiologia* 589, 265–272. <https://doi.org/10.1007/s10750-007-0740-4>.
- Xie, Y.H., Deng, W., Wang, J.D., 2007. Growth and root distribution of *Vallisneria natans* in heterogeneous sediment environments. *Aquat. Bot.* 86 (1), 9–13. <https://doi.org/10.1016/j.aquabot.2006.08.002>.
- Yan, C., Jin, X., Zhao, J., Shen, B., Li, N., Huang, C., Xiong, Z., 2005. Ecological protection and sustainable utilization of Erhai Lake, Yunnan. *J. Environ. Sci.* 26, 38–42. <https://doi.org/10.13227/j.hjkx.2005.05.008> (in Chinese with English abstract).
- Yang, Y.Q., Yu, D., Li, Y.K., Xie, Y.H., Geng, X.H., 2004. Phenotypic plasticity of two submersed plants in response to flooding. *J. Freshw. Ecol.* 19 (1), 69–76. <https://doi.org/10.1080/02705060.2004.9664514>.
- Yuan, C., Zhu, T., Cao, T., Xi, Y., Zhang, X., Ni, L., 2019. Antioxidant systems of aquatic macrophytes in three life forms: a case study in Lake Erhai, China. *J. Oceanol. Limnol.* 37 (2), 665–674. <https://doi.org/10.1007/s00343-019-8025-0>.
- Yuan, D.Y., Meng, X., Duan, C.Q., Wei, Z.H., Gao, W., Chang, J.J., Lv, X.J., Pan, Y., 2018. Effects of water exchange rate on morphological and physiological characteristics of two submerged macrophytes from Erhai Lake. *Ecol. Evol.* 8 (2), 12750–12760. <https://doi.org/10.1002/ece3.4703>.
- Zhu, G.R., Li, W., Zhang, M., Ni, L.Y., Wang, S.R., 2012. Adaptation of submerged macrophytes to both water depth and flood intensity as revealed by their mechanical resistance. *Hydrobiologia* 696 (1), 77–93. <https://doi.org/10.1007/s10750-012-1185-y>.