PRIMARY RESEARCH PAPER

Stay dormant or escape sprouting? Turion buoyancy and sprouting abilities of the submerged macrophyte *Potamogeton crispus* L.

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Abstract The ability of asexual propagules to disperse is an important ecological determinant of the spread and establishment of many aquatic species. However, few previous studies have addressed the relationship between the asexual propagule buoyancy and sprouting abilities in submerged macrophytes. For this reason, turions of *Potamogeton crispus* samples were collected from Lake Liangzi, and an incubator sprouting experiment was conducted. Our results revealed that the floating turions showed higher sprouting rates than that of sinking turions, indicating the former ones are possibly with high levels of primary metabolites. The higher N and P concentrations in the floating turions caused lower C:N, C:P, and N:P ratios in these turions compared with sinking turions, which confirmed the activation of floating turions. The free amino acid and soluble carbohydrate concentrations were also higher in floating turions than those in sinking turions. Our results also revealed that turion leaf porosity rather than starch concentration may determine the density of *P. crispus* turions. This study makes a contribution to our understanding of how the internal characteristics of turions can (at

least partly) determine dispersal outcomes and offers new insights into the dispersal and sprouting of asexual propagules of submerged macrophytes.

Keywords Density · Floating · Porosity · *Potamogeton crispus* L. · Sinking · Turion

Introduction

The ability of asexual propagules to disperse is an important ecological determinant of the spread and establishment of plant species (Riis & Sand-Jensen, 2006; Klimešová & Klimeš, 2007). Efficient and successful dispersal is a key factor that regulates the temporal and spatial dynamics of plant communities, and influences the management of habitat conservation and restoration, especially in aquatic habitats (Boedeltje et al., 2003; Riis & Sand-Jensen, 2006; Chambert & James, 2009). Therefore, an improved understanding of the determinants of the dispersal and survival of propagules is of both scientific and practical interest.

Compared with terrestrial plant species, most aquatic plant species reproduce asexually and disperse via hydrochory (Boedeltje et al., 2003; Chambert & James, 2009; Sarneel, 2012). The asexual propagules of aquatic plants are highly suited for dispersal within or between water bodies (Santamaría, 2002). For example, the stem fragments of macrophytes can disperse over long distances via water flow (Riis & Sand-Jensen, 2006). Nevertheless, recent studies show an extremely limited

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genotypic variation among the populations of aquatic invading species worldwide and indicate that these species invade and become established in new habitats via asexual propagules (Okada et al., 2009; Zhang et al., 2010). Therefore, the buoyancy (sinking/floating) of the propagules plays an important role in propagule dispersal, survival and establishment for aquatic plant species (Boedeltje et al., 2003; van den Broek et al., 2005; Riis & Sand-Jensen, 2006; Chambert & James, 2009). In particular, propagules may disperse over longer distances by floating or may be protected from adverse conditions by sinking to the bottom of the water body (Chambert & James, 2009). However, few studies have addressed the relationship between buoyancy and survival of the asexual propagule of aquatic plants.

Turions are asexual dormant organs that become detached from perennial aquatic plants at the end of the growth season (Sculthorpe, 1967). Turions are tough, sturdy organs and are formed by specialized shoot apices. They may be spherical or rhomboid in shape or may be greatly enlarged (Adamec, 2011). Previous studies concluded that the formation of turions is triggered by high rates of photosynthesis or stress factors (e.g., low temperatures or shortages of nutrients). In either case, turions store large amounts of carbohydrates, starch (9–70% dry weight [DW]), free sugars (in total 7–14% DW) (Winston & Gorham, 1979; Kunii, 1989; Ley et al., 1997; Adamec, 2003; Weber & Noodén, 2005; Xie & Yu, 2011), and mineral nutrients (N and P) (Adamec, 2010). These high levels of reserves could facilitate propagule survival and establishment over icy winter. Therefore, storage is the primary function of turions in aquatic plants (e.g., for overwintering). Moreover, some turions can also disperse via hydrochory, which is favored in aquatic habitats (Boedeltje et al., 2003). A few previous studies reported that the concentration of starch may play a role in determining the buoyancy of asexual propagules during certain periods because starch is relatively dense (1.44 g cm^{-3}) and may alter the density of the turions. However, other factors, such as the tissue porosity (lacunal volume), may be more important during the sprouting phase and may determine the density of the turion (Weber & Noodén, 2005). Unfortunately, our understanding of how these factors affect the sinking/floating of turions remains limited, even though the occurrence is very evident in natural habits.

Potamogeton crispus L. (curly pondweed) is a perennial submerged aquatic macrophyte native to Eurasia and is dominant in freshwater regions throughout China (Xie et al., 2003; Xie & Yu, 2011). However, the macrophyte is now considered one of the most harmful weed species: it forms thick monospecific stands, degrades the water quality (the aboveground portions of *P. crispus* decompose in the summer), and reduces the relative abundance and richness of other local species in South Africa and North America (Rogers & Breen, 1980; Bolduan et al., 1994; James et al., 2002; Woolf & Madsen, 2003). Unlike other aquatic macrophytes, *P. crispus* simultaneously flowers and produces bur-like turions almost continuously from late May to late October in temperate regions in eastern Asia (Sastroutomo, 1981; Wu et al., 2009). The dominant method of propagation for this species is the dispersal of turions, as the viability and germination of the seeds is reportedly very low (approximately 0.001%) (Rogers & Breen, 1980; Chambers et al., 1985; Jian et al., 2003; Woolf & Madsen, 2003). Mature turions are usually 10–17 mm long and 8–14 mm wide (Bolduan et al., 1994). Under field conditions, newly formed turions are green and demonstrate a high sprouting rate (100%), whereas the turions turn brown and show a relatively lower sprouting rate after the period of summer dormancy. In contrast, the turions from the previous growing season have a low ability to sprout and eventually decompose (Sastroutomo, 1981; Bolduan et al., 1994; Woolf & Madsen, 2003). It is noteworthy that *P. crispus* turions from Southern China do not behave as typical dormant temperate plant turions. They re-sprout as early as in summer to insure two generations per season. Some mature turions can float on water surface and be dispersed by wind and wave action (Bolduan et al., 1994). Previous studies also suggest that the attached leaves can act as floats to aid the dispersal of the turions (Sastroutomo et al., 1979).

Venable & Lawlor (1980) predicted that "if a plant makes two kinds of seeds with differing dispersibility, reproduction is maximized if the low dispersal seeds have delayed germination and the high dispersal seeds have quick germination." In aquatic environments, a similar risk reduction tendency would exhibit in asexual propagule dispersal of submerged macrophytes, as the asexual propagule contributes significantly to the long-distance dispersal (Boedeltje et al., 2003). However, to the best of our knowledge, no study has focused on the asexual propagules of submerged macrophytes for testing such a tendency. In this study,

we examined the relationship between sprouting abilities and buoyancy (ability to disperse) using *P. crispus* turions. We hypothesize that floating turions (with possibilities of longer distance dispersal) may have higher sprouting rates than sinking turions; if so, the internal characteristics related to primary metabolism would be higher in floating turions compared with that in sinking turions.

Materials and methods

Study site and field turion sampling

This study was conducted at Liangzi Lake, Hubei Province, China (30°5′–30°18′N, 114°21′–114°39′E); *P. crispus* is a common submerged macrophyte at this lake (Xie et al., 2003; Xie & Yu, 2011). The water level of the lake remained relatively stable during the early summer (Jian et al., 2003). From 19 to 20 June 2010, *P. crispus* turions were collected from nine field sites in which the vegetative cover was dominated by *P. crispus*; the water depths ranged from 1.2 to 2.7 m. Detailed information on the sampling sites is provided in Table 1. At each sampling site, 110 mature turions with fine structure (not grazed or broken) and green color (55 sinking and 55 floating) were collected from different individuals to avoid the influence of the same plant clone. Upon collection, the freshly collected turions were rinsed with a light jet of lake water. A total of 720 turions (360 sinking and 360 floating) were placed in light incubators for a sprouting experiment. A total of 270 turions (135 sinking and 135 floating) were used for measurements of density and tissue porosity and then dried at 80°C for 72 h to obtain the dry-weight biomass.

Sprouting experiment

The experiment was conducted at the National Field Station of Freshwater Ecosystem of Liangzi Lake over a period of 4 weeks. Turions of the same type (sinking or floating) and of similar size were placed in plastic bowls (diameter 10 cm, height 10 cm), with five sinking or five floating turions per bowl. The bowls for the nine sampling sites were filled with lake water and then were randomly placed in a light incubator (light:dark period ratio: 16 h:8 h). The experiment was repeated 8 times, and consequently, eight light incubators were used. The water was changed every 3 days. The light intensity was 110 ± 5 μmol m^{-2} s^{-1}, and the temperature was 15 ± 1°C. The light and temperature conditions in the incubators were consistent with the optimal sprouting conditions for *P. crispus* turions in China (Wu et al., 2009).

Measurements and analyses

Upon collection, the sediment samples of each sampling sites were dried, powdered, and digested with a K-435 digestion unit (Büchi® Labortechnik AG, Flawil, Switzerland). The total nitrogen (N) concentration was measured using a KjelFlex B-324 nitrogen analyzer (Büchi® Labortechnik AG, Flawil, Switzerland). The sediment samples were digested using the HClO$_4$–H$_2$SO$_4$ method (Bray & Kurz, 1945), and their total phosphorus (P) concentration was measured using a IL-500P phosphorus analyzer (Hach® Company, Loveland, CO, USA). The water samples were analyzed using an ion chromatography system (ICS-1000, Dionex, Sunnyvale, CA, USA) to determine the NH$_4^+$, NO$_3^-$, and PO$_4^{3-}$ concentrations. The measurements of the N and P concentrations were replicated three times (Table 1).

Turions from each site were analyzed to study the relationship between the turion density (ρ, g cm^{-3}) and the chemical parameters (i.e., total carbon [C], N, P, and starch). The turion density was expressed as

$$\rho = M_f / V_t, \tag{1}$$

where M_f is the fresh weight of the turion and V_t is the volume of the turion. The V_t was calculated using a 50 ml glass cylinder at 20°C as follows: first, the weight of the cylinder filled with 50 ml of ultra-pure water (M_{w1}) was recorded; second, one turion was placed into the cylinder, and volume of water in the cylinder was restored to 50 ml, and the weight (M_{w2}) of the cylinder and its contents was recorded. The turion volume was expressed as

$$V_t = \Delta V_w = (M_{w1} + M_{w2} - M_f)/\rho_w, \tag{2}$$

where ΔV_w is the volume of water restored and ρ_w is the density of water at 20°C (0.998 g cm^{-3}).

After the turion volume was measured, the turions were divided into hardened leaves and hardened stems. The volumetric air-filled porosity was then measured using the differential mass volume method. After the weight of a 50 ml cylinder filled with turion

Table 1 Principal characteristics of the nine sampling sites

Sampling site	Water depth (m)	Water						Sediment		
		pH	Dissolved oxygen (mg l^{-1})	Turbidity (NTU)	NH$_4$-N (mg l^{-1})	NO$_2$-N (mg l^{-1})	PO$_4$-P (µg l^{-1})	TN (mg g^{-1} dw)	TP (mg g^{-1} dw)	Organic matter (%)
1	30°16′26″N, 114°35′44″E	1.8	6.54	7.61	3.31	0.10–0.22	0.11–0.25	9.08–9.22	1.71–2.33	0.11–0.21	8.40–10.78
2	30°17′1″N, 114°35′46″E	2.5	7.25	7.20	3.28	0.06–0.16	0.27–0.36	8.52–8.98	1.90–2.11	0.14–0.23	11.20–14.69
3	30°15′56″N, 114°35′35″E	2.0	7.47	7.10	3.58	0.04–0.18	0.13–0.29	7.50–7.81	1.96–1.98	0.09–0.13	8.34–8.65
4	30°17′32″N, 114°34′50″E	1.5	7.54	7.04	5.05	0.12–0.27	0.24–0.39	8.36–8.51	2.06–2.83	0.11–0.19	12.88–13.82
5	30°17′47″N, 114°35′03″E	1.2	7.66	7.14	5.52	0.23–0.44	0.69–0.84	4.50–4.86	2.00–2.47	0.37–0.53	10.60–12.59
6	30°15′47″N, 114°34′32″E	1.6	7.73	7.03	4.54	0.04–0.06	0.12–0.26	3.76–4.67	0.53–0.86	0.03–0.07	4.80–6.85
7	30°16′13″N, 114°33′26″E	2.2	7.21	7.10	3.28	0.16–0.23	0.13–0.30	5.90–6.03	2.49–3.05	0.46–0.52	13.02–18.23
8	30°14′45″N, 114°33′36″E	2.7	7.65	6.93	3.45	0.08–0.15	0.12–0.19	7.33–7.68	1.42–1.89	0.16–0.24	9.64–10.96
9	30°15′12″N, 114°34′33″E	1.7	7.65	6.92	3.93	0.02–0.08	0.11–0.17	4.56–5.39	0.55–0.95	0.04–0.09	3.33–4.65

leaves/stems and ultra-pure water (M_1) was recorded, the cylinder was placed in a desiccator connected to a vacuum pump. Suction was continued for 10–20 s and repeated 10 times. After the above procedure, ultra-pure water was carefully restored to a volume of 50 ml, and the cylinder was weighted again (M_2). The volumetric air-filled porosity was expressed as

$$\Delta V_{\mathrm{w}}/V_{\mathrm{t}} = [(M_2 - M_1)/\rho_{\mathrm{w}}]/V_{\mathrm{t}} \times 100\%. \qquad (3)$$

After the turions were dried, the plant tissues were then powdered for chemical analyses. The C and N concentrations were analyzed using a Euro EA3000 elemental analyzer (EuroVector® Instruments and Software, Milan, Italy). The turion samples were digested using the HCLO$_4$–H$_2$SO$_4$ method (Bray & Kurz, 1945) and analyzed using an IL-500P phosphorus analyzer (Hach® Company, Loveland, CO, USA) to determine the P concentration. The perchloric acid/anthrone method (Morris, 1948) was used to determine the concentration of soluble sugar (SS) and starch. FAA measurements were performed with the ninhydrin method (Yemm & Cocking, 1955).

Statistical analyses

All the experimental data were transformed using a log(x) function to satisfy the conditions of the homogeneity of variance or normal distribution of residuals. The transformed data were then analyzed. The turion sprouting rates were analyzed using one-way ANOVA, with the sinking and floating of turions as the fixed factors, and the sampling sites and light incubators as the random factors. The correlations between the turion density and internal characteristics (e.g., elemental concentrations and tissue porosity) were analyzed using a Pearson correlation. The differences in the internal characteristics between the sinking and floating turions were analyzed using a nested ANOVA, with the turion sinking and floating (turion type) as the between-subject main effect and the sampling sites nested within the between-subject main effect. All the data were analyzed using the SPSS 19.0 program (SPSS, Chicago, IL, USA).

Results

At the end of the sprouting experiment, the sprouting rates of the floating turions were significantly higher
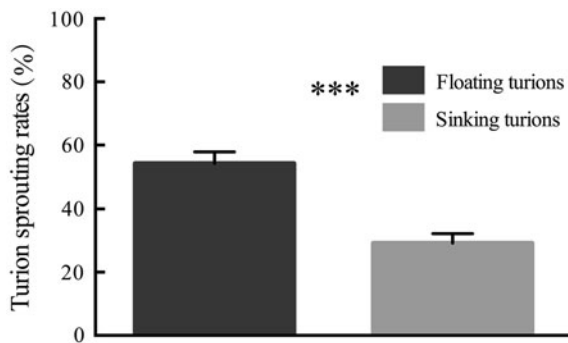
Fig. 1 *Potamogeton crispus* turion sprouting rates for sinking and floating turions from nine sampling sites in eight light incubators. The data are presented as the mean $\pm$ SE ($n = 72$). The results were analyzed using one-way ANOVA. ***$P < 0.001$

than that of the sinking turions (Fig. 1) ($F_{1,112} = 50.8$, $P < 0.001$). Although the sprouting rates differed significantly among the different sampling sites ($F_{8,112} = 13.7$, $P < 0.001$) the sprouting rates among the light incubators were similar ($F_{7,112} = 1.86$, $P = 0.082$). The interactions between the sampling site and turion density (floating and sinking) were also significant ($F_{8,112} = 2.81$, $P = 0.007$).

For the freshly field-sampled turions, the floating turions had smaller dry weight (288 ± 11.6 mg) than sinking turions (375 ± 16.0 mg) ($n = 270$, $P < 0.001$). The C and starch concentrations showed no significant correlations with the turion density (Fig. 2a, d), and the concentrations in the sinking and floating turions were similar, but differed significantly within the sampling sites (Table 2). For all the turion samples, the N and P concentrations in turions were significantly correlated with the turion density (Fig. 2b, c), and the N and P concentrations were higher in the floating turions than in the sinking turions (Table 2). The N concentration differed significantly among the sampling sites (Table 2). Moreover, the C:N, C:P, and N:P ratios were lower in the floating turions than the sinking turions at all the sampling sites (except for C:N ratio within sampling sites, Table 2). However, the FAA and SS concentrations were significantly higher in the floating turions than the sinking turions (Table 2). The sampling site effect was only significant on SS concentration (Table 2).

The tissue porosity of the turion leaves had a strongly negative correlation with the turion density (Fig. 2e): for all the sampling sites, higher leaf porosities were observed in the turions with low

densities (Table 2). However, the tissue porosity of the turion stem showed no such correlation with the turion density (Fig. 2f; Table 2).

Discussion

In our experiment, the sprouting rates of the floating turions (approximately 57%) were significantly higher than those of the sinking turions (approximately 30%), which confirmed our hypothesis. This finding is consistent with field results showing that most of the sinking turions are dormant on the bottom of the water body during the summer (Sastroutomo, 1981; Bolduan et al., 1994; Woolf & Madsen, 2003), however, turions sprouted occasionally when they were floating on the water surface (Quade et al., 1994; and our field observations). Previous studies revealed that the higher temperature (30–35°C) interacting with light pretreatments might facilitate the breaking of turions' dormancy (Sastroutomo, 1981; Jian et al., 2003). In addition, it is also worth noting that the floating turions had smaller individual sizes (dry weight) than sinking turions, indicating much of the floating turions were formed at a higher position in water column than sinking turions because plant heights were significantly negative correlated with the turion sizes (Xie & Yu, 2011). Therefore, it is possible that the floating turions may sprout faster than the sinking turions due to receiving higher temperature and more light on the water surface. Indeed, wind and water flow can move floating turions to shallow littoral areas (<30 cm deep), however, little or no *P. crispus* is present in these areas during peak growth due to the adverse ice-environment in the coming winter (Bolduan et al., 1994; Quade et al., 1994). All these results implied the low establishing abilities of floating turions. Furthermore, among the floating turions, approximately 20% of them sank during the 4-week experimental period. A possible explanation of this result is that the density of the turions is near the density of water (mean density 0.98 g/cm^3), so that small changes, such as changes in the ambient air pressure due to low air temperatures in our incubator, could affect the floating or sinking of the turions during their dispersal (Weber & Noodén, 2005).

Moreover, our results show that the N and P concentrations were significantly correlated with the turion density, although the correlation coefficients
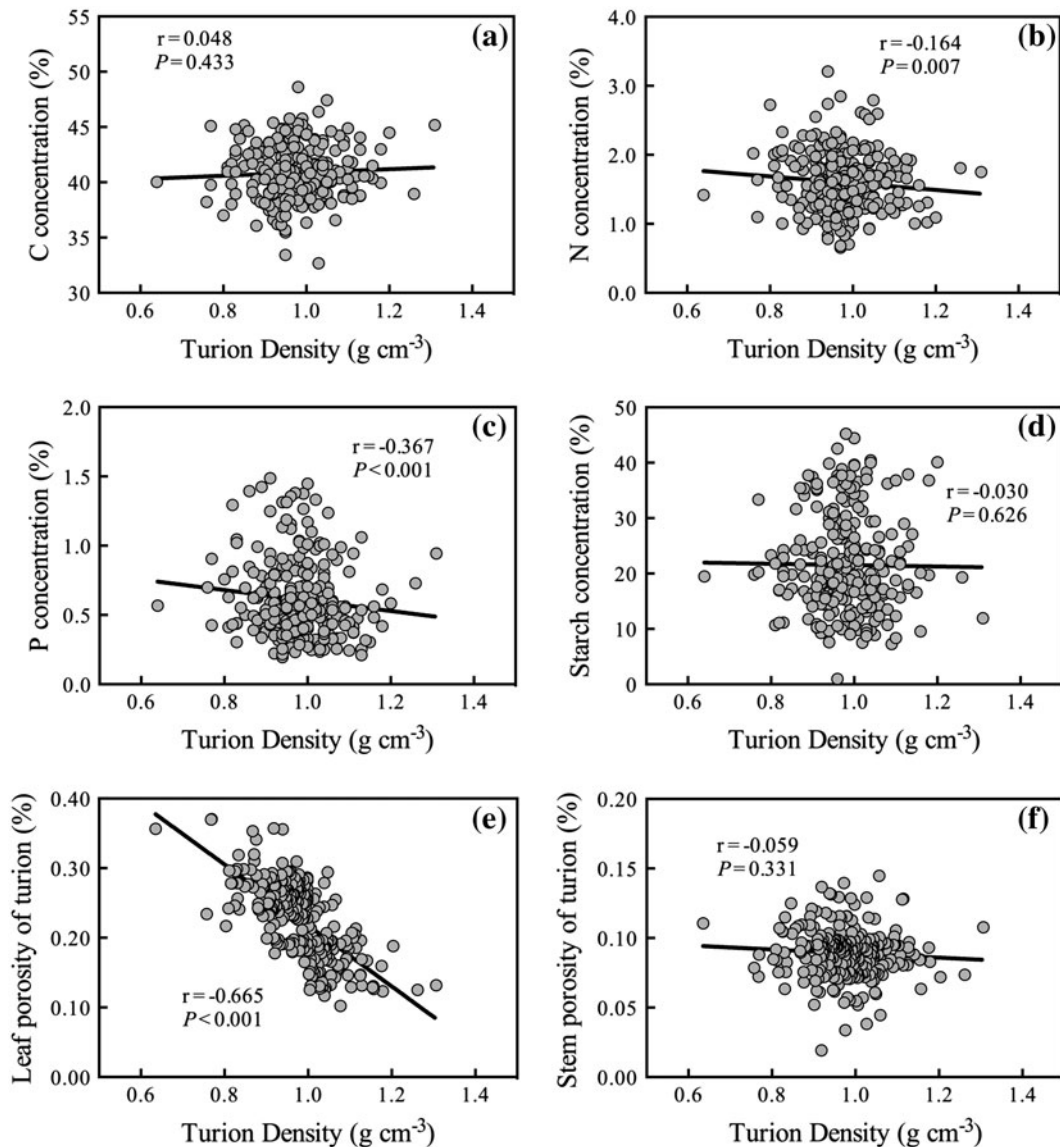
Fig. 2 Relationships of the C (**a**), N (**b**), P (**c**), starch (**d**) concentrations, leaf porosity (**e**), and stem porosity (**f**) to the density of the *Potamogeton crispus* turions from nine sampling sites. *R* values and *P* values of Pearson correlations are shown ($n = 270$)

(*r* values) were relatively low. These correlations between turion density and the N and P concentrations may not themselves constitute conclusive evidence that the higher N and P concentrations cause turion flotation. However, these negative effects of the N and P concentrations on the turion density produced reductions in the C:N, C:P, and N:P ratios in the low density turions (floating turions). The high N and P concentrations in floating turions would support their rapid sprouting and growth, because more N and P (especially more P) are allocated to RNA for synthesis

of proteins required for growth (Elser et al., 1996; Elser et al., 2000; Rivas-Ubach et al., 2012). For example, previous studies determined that the turion sprouting process (starch breakdown and growth of new photosynthetic organs) requires the availability of N source to achieve the necessary energy metabolism and biosynthesis reactions (Appenroth et al., 1992; Appenroth & Ziegler, 2008). In the present study, high FAA and SS concentrations indicated high primary metabolites in the floating turions. The high FAA concentrations indicated the degradation of

Table 2 The internal characteristics of the floating and sinking turions

Dependent variables	Sinking turion	Floating turion	Turion type			Sampling site (turion type)		
			d.f.	F	P	d.f.	F	P
C concentration (%)	40.87 ± 0.193	40.84 ± 0.221	9,252	0.411	0.929	8,9.026	16.76	**<0.001**
N concentration (%)	1.53 ± 0.032	1.66 ± 0.039	9,252	2.449	**0.011**	8,9.004	8.263	**0.002**
P concentration (%)	0.52 ± 0.018	0.71 ± 0.027	9,252	4.198	**<0.001**	8,9.003	0.733	0.664
Starch concentration (%)	21.34 ± 0.792	21.75 ± 0.718	9,252	1.626	0.108	8,9.006	14.203	**<0.001**
C:N ratio	28.32 ± 0.665	26.38 ± 0.644	9,252	2.848	**0.003**	8,9.004	8.595	**0.002**
C:P ratio	91.93 ± 3.156	70.48 ± 2.913	9,252	4.085	**<0.001**	8,9.003	0.784	0.629
N:P ratio	3.03 ± 0.099	2.73 ± 0.104	9,252	2.052	**0.034**	8,9.005	2.598	0.088
FAA concentration (mg g^{-1})	0.22 ± 0.003	0.26 ± 0.002	9,252	19.251	**<0.001**	8,9.001	0.023	1.000
SS concentration (%)	4.85 ± 0.171	6.10 ± 0.240	9,252	2.221	**0.021**	8,9.005	9.891	**0.001**
Leaf porosity of turion (%)	0.19 ± 0.004	0.26 ± 0.003	9,252	17.472	**<0.001**	8,9.001	0.126	0.996
Stem porosity of turion (%)	0.09 ± 0.001	0.09 ± 0.002	9,252	1.367	0.203	8,9.008	2.823	0.072

Absolute values (mean ± SE) are shown with F values and P values for the nested ANOVA with the turion type (sinking and floating) as the between-subject main effect and the sampling sites nested within the between-subject main effect. All the data were transformed using a log(x) function. Significant P values (<0.05) are indicated in bold font

nitrogenous compounds (e.g., non-structural proteins) in turions (Cao et al., 2011). The SS storage was significantly correlated with the sprouting capacities in asexual propagules (e.g., tubers, rhizomes, and turions) of some aquatic plants (Suzuki & Stuefer, 1999; Spencer et al., 2001; Deng et al., 2013).

Large proportions of C (primarily starch) were found in turions of *P. crispus* in this study, which were consistent with the previous results that the principal function of nutrient reserves in turions may be to promote plant survival during dormancy (Woolf & Madsen, 2003; Xie & Yu, 2011). However, neither C nor starch concentrations showed significant effect on the density or sprouting of turions. This result is also consistent with recent studies that the starch reserves do not directly drive propagule sprouting (Deng et al., 2013), but must be transformed to SS for utilization by the plant (Karunagaran & Ramakrishna Rao, 1991; Hajirezaei et al., 2003). Indeed, starch content usually decreased over several days/hours after the activation of α-amylase and starch phosphorylase (Appenroth & Gabrys, 2001; Harada & Kimiharu, 2003; Appenroth et al., 2011). Furthermore, previous studies also observed that high starch content may not necessarily cause the sinking of turions. For example, turions of *Aldrovanda vesiculosa* sink gradually to the bottom in autumn which is not only caused by their high starch content but also, presumably, by their expulsion of excess gases (Adamec, 1999). Due to high respiration,

which may increase the gas concentration in the leaf lacunae of turions, sprouting turions rise to the water surface in next April to May (Adamec, 2011). Therefore, a critical anatomical organ may exist and control the density (buoyancy) of the turion (Adamec, 1999; Weber & Noodén, 2005; Witty, 2009).

In this study, the turion leaf porosity was significantly correlated with the turion density, indicating that the leaf of turions could be the organ that determines the buoyancy of the turions. Adamec (2008, 2011) discovered that dark respiration is higher in sprouting turions. This process may increase the amount of gas in the leaf lacunae of the turions and facilitate floating; in contrast, the dark respiration of dormant turions is usually very low. On the other hand, leaves of turion were formed from leaves of mother plants (Wehrmeister, 1978). As the temperature rises in summer, the dark respiration of mother plants also increases (Barko & Smart, 1981), which might also increases the gas in turion leaves. However, the mechanism governing the formation of the porous tissue is currently unknown, and further in-depth study is required.

In conclusion, our results show that the floating turions (with high dispersal ability) had higher sprouting rates than the sinking turions, indicating the optimal dispersal-sprouting strategy of *P. crispus*. In addition, after measuring the internal characteristics in turions, we found that the floating turions are

possibly with high levels of primary metabolites, causing a higher porosity of the tissues (leaves) of the floating turions, thus allowing these turions to float. Although the extent to which the sinking and floating of turions contributes to the dispersal of *P. crispus* is uncertain, this study provides evidence that anatomical structure may cause the sinking or floating of *P. crispus* turions and may, thus, produce different patterns of propagule dispersal. Our results offer new insights into the expansion of the submerged macrophyte population.

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