

# Size-related auto-fragment production and carbohydrate storage in auto-fragment of *Myriophyllum spicatum* L. in response to sediment nutrient and plant density

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Received: 26 April 2010 / Revised: 6 September 2010 / Accepted: 13 September 2010 / Published online: 25 September 2010  
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**Abstract** Size-related asexual reproduction of submersed macrophytes is still poorly understood. Here, we investigate how size-related auto-fragmentation in *Myriophyllum spicatum* L. responds to sediment nutrients and plant density. An experiment was carried out with sediments containing two different nutrient levels and with two levels of plant density. The results show that sediment nutrients and plant density brought about a strong dependency of auto-fragment production and the amount of total non-structural carbohydrate (TNC) storage in auto-fragments on individual plant size (total plant biomass). However, these two factors acted differently on size dependency. Sediment nutrients positively affected auto-fragment production and the amount of TNC in auto-fragments of *M. spicatum*. High concentrations of sediment nutrients significantly increased these two traits in absolute value and the value relative to plant size. Although the auto-fragment biomass and the amount of TNC in auto-fragments did not differ between density treatments when plant size was considered, the absolute values of these two traits were much larger in the low

plant density treatment than in the high plant density treatment, which suggested an indirect negative effect of plant density on the auto-fragmentation of *M. spicatum*. In addition, higher percentages of large auto-fragments ( $>100$  mg) were produced by plants that grew in nutrient poor sediment and low plant density environment than plants in nutrient rich sediment and high plant density environment. These results do not solely highlight a size-dependent effect, but also a size-independent effect of auto-fragment production and the amount of TNC in auto-fragments of *M. spicatum*. Furthermore, such size-independent effects can be explained by the significant biomass partitioning differences and the similar TNC-concentrations in auto-fragments under different environmental conditions.

**Keywords** Auto-fragmentation · Carbohydrate storage · *Myriophyllum spicatum* L. · Plant density · Plant size · Sediment nutrients

## Introduction

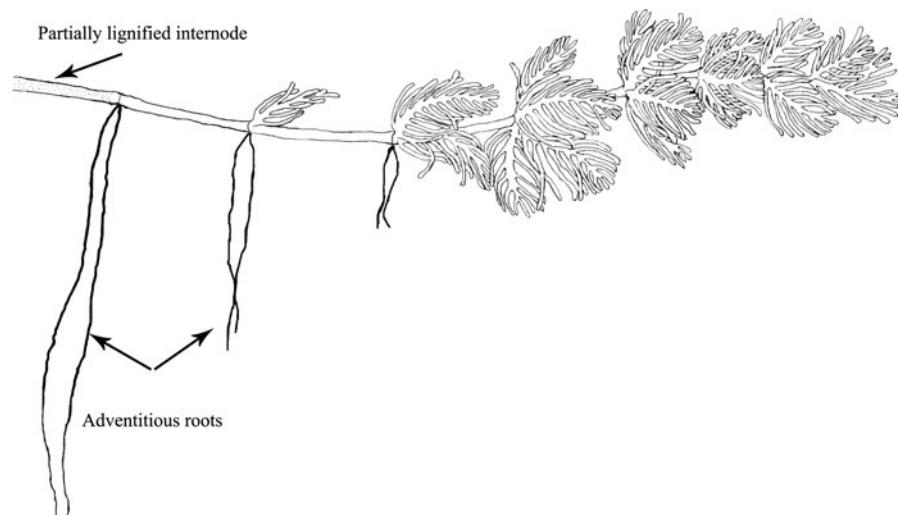
The effect of plant size on reproductive variation has been shown empirically in numerous studies (Giesel, 1976; Samson & Werk, 1986; Hartnett, 1990; Sugiyama & Bazzaz, 1998). Many studies have demonstrated a size-dependent effect on reproductive output for ephemeral and annual plants, such that a positive relationship exists between seed output and

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Handling editor: S.M. Thomaz

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**Fig. 1** Schematic representation of the auto-fragment of *M. spicatum*



plant size (e.g., Samson & Werk, 1986; Sugiyama & Bazzaz, 1998). Recent studies have demonstrated that shifts in plant reproduction are not exclusively driven by variation in size, but can also be based on the plasticity of a plant's response to a changing environment (also called size-independent effect or true plasticity) (Weiner, 2004; Niu et al., 2009). Unfortunately, except for a few cases (e.g., Hartnett, 1990; Verburg et al., 1996; Puijalon et al., 2008b), this size-dependent or size-independent effect on asexual propagation reproduction has received little attention in clonal perennials (Schmid et al., 1995; Thompson & Eckert, 2004), particularly in aquatic habitats where vegetative reproduction is more frequent (Santamaría, 2002).

Nutrient availability and plant density both are important factors that lead to size differences (Enquist et al., 1998; Sugiyama & Bazzaz, 1998; Müller et al., 2000), and variation in reproduction (Antonovics & Levin, 1980; Sultan & Bazzaz, 1993; Dong et al., 1997; Mandák & Pyšek, 1999; Winkler & Stöcklin, 2002; Dorken & Barrett, 2004; Xiao et al., 2006). However, our understanding of how nutrient availability and plant density's effects on plant size contribute to reproduction is still limited. In aquatic macrophytes, relatively few studies have implied that bigger plant size is correlated with increased asexual reproduction. For instance, Smith et al. (2002) reported that the lack of nitrogen in sediment would increase both the biomass and the number of *Myriophyllum spicatum* L. auto-fragments. However, the number and biomass of auto-fragments

in this species were decreased when competition was present (Wang et al., 2008).

*Myriophyllum spicatum* L. is a submersed aquatic macrophyte that is native to Europe and Asia (Couch & Nelson, 1985) and is now considered to be one of the most troublesome weeds in North America (Grace & Wetzel, 1978; Smith & Barko, 1990; Buchan & Padilla, 2000) and North Africa (Ali & Soltan, 2006). Invasion by this species has various ecological and economic consequences, such as interference with water-based recreation, disruption of water flow (Boyle et al., 1999), and a decrease in the relative abundance and richness of native species in communities (Madsen, 1997). The asexual propagation of *M. spicatum* is predominantly via self-form stem segments (also called auto-fragments) that are produced through the development of an abscission layer (Smith & Barko, 1990; Madsen, 1997; Santamaría, 2002). During the growing season, a zone of “partially lignified cells” are formatted at the last node and cause the abscission of auto-fragment (Aiken et al., 1979). The auto-fragments are usually 10–20 cm long, and often develop roots at the nodes (Grace & Wetzel, 1978; Aiken et al., 1979) (Fig. 1). This means of dispersal can provide the species with an efficient mechanism for population expansion into different water bodies (Madsen & Smith, 1997), resulting in the species rapidly spreading into previously un-invaded places (Buchan & Padilla, 2000). Previous studies have demonstrated that *M. spicatum* obtains most of its nutrients from the sediment and that the production of auto-fragments is also correlated with the canopy

biomass and nitrogen content in the sediment (Barko & Smart, 1986; Madsen, 1997; Smith et al., 2002). These results suggest that nutrient availability and/or plant density may influence plant size and may have consequences for auto-fragmentation.

Field observation and experimental studies have indicated that auto-fragments of *M. spicatum* have greater regeneration, colonization, and overwintering abilities than stem fragments of this species (Kimbrel, 1982; Barrat-Segretain, 1996; Barrat-Segretain et al., 1999). This is likely due to the high concentration of total non-structural carbohydrate (TNC) in the auto-fragments (Kimbrel, 1982; Smith et al., 2002). In aquatic plants, storage of TNC in multiple structures, such as stems (Best, 1977), winter buds (Titus & Adams, 1979), and rhizomes (Vojtíšková et al., 2006), may serve to reduce offspring mortality and increase re-growth in habitats with frequent disturbances (Madsen, 1997). Additionally, environmental factors may also trigger storage in different organs (Suzuki & Stuefer, 1999). For instance, Puijalon et al. (2008a) found that when in stressful habitats, *Berula erecta* (Hudson) Coville allocates more dry mass to stems (storage organs) compared to plants in favorable habitats. These results highlight a potential trade-off between current growth and resource storage in asexual propagules among different environmental conditions.

In this article, we study size-related auto-fragmentation of *M. spicatum* under different levels of sediment nutrients and different levels of plant density. The following questions were addressed: (1) Does *M. spicatum* adjust its size (biomass accumulation) and biomass partitioning in response to sediment nutrient and plant density? (2) Are auto-fragmentation differences among the treatments caused only by plant size, or is there a size-independent effect due to plant biomass partitioning?

## Materials and methods

### Material

This study was conducted at The National Field Station of Freshwater Ecosystem of Liangzi Lake, Hubei Province, China ( $30^{\circ}5'$ – $30^{\circ}18'$ N,  $114^{\circ}21'$ – $114^{\circ}39'$ E). In the spring of 2008, 300 apical shoots (5 cm long) of *M. spicatum* were collected from different individuals

in Liangzi Lake. The apical shoots were transplanted into containers filled with sand and water at a 20-cm depth in a greenhouse for approximately 2 weeks until the plants had developed root systems.

### Experimental design

This experiment lasted for 5 months (from 01 Apr. to 01 Sep., 2008). After developed roots, the apical shoots (similar in both initial biomass and height,  $0.73 \pm 0.02$  g wet weight,  $18.46 \pm 0.27$  cm, mean  $\pm$  s.e.) were cultivated in plastic boxes ( $44 \times 30 \times 15$  cm, length  $\times$  width  $\times$  height) filled with approximately 7 cm (16 kg) of substratum. Plastic boxes of different treatments were then placed into separate circular fiberglass tanks (radius of 50 cm  $\times$  100 cm deep) and filled with 100 cm of lake water [Total Nitrogen (TN)  $0.71 \text{ mg l}^{-1}$ , Total Phosphorus (TP)  $0.04 \text{ mg l}^{-1}$ ]. Thirty-two outdoor tanks were used at the start of the experiment. Two different nutrient sediments were tested: nutrient rich sediment (pure muddy substratum),  $2.94 \text{ mg g}^{-1}$  TN,  $0.13 \text{ mg g}^{-1}$  TP, and 12.5% organic matter; and nutrient poor sediment (8 kg clay sediment being mixed into 8 kg muddy substratum),  $1.65 \text{ mg g}^{-1}$  TN,  $0.10 \text{ mg g}^{-1}$  TP, and 10.4% organic matter. Both clay and muddy substratum were obtained from Liangzi Lake. Within each sediment nutrient treatment, two different plant densities were also tested: low density, two plants per box (equivalent to  $15 \text{ plants m}^{-2}$ ) and high density, 10 plants per box (equivalent to  $75 \text{ plants m}^{-2}$ ). These densities approximate those occurring in natural populations of *M. spicatum* in Hubei Province, China (Yu & Li, 2000). In the high density treatment, plants were placed 10 cm apart. Each treatment was replicated eight times.

Water temperature and light intensity were recorded at noon every 3 days (water temperature  $28.79 \pm 4.98^\circ\text{C}$ ; light intensity  $2,250 \pm 48.63 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , mean  $\pm$  s.e.). In order to reduce the influence of alga on the plant growth, tank water was changed weekly.

### Plant harvest

Auto-fragments were collected and recorded when they were abscised from the parental plants every 3 days. At the end of the experiment, the individual plants were segmented and categorized into roots, above-ground tissues and auto-fragments. All plant

tissue samples were dried at 80°C for 72 h and then weighed to obtain dry-weight biomass.

### Chemical analysis

Upon collection, water and sediment samples were digested with a K-435 digestion unit (Büchi® Labor-technik AG, Flawil Switzerland), and analysed for TN and TP concentrations on a KjelFlex B-324 nitrogen analyzer (Büchi® Labortechnik AG, Flawil Switzerland) and a IL-500P phosphorus analyzer (Hach® Company, Loveland USA). TN and TP concentrations were replicated three times.

The Perchloric acid/anthrone method (Morris, 1948) was used for analyzing the soluble sugars and starch fractions of TNC. This technique has proven to be robust and results from this method are known to be correlated with results obtained using other analytical approaches (Rose et al., 1991). Dried plant tissues were powdered, and 20 mg samples were used for each TNC analysis. Soluble sugars were extracted from the dried material with ethanol (80%) at 80°C, and then centrifuged. After three extraction processes, the supernatant was used for soluble sugar determination and the residue was hydrolyzed with perchloric acid (35%) for starch determination. The TNC concentration was calculated by summing the sugar and starch concentrations and then multiplying by the dry mass of a sample.

### Statistical analysis

Differences in plant growth traits (i.e., total plant biomass, root:shoot ratio) and auto-fragmentation traits (i.e., auto-fragment biomass, TNC-concentration in auto-fragments, amount of TNC in the auto-fragments) between the nutrient and plant density treatments were analyzed with two-way ANOVA with Bonferroni correction ( $\alpha = 0.05$ ). Analyses of covariance (ANCOVAs) with total plant biomass as covariate were used to test the effect of treatment on the auto-fragment biomass and the amount of TNC in the auto-fragments for plant size (total plant biomass). Sediment nutrients and plant density were treated as fixed factors in these ANCOVAs. If the data did not satisfy homogeneity of variances or normal distribution of residuals, they were log and arcsin transformed to analysis. All of the data were

analyzed using the SPSS 13.0 software package (SPSS, Chicago, IL, USA).

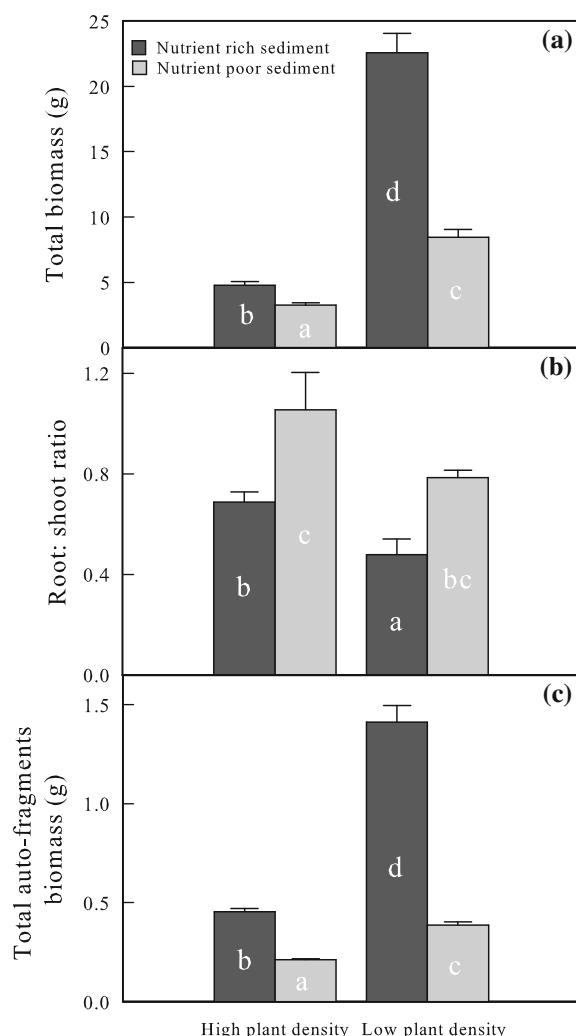
## Results

### Plant growth

After 5 months' growth, we detected statistically significant effects of sediment nutrients and plant density on total plant biomass, and the interactions of sediment nutrient and plant density were also significant. Plants that grew in nutrient rich sediment and low plant density environment had higher biomass than the ones in nutrient poor sediment and high plant density environment (Fig. 2a; Table 1). Moreover, plants were seen to adjust their biomass partitioning in response to sediment nutrient increasing and not the plant density treatment. The plants that grew under nutrient rich sediment produced more above-ground biomass than the plants from nutrient poor sediment. Similarly, there was a significant difference in the ratio of roots to shoots between different plant densities. However, the interaction of sediment nutrient and plant density was not significant (Fig. 2b; Table 1).

### Production of auto-fragments

Biomass of auto-fragments was highly significantly correlated with total plant biomass (Fig. 3a). Auto-fragment biomass of *M. spicatum* was higher under nutrient rich treatment than nutrient poor treatment, regardless of how it was measured (absolute value or relative to the plant size). Although the absolute value of auto-fragment biomass was higher under low density treatment than high density treatment, auto-fragment biomass between plant density treatments was similar when considered relative to plant size (Figs. 2c, 3a; Table 2). Moreover, auto-fragment size varied between treatments. Plants that grew in nutrient poor sediment produced higher percentages of large auto-fragments (>100 mg) than the ones in nutrient rich sediment. Higher percentages of large auto-fragments (>100 mg) were produced by plants that grew in a low plant density environment, although only significant at the  $\alpha = 0.10$  level (Fig. 4).



**Fig. 2** Differences in total plant biomass (a), root:shoot ratio (b) and total auto-fragment biomass (c) between the sediment nutrients and plant density treatments, respectively. Data are presented as means  $\pm$  s.e. ( $n = 8$ ). Bars sharing the different letters indicate significant differences among the treatments ( $P < 0.05$ , two-way ANOVA with Bonferroni correction). Data were transformed using the log ( $x$ ) function

#### Auto-fragments TNC storage

There were no significant TNC-concentration differences in the auto-fragments in either treatments (Fig. 5a; Table 1) and the TNC-concentration was not significantly correlation with plant size (Pearson correlation,  $P = 0.821$ ). But the amount of TNC in the auto-fragments varied between different treatments (Fig. 5b; Table 1) and had a high correlation with total plant biomass (Fig. 3b). Similar to auto-fragment

biomass, sediment nutrient enrichment had significantly increased the amount of TNC in auto-fragments when considered relative to plant size, but plant density had no significant effect on the amount of TNC in auto-fragment (Fig. 3b; Table 2).

## Discussion

### Production of auto-fragments

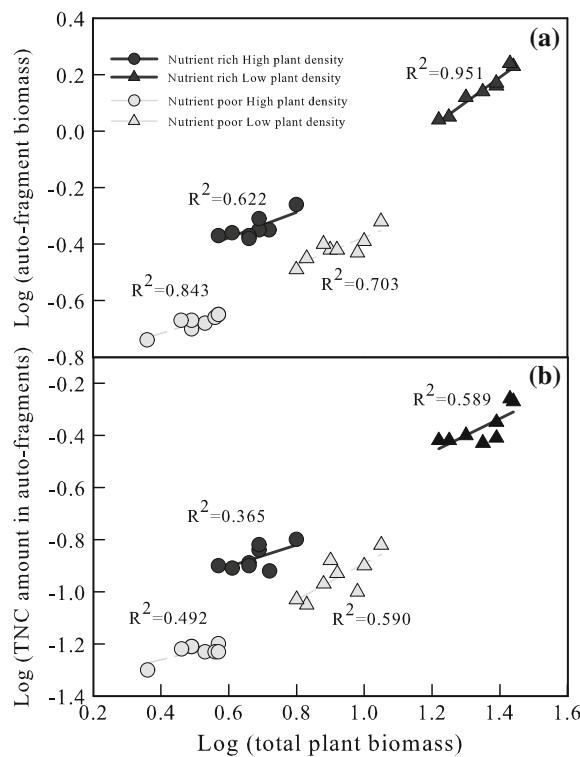
Auto-fragment biomass was found to be positively correlated with individual plant size. This is consistent with previous studies in clonal plants, which demonstrated that asexual propagule production was size dependent (Schmid et al., 1995; Méndez & Karlsson, 2005). However, our observation was opposite to that of Smith et al. (2002), who found that *M. spicatum* growing in low nitrogen sediments produced auto-fragments that were larger in size than, and twice as numerous as, plants growing in high-nitrogen sediments. Smith et al. (2002) observed smaller plant sizes under high-nitrogen conditions because of the ionic stresses on the plants, while we observed larger plant sizes under nutrient rich conditions. Both experiments, however, indicated auto-fragment production is correlated with plant size. It is generally known that ammonium stress in plants is accompanied by a reduction in growth (Nimptsch & Pflugmacher, 2007). Such an effect can be explained by the elevated energy consumption caused by  $\text{NH}_4^+$  transport costs (Britto et al., 2001) and ammonia promoting oxidative stress (Nimptsch & Pflugmacher, 2007). In our experiment, however, we did not observe ionic stresses on the plant growth. It is possible that enrichment of multiple nutrients in the sediment (especially phosphorus) may mitigate ammonium stresses and increase the growth of *M. spicatum*, which is supported by the previous studies in both freshwater and marine submersed plants (Barko & Smart, 1986).

Additionally, our results suggest a positive effect of sediment nutrients on auto-fragment production. This means that in addition to the size-dependent effect, the auto-fragmentation of *M. spicatum* may also respond to heterogeneous environments via a size-independent mechanism. The results we obtained in *M. spicatum* are in agreement with results demonstrated in terrestrial plants showing that biomass partitioning due to a

**Table 1** *F* and *P* values of sediment nutrient and plant density for total biomass, root:shoot ratio, total auto-fragment biomass, TNC concentration in auto-fragment and amount of TNC in auto-fragments (Bonferroni correction,  $\alpha = 0.05$ )

	d.f.	Sediment nutrient (S)		Plant density (P)		S × P	
		F	P	F	P	F	P
Total biomass	1, 28	115.579	<0.001	384.758	<0.001	21.651	<0.001
Root:shoot ratio	1, 28	18.556	<0.001	8.857	0.006	0.795	0.380
Total auto-fragment biomass	1, 28	620.210	<0.001	439.833	<0.001	39.844	<0.001
TNC concentration in auto-fragment	1, 28	2.860	0.102	1.907	0.178	0.051	0.823
Amount of TNC in auto-fragments	1, 28	496.606	<0.001	351.200	<0.001	27.622	<0.001

All data were transformed using log (*x*) function



**Fig. 3** Relationship of total auto-fragment biomass (a) and the amount of TNC in auto-fragments (b) to total plant biomass between different levels of sediment nutrients and different plant density treatments, respectively. Data were transformed using the log (*x*) function, and are shown on a log–log scale ( $n = 8$ )

heterogeneous environment affects the reproduction of plants (Sugiyama & Bazzaz, 1998; Müller et al., 2000; Niu et al., 2009). In our experiment, more above-ground biomass of *M. spicatum* was produced under nutrient rich treatments, whereas in nutrient poor conditions shoot development was constrained

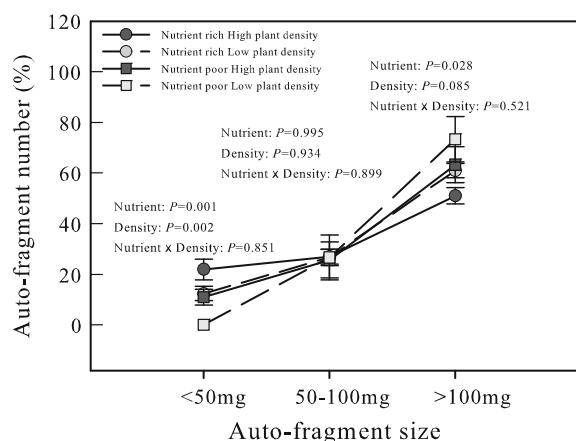
because a greater proportion of the biomass is allocated to the root system (Xie et al., 2007). Large above-ground biomass of *M. spicatum* contributes more to auto-fragment production. For instance, auto-fragments are formed entirely in the upper canopy of *M. spicatum* (Madsen, 1997). Moreover, plants tend to allocate more biomass to branches when grown in more favorable environments (Bonser & Aarssen, 2003), the lateral branches of *M. spicatum* (potential fragments) may abscise and turn into auto-fragments. Although evidence suggested that auto-fragments tend to abscise in unfavorable conditions (Smith et al., 2002), *M. spicatum* decreases investment in branch number in these conditions (e.g., low light environments, Starand & Weisner, 2001), which may cause the low production of auto-fragments in nutrient poor and high plant density environments.

The absolute auto-fragment biomass was much larger with low plant density treatments than high plant density treatments. This result is consistent with a previous competition study in *M. spicatum* (Wang et al., 2008). Most terrestrial and aquatic plants can utilize more resources than suppressed plants in a high density population, and reproductive output varies with plant size due to developmental constraints (Sugiyama & Bazzaz, 1998). Indeed, plants from the low density treatment produced more above-ground biomass than plants from the high density treatment. It is common that perennial clonal plants may not produce asexual propagules in habitats such as high plant density because of low asexual propagule survival; instead, they increase vegetative size in response to enhanced competition and nutrient influxes (Takada & Nakajima, 1996; Jongejans et al., 2006). However, auto-fragment production was similar when considered relative to plant size between

**Table 2** Analysis of covariance of total auto-fragment biomass and amount of TNC in auto-fragments with sediment nutrient (N) and plant density (D) as fixed factors and total plant biomass (C) as covariate

Source	Total auto-fragment biomass			Amount of TNC in auto-fragments		
	d.f.	F	P	d.f.	F	P
Total plant biomass (C)	1,25	94.907	<0.001	1,27	27.150	<0.001
Sediment nutrient (N)	1,25	204.629	<0.001	1,27	81.189	<0.001
Plant density (D)	1,25	4.230	0.050	1,27	3.087	0.090
N × D	1,25	3.455	0.075	1,27	4.194	0.050
C × N	1,25	5.320	0.030	—	—	—
C × D	1,25	5.993	0.022	—	—	—

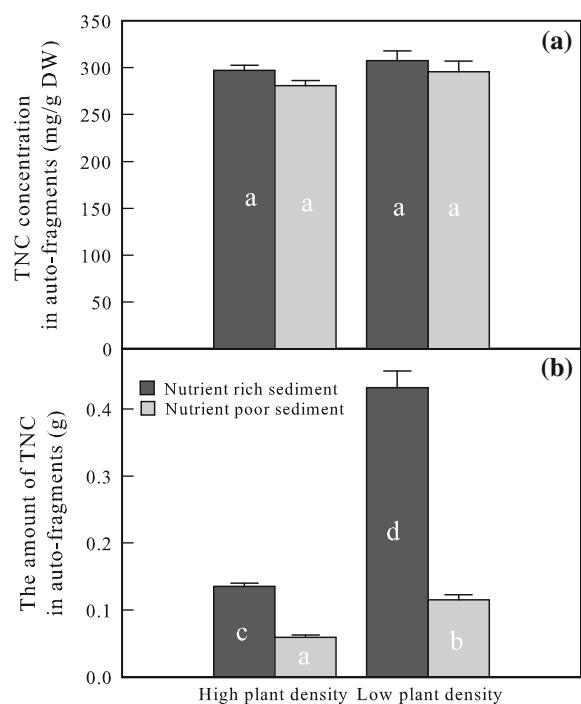
Non-significant interaction terms were eliminated from the analysis to obtain the final model. Data were transformed using the log ( $x$ ) function



**Fig. 4** Percentages of auto-fragment number (%  $\pm$  s.e.,  $n = 8$ ) classified with different dry mass under different nutrient sediments and at different plant densities, respectively. The  $P$  values of two-way ANOVA are shown. All data were transformed using the arcsin ( $x$ ) function

plant density treatments, but the interactions between plant size and the plant density treatments were significant. Therefore, plant density's effect on size dependency of auto-fragmentation is a result of the effect of size differences on the relationship between auto-fragmentation and plant size. These results are consistent with previous studies in sexual reproductive allocation (Sugiyama & Bazzaz, 1998).

Individual auto-fragment biomass varied among treatments. Since survival of a propagule (i.e., auto-fragment) is key to a plant's ability to reproduce optimally in various environments (Takada & Nakajima, 1996), being able to produce larger fragments would be an advantage as these have a higher probability of surviving and allow for greater dispersal distances



**Fig. 5** TNC-concentration (a) and amount of TNC in auto-fragment (b) in nutrient and plant density treatments, respectively. Data are presented as means  $\pm$  s.e. ( $n = 8$ ). Bars sharing different letters indicate significant differences among the treatments ( $P < 0.05$ , two-way ANOVA with Bonferroni correction). Data were transformed using the log ( $x$ ) function

(Barrat-Segretain et al., 1999; Wu et al., 2007). It is reasonable to think that when grown in nutrient poor sediments, *M. spicatum* tended to produce larger auto-fragments; whereas under high plant density environments, auto-fragment development was constrained

due to the high intra-specific competition and therefore more small auto-fragments were produced.

Once established in a favorable environment, *M. spicatum* grows rapidly and reaches a large size, which provides for a large propagule pool (Kautsky, 1988). The idea that competitive ability and colonization capacity are positively related may explain the expansion and success of some invasive species (Boedeltje et al., 2008). Both field observations and experiments have demonstrated that auto-fragments of *M. spicatum* have high survival rates and possess great regeneration and colonization abilities (Kimbrel, 1982; Barrat-Segretain, 1996; Madsen, 1997; Barrat-Segretain et al., 1999), which allow the auto-fragments of this species to spread rapidly and over long distances. Such strategies may increase asexual propagule pressure of *M. spicatum* on local habitats and trigger invasion by this species, especially in eutrophic and unvegetated habitats in North America (Nichols & Shaw, 1986; Smith & Barko, 1990). Furthermore, as a competitive species (Kautsky, 1988), *M. spicatum* allocates more biomass into vegetative structures in nutrient rich sediments or at low plant density than plants in nutrient poor sediments or at high plant density. This strategy may allow them to capture more light and reduce competition for soil nutrients with neighboring plants (Smith & Barko, 1990; Madsen, 1997), resulting in a displacement of native species. In contrast to North America, *M. spicatum* is not considered to be a serious troublesome weed in China (Yu & Li, 2000). Although the climate and soils in eastern Asia are comparable to that of North America (Guo et al., 2006), the relatively lower amounts of local diversity coupled with slower species turnover might increase the amount of resources (i.e., sediment nutrients and light) available to *M. spicatum* in North America which would allow for its rapid colonization and expansion.

#### TNC storage in auto-fragments

Carbohydrate storage plays an important role in vegetative propagule performance (e.g., survival, sprouting and spread) (Spencer & Ksander, 1996; Suzuki & Stuefer, 1999; Werger & Huber, 2006). *M. spicatum* translocated and stored a large amount of TNC (soluble sugars and starch) in its auto-fragments, similar to results found in earlier studies (Kimbrel, 1982; Madsen, 1997; Smith et al., 2002). The amount

of TNC in the auto-fragments was dependent on the total plant biomass. This is in agreement with previous reports of other aquatic plants; storage in propagules under different environments is regulated by propagule production and may in turn be limited by plant size (Hangelbroek et al., 2003). This is because plants of different sizes are likely to vary with their investments in photosynthetic tissue and enzymatic machinery, which would influence the supply of carbohydrates to newly growing propagules (Hangelbroek et al., 2003). However, it is possible that in any environment *M. spicatum* will exhibit the optimal storage strategy (i.e., stored sufficient amount of TNC, Smith et al., 2002) in auto-fragments to gain the maximum fitness of its propagules. This strategy would likely help its offspring adapt to different environments and prevent extinction of its population. It is speculated that, in the field, when *M. spicatum* grows under an adverse environment, the plant is small and more TNC is allocated to its own growth (e.g., root, stem and inflorescence) (Smith et al., 2002). However, when *M. spicatum* grows under more favorable environments, the plant is larger and more resources are allocated to auto-fragment production.

It is most likely that large TNC storage in auto-fragments, especially starch (Madsen, 1997; Smith et al., 2002), might provide quick sinking and regrowth of auto-fragments over a short period (Weber & Noodén, 2005; Wu et al., 2007). Overall, the higher TNC potentially enhances the survival of the auto-fragments during the dispersal process (Kimbrel, 1982; Madsen, 1997; Madsen & Smith, 1997), increases their competitive ability during early recruitment and establishment processes, and ensures their overwintering (Kimbrel, 1982). Moreover, a benefit of fragmentation is lowered respiratory costs, which increases the proportion of currently fixed carbon that is available for growth of the parental plants and the production of new auto-fragments, as demonstrated in terrestrial clonal plant (Hay & Kelly, 2008).

#### Conclusion

In this study, nutrient-rich sediment and low-plant density both triggered an increase in individual plant size (total biomass) and a decrease in root:shoot ratio of *M. spicatum*. Auto-fragment production was highly

correlated with the individual plant size. Increased sediment nutrients significantly increased the auto-fragment biomass both in absolute value and relative to the plant size, which indicate a positive effect on auto-fragmentation. Plant density, however, affected the size dependency of auto-fragment biomass through the effects of size difference (an indirect negative effect). In addition, individual auto-fragment sizes were significantly influenced by sediment nutrients and plant density.

Similar to auto-fragment production, the amount of TNC storage in auto-fragments was also strongly correlated with individual plant size due to similar TNC-concentrations in auto-fragments among these treatments. Sediment nutrients increased the amount of TNC storage in auto-fragments; whereas plant density caused an indirect and negative effect on the amount of TNC storage in auto-fragments, which suggest a size-independent effect on the amount of TNC storage in auto-fragments.

In this article, we only investigated auto-fragmentation that occurred in still water. In the field, however, running water usually accelerates the auto-fragmentation process. Therefore, in order to elucidate the various interactions between plant size and environmental factors in auto-fragmentation, further analysis should be performed in the field.

**Acknowledgments** We thank Wen Xiong, Ligong Wang, Yuqin Han, Keyan Xiao and Manghui Tu for laboratory/field assistance and helpful discussion. We also greatly appreciate Dr. Sidinei M. Thomaz and two anonymous reviewers for valuable comments on an early version of the manuscript. This research was supported by the National Natural Science Foundation of China (30770363 and 30930011).

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