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Effects of water nutrients on regeneration capacity of submerged aquatic plant fragments

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Abstract – Aquatic plants play a substantial role in almost all freshwater habitats throughout the world. Even though submerged aquatic plants dominantly spread by the dispersal of vegetative plant fragments, most aquatic plant species show a broad distribution range. Here we studied the differences in the regeneration capacity and the regeneration type of fragments (by root and/or shoot growth) of eight submerged plant species (*Ceratophyllum demersum*, *Egeria najas*, *Elodea canadensis*, *Elodea nuttallii*, *Hydrilla verticillata*, *Myriophyllum aquaticum*, *Myriophyllum heterophyllum* and *Myriophyllum spicatum*) under different water nutrients in sediment-free conditions. Overall, *M. spicatum* showed the highest regeneration ($82 \pm 2\%$) in this study, followed by *C. demersum* ($73 \pm 2\%$) and *M. aquaticum* ($47 \pm 4\%$), whereas *M. heterophyllum* showed the lowest ($1 \pm 1\%$). The shoot fragments of *E. canadensis*, *H. verticillata*, *E. najas* and *E. nuttallii* regenerated by 40 ± 2, 23 ± 2, 16 ± 2 and $7 \pm 1\%$. The nitrate concentration affected the regeneration capacities of *E. najas* ($P = 0.05$), *M. spicatum* ($P = 0.013$) and *C. demersum* ($P = 0.001$), whereas phosphate had no significant effect. Additionally, the different nutrient concentrations had a significant effect on the portion of the regeneration types within *E. canadensis*, *E. nuttallii* and *H. verticillata*. Summarizing, submerged plants differ significantly in their regeneration capacity, and water nutrients have a potential effect on the regeneration of submerged plant fragments. This might influence the further colonization and spread of the species under field conditions.

Key words: Colonization / regeneration / dispersal / spread / submerged macrophytes

Introduction

Aquatic plants play a substantial role in almost all freshwater habitats throughout the world (Sculthorpe, 1967; Cook, 1985). Most aquatic plant species show a broad distribution range and can be found in different types of freshwaters (Cook, 1985; Santamaria, 2002). In contrast to terrestrial plants, aquatic plants dominantly spread by dispersal of vegetative plant fragments, and propagules significantly contribute to the maintenance of the population of a given species (Barrat-Segretain, 1996), while the dispersal by seeds play only a minor role for the maintenance of the species population (Barrat-Segretain, 1996). In general, seed production is rare for some species (*e.g.*, *Myriophyllum aquaticum* (Vell.) Verdc., Orchard, 1979), but even for species with a high number of produced seeds (*e.g.*, *Potamogeton* spec., Wiegleb and Brux, 1991), seed germination is rare in their native range. Several other species, such as non-native *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) St. John, did not produce any seeds in their introduced range in Europe (Cook and Urmi-König, 1985). But even though seed production is absent among invasive alien submerged species in Europe, these species became invasive in their alien range, for which highly effective vegetative dispersal was the key factor.

Aquatic plant fragments are produced in different ways. Stem fragments can be produced either through autofragmentation, a self-induced abscission of shoot fragments, which has been reported for *e.g.*, *Myriophyllum spicatum* L. (Xie and Yu, 2011), or by allofragmentation, when the production of shoot fragments is caused by disturbances such as increased flow velocity and sediment mobility during flood events, perturbations by water birds or fishes or human activities (Madsen and Smith, 1989; Riis *et al.*, 2009).

Once produced, the plant fragments float freely at the water surface. The fragments are transported by water drift, water fowls or human activities (such as boating), and are able to partly withstand desiccation (Barnes *et al.*, 2013). First root production of free-floating plant fragments has been observed for at least *M. spicatum* before

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the plant fragments get in contact with the sediment (Aiken *et al.*, 1979).

Despite the known fact, that most aquatic plants show high vegetative dispersal and regeneration by stem fragments, less is known about the influencing parameters for the regeneration of aquatic plant fragments.

Nutrients are a major factor for submerged plant growth in general (Sand-Jensen, 1989). Submerged plants use both shoots and roots for the nutrient uptake, but differences in the nutrient uptake capacities by roots and shoots between the species occur (Eugelink, 1998). Thus, it seems likely that nutrients affect the regeneration capacity of stem fragments of submerged plants. Here, we studied the general regeneration capacity, which means the capacity of fragments to grow new shoots and/or roots, of eight submerged plant species (*E. canadensis*, *E. nuttallii*, *Egeria najas* Planch., *Hydrilla verticillata* Royle, *Ceratophyllum demersum* L., *M. spicatum*, *Myriophyllum heterophyllum* Michx. and *M. aquaticum*), their form of regeneration, their regeneration time and the effect of different macronutrient concentrations when cultivated under sediment free conditions.

Material and methods

Plant material

Plant material was collected from different waters. *Elodea canadensis*, *E. nuttallii*, *M. heterophyllum*, *C. demersum*, *M. spicatum* and *M. aquaticum* were sampled in waters close to the University of Düsseldorf. *Egeria najas* was taken from a commercial plant trader and *H. verticillata* originated from the plant stock of the National Institute of Water and Atmospheric Research in Hamilton, New Zealand.

Prior to the experiment, the plants were cultivated for at least 4 weeks under controlled laboratory conditions. Plants grew separately in 30 L aquaria (35 × 28 × 30.5 cm) (two aquaria for each species) filled with a 5 cm thick layer of a nutrient-rich sediment and 1 cm of washed sand on top in artificial water for general aquatic plant purposes with a DIC of 0.85 mM according to Smart and Barko (1985). Water was filtered with a Trixie 200 (Fa. Trixie, Tarp, Germany) filter to avoid phytoplankton growth. Plants were exposed without self-shading to light intensities of $\sim$80 µmol photons $m^{-2}.s^{-1}$ in a 16/8 light/dark cycle at room temperature of 22 °C.

The sizes of plant fragments used were chosen due to data from the literature (Langeland and Sutton, 1980; Fritschler, 2008; Hussner, 2008) and previous experiments. Shoot fragments with five nodes for the four Hydrocharitaceae (*E. canadensis*, *E. nuttallii*, *E. najas* and *H. verticillata)* and shoot fragments with only one node for *M. spicatum*, *M. heterophyllum*, *M. aquaticum* and *C. demersum* were used for the experiments. Plant fragments were cut from the upper $\sim$35 cm of similar sized shoots of $\sim$ 50–60 m in length, excluding the three cm of the apical bud.

Fig. 1. The four regeneration types differentiated within this study: (a) by new roots with a length of > 1 cm, (b) by new roots with a length of > 1 cm and a shoot of < 1 cm, (c) by new shoots with a length of > 1 cm and a root of < 1 cm, and (d) by new shoots with a length of > 1 cm.

Experimental study

Experimental setup

We studied the effect of nitrate and phosphate concentrations on regeneration in a two-factorial design with three different levels of both nitrate and phosphate. The nutrients were added to the used Smart and Barko (1985) solution to a final concentration of 0.4, 2.0 and 8.0 mg L^{-1} NO_3-N, respectively, 0.02, 0.1 and 0.5 mg L^{-1} PO_4-P, resulting in nine different nutrient treatments. For each species and treatment, three 1.1 L glass beakers were filled with 600 mL media and 20 shoot fragments were put into each glass beaker. The glasses were exposed to room temperature ($\sim$23 °C) for a 16 h light period of $\sim$80 µmol photons $m^{-2}.s^{-1}$ at the top of the glass beakers. The media was changed twice a week to minimize changes in both nutrients and DIC content during the study time. At this time, the regenerated shoot fragments were counted. Regenerated shoots were removed from the glass beakers during the media change. The experiments lasted 32 days.

Regeneration types

During this experiment, we differentiated between four different types of regeneration (Fig. 1). The plants regenerated either by the production of new roots, new shoots or both. Thus, we differentiated between regeneration (type a) by new roots with a length of > 1 cm, (type b) by new roots with a length of > 1 cm and a shoot of < 1 cm, (type c) by new shoots with a length of > 1 cm and a root of < 1 cm, and (type d) by new shoots with a length of > 1 cm (Fig. 1). Every regenerated fragment was classified into one of these four groups. We did not find any fragments with beginning root or shoot production, which did not match the criteria of one of these groups within the study period. The regeneration time was defined as period of time from the initial of the experiment to the day, when the regenerated fragments match one of the four criteria.

Statistical analysis

The data sets of each species were analyzed using IBM SPSS 21.0. Data were tested for normality and homogeneity of variances to meet the assumptions of analysis of variance (ANOVA). Differences between the species and between and within the species treatments were analyzed with ANOVA. A three-way ANOVA followed by Tukey's honestly significant difference (HSD) test was conducted to analyze the effect of nitrate and phosphate on the regeneration capacity of each species.

Results

General observations

During the 35 days we did not find any evidence for mortality of the used plant fragments. Even plant fragments with beginning senescence (*e.g.*, chlorophyll degradation) regenerate. The regeneration of the fragments almost occurs within the first 3 weeks after the start of the experiment, when $>90\%$ of the documented regeneration occurs within the first 22 days in all species, except for *E. canadensis* and *E. nuttallii* (18 and 15 days, respectively) (Figs. 2(a) and (b)).

The species differed significantly in their overall regeneration capacities ($P < 0.0001$). Despite the fact, that the used fragments were much smaller for the three *Myriophyllum* species and *C. demersum* than for the Hydrocharitaceae (*E. canadensis*, *E. nuttallii*, *E. najas* and *H. verticillata*), *M. spicatum* showed the highest regeneration capacity ($82 \pm 2\%$) in this study, followed by *C. demersum* (73 ± 2) and *M. aquaticum* ($47 \pm 4\%$), while *M. heterophyllum* showed the lowest ($1 \pm 1\%$). The shoot fragments of *E. canadensis*, *H. verticillata*, *E. najas* and *E. nuttallii* regenerated by 40 ± 2, 23 ± 2, 16 ± 2 and $7 \pm 1\%$ (Table 1).

Effects of nutrients on regeneration capacity and regeneration type

Overall, the regeneration capacities within the nine treatments differed significantly within *C. demersum*, *M. spicatum* and *H. verticillata* (all $P < 0.05$) (Table 1). The nitrate concentration affected the regeneration capacities of *E. najas* ($P = 0.05$), *M. spicatum* ($P = 0.013$) and *C. demersum* ($P = 0.001$) (Table 1, Figs. 2(a) and (b)), but there was no effect on regeneration time. In *H. verticillata*, a tendency for increased regeneration was found under both increasing phosphate and nitrate concentrations, but which was not significant (Fig. 2(a)). Generally, we found no interaction of nitrate $\times$ phosphate on regeneration capacity of species.

The species differed significantly in their regeneration types. Within the four studied Hydrocharitaceae, the portion of regeneration by the concurrent growth of both new roots and shoots (regeneration types b and c in Fig. 1) was highest in *E. najas* (Table 1), whereas *E. canadensis*, *E. nuttallii* and *H. verticillata* showed significantly higher portion of regeneration by new shoot growth with or without the concurrent onset of root growth (types c and d, Fig. 1). In contrast to *M. heterophyllum*, which only regenerated by roots and roots with onset of shoot growth, *M. spicatum* and *M. aquaticum* mainly regenerated by new shoots. *Ceratophyllum demersum* regenerated only by the production of new shoots and its regeneration capacity increased with increasing nitrogen concentrations (Table 1, Fig. 2(b)).

The different nutrient concentrations had a significant effect on the portion of the regeneration types within the found regeneration in *E. canadensis*, *E. nuttallii* and *H. verticillata*. In *H. verticillata* ($P = 0.001$), the regeneration by shoots decreased with decreasing nitrate concentration. In *E. canadensis* ($P = 0.039$) and *E. nuttallii* ($P = 0.037$), the phosphate concentration affected the regeneration by roots and roots with beginning shoot growth, respectively (Table 1).

Discussion

The ability to regenerate from plant fragments is of major importance for both maintenance and dispersal of submerged aquatic plants (Barrat-Segretain, 1996). Here, we found differences in the general regeneration rates and regeneration type of eight submerged plants under sediment-free conditions. Even though the regeneration capacities here were lower than in some prior studies with sediment (Langeland and Sutton, 1980; Barrat-Segretain and Cellot, 2007), regeneration capacities of up to $>80\%$ were found. The lower regeneration capacities were possible at least partly caused by the exclusion of fragments with apical buds from our experiment, which are known for their high regenerative capacity (Riis *et al.*, 2009; Vari, 2013). As the present study was carried out during summer, it can be ruled out that seasonal effects played a role (Vari, 2013). The average regeneration time was 2–3 weeks in this study, which is similar to the rooting times reported for other submerged species (Vari, 2013). *Elodea canadensis* and *E. nuttallii* showed the shortest regeneration time, which might influence the establishment rate of produced plant fragments. Even though changes in DIC and pH were kept to a minimum due to the water changes twice a week, it cannot be ruled out that CO_2 depletion occurs within the treatments. But due to the fact that all species are able to use HCO_3^- as an additional carbon source (Bowes, 2011), the potential effect of CO_2 depletion in this study seems to be minor.

We documented that at least for *E. najas*, *M. spicatum* and *C. demersum* the regeneration capacities were influenced by nutrient availability. *Myriophyllum* species and *C. demersum* showed the highest regeneration capacities, even though the used fragments were much shorter than those of the Hydrocharitaceae, which are known for their increasing regeneration capacity with increasing fragment size (Langeland and Sutton, 1980). While *M. spicatum*

Table 1. Portion of the regeneration types within the regeneration found and total regeneration within the different treatments. Means ± SE are given.

		PO_4-P (mg L^{-1})	0.5	0.1	0.02	0.5	0.1	0.02	0.5	0.0	0.02	
	Regeneration type	NO_3-N (mg L^{-1})	8	8	8	2	2	2	0.4	0.4	0.4	Total
Myriophyllum spicatum	Root > 1 cm		6.3 ± 0.4	7.0 ± 4.1	9.0 ± 2.1	8.0 ± 4.1	13.8 ± 4.2	4.2 ± 2.1	5.3 ± 5.3	3.7 ± 3.7	1.8 ± 1.8	6.5 ± 1.1
	Root > 1 cm, shoot < 1 cm		2.2 ± 2.2	2.2 ± 2.2	4.4 ± 2.2	0 ± 0	0 ± 0	6.3 ± 3.6	5.3 ± 5.3	1.9 ± 1.9	3.6 ± 1.8	29.0 ± 0.8
	Shoot > 1 cm, root < 1 cm		10.0 ± 5.1	17.2 ± 9.2	25.4 ± 10.5	26.5 ± 3.9	17.5 ± 3.9	12.4 ± 3.7	13.5 ± 2.6	18.0 ± 6.3	21.8 ± 3.2	18.0 ± 2.0
	Shoot > 1 cm		81.5 ± 6.1	73.6 ± 6.1	61.1 ± 9.5	65.5 ± 1.2	68.7 ± 8.3	77.2 ± 8.5	76.0 ± 9.3	76.4 ± 6.6	72.8 ± 5.3	72.5 ± 5.3
	Total regeneration (%)		80.0 ± 5.0	80.0 ± 7.6	73.3 ± 1.7	86.7 ± 6.0	66.7 ± 10.1	81.7 ± 1.7	88.3 ± 4.4	91.7 ± 1.7	91.7 ± 1.7	82.2 ± 2.1
Myriophyllum heterophyllum	Root > 1 cm		0 ± 0	50.0 ± 40.8	0 ± 0	100.0 ± 0.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	75.0 ± 9.6
	Root > 1 cm, shoot < 1 cm		0 ± 0	50.0 ± 40.8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	25.0 ± 9.6
	Shoot > 1 cm, root < 1 cm		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Shoot > 1 cm		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Total regeneration [%]		3.3 ± 3.3	3.3 ± 1.7	0 ± 0	3.3 ± 1.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.1 ± 0.6
Myriophyllum aquaticum	Root > 1 cm		0 ± 0	0 ± 0	0 ± 0	2.0 ± 2.0	2.1 ± 2.1	0 ± 0	5.6 ± 5.6	0 ± 0	0 ± 0	1.1 ± 0.7
	Root > 1 cm, shoot < 1 cm		0 ± 0	0 ± 0	0 ± 0	4.7 ± 2.5	0 ± 0	0 ± 0	0 ± 0	3.3 ± 3.3	5.6 ± r5.6	1.5 ± 0.8
	Shoot > 1 cm, root < 1 cm		3.0 ± 3.0	5.6 ± 5.6	14.8 ± 7.4	29.3 ± 9.2	13.1 ± 7.2	24.9 ± 8.1	26.3 ± 7.0	28.0 ± 1.0	21.4 ± 14.9	18.5 ± 2.9
	Shoot > 1 cm		97.0 ± 3.0	94.4 ± 5.6	85.2 ± 7.4	64.0 ± 6.3	84.8 ± 9.0	75.1 ± 8.1	68.1 ± 5.2	68.7 ± 4.3	73.0 ± 11.5	78.9 ± 3.0
	Total regeneration (%)		21.7 ± 16.7	38.3 ± 13.0	55.0 ± 10.0	71.7 ± 7.3	50.0 ± 15.0	53.3 ± 10.9	33.3 ± 6.0	60.0 ± 7.7	38.3 ± 6.0	46.9 ± 4.1
Elodea canadensis	Root > 1 cm		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Root > 1 cm, shoot < 1 cm		0 ± 0	5.6 ± 5.6	22.2 ± 14.7	3.3 ± 3.3	0 ± 0	0 ± 0	6.1 ± 6.1	0 ± 0	27.3 ± 3.9	7.2 ± 2.5
	Shoot > 1 cm, root < 1 cm		59.1 ± 15.1	55.1 ± 16.5	57.6 ± 7.6	63.3 ± 18.6	74.1 ± 9.0	73.9 ± 3.9	74.2 ± 0.8	67.2 ± 4.3	67.1 ± 8.9	65.8 ± 3.4
	Shoot > 1 cm		40.9 ± 15.1	39.4 ± 13.5	20.2 ± 10.3	33.3 ± 16.7	25.9 ± 9.0	26.1 ± 3.9	19.7 ± 5.3	32.8 ± 4.3	5.6 ± 5.6	27.1 ± 3.5
	Total regeneration (%)		41.7 ± 9.3	40.0 ± 5.0	35.0 ± 10.4	34.4 ± 10.3	40.0 ± 2.9	40.0 ± 5.8	45.0 ± 5.0	26.7 ± 7.3	30.0 ± 2.9	37.0 ± 2.3
Elodea nuttallii	Root > 1 cm		50.0 ± 40.8	0 ± 0	0 ± 0	33.3 ± 19.2	33.3 ± 33.3	0 ± 0	55.6 ± 29.4	16.7 ± 16.7	66.7 ± 33.3	32.6 ± 8.3
	Root > 1 cm, shoot < 1 cm		0 ± 0	0 ± 0	0 ± 0	11.1 ± 11.1	0 ± 0	0 ± 0	0 ± 0	33.3 ± 33.3	0 ± 0	6.1 ± 4.3
	Shoot > 1 cm, root < 1 cm		0 ± 0	50.0 ± 40.8	50.0 ± 40.8	0 ± 0	66.7 ± 33.3	0 ± 0	0 ± 0	33.3 ± 16.7	33.3 ± 33.3	27.3 ± 8.3
	Shoot > 1 cm		50.0 ± 40.8	50.0 ± 40.8	50.0 ± 40.8	55.6 ± 22.2	0 ± 0	66.7 ± 33.3	11.1 ± 11.1	16.7 ± 16.7	0 ± 0	34.1 ± 8.1
	Total regeneration (%)		3.3 ± 1.7	3.3 ± 1.7	6.7 ± 4.4	15.0 ± 0.0	8.3 ± 1.7	3.3 ± 1.7	6.7 ± 4.4	8.3 ± 1.7	6.7 ± 1.7	6.9 ± 1.0
Hydrilla verticillata	Root > 1 cm		5.6 ± 5.6	11.1 ± 11.1	4.8 ± 4.8	0 ± 0	0 ± 0	0 ± 0	13.3 ± 13.3	0 ± 0	33.3 ± 33.3	7.6 ± 4.1
	Root > 1 cm, shoot < 1 cm		16.7 ± 16.7	0 ± 0	4.8 ± 4.8	6.7 ± 6.7	8.3 ± 8.3	0 ± 0	20.0 ± 11.5	0 ± 0	6.7 ± 6.7	7.0 ± 2.6
	Shoot > 1 cm, root < 1 cm		5.6 ± 5.6	25.0 ± 14.4	24.2 ± 5.5	13.3 ± 6.7	26.9 ± 16.1	16.7 ± 16.7	40.0 ± 23.1	83.3 ± 16.7	13.3 ± 13.3	27.6 ± 5.8
	Shoot > 1 cm		72.2 ± 11.1	63.9 ± 7.3	66.3 ± 5.2	80.0 ± 0.0	64.8 ± 10.2	83.3 ± 16.7	26.7 ± 6.7	16.7 ± 16.7	46.7 ± 29.1	57.9 ± 5.8
	Total regeneration (%)		21.9 ± 6.6	23.0 ± 3.5	26.2 ± 5.9	33.3 ± 8.3	28.0 ± 8.5	8.3 ± 1.7	25.0 ± 0.0	8.3 ± 1.7	11.7 ± 6.7	20.6 ± 2.3
Egeria najas	Root > 1 cm		22.2 ± 11.1	0 ± 0	8.3 ± 8.3	0 ± 0	11.1 ± 11.1	0 ± 0	16.7 ± 16.7	0 ± 0	0 ± 0	6.7 ± 2.8
	Root > 1 cm, shoot < 1 cm		46.7 ± 10.2	93.3 ± 6.7	72.5 ± 11.5	66.7 ± 33.3	88.9 ± 11.1	88.9 ± 11.1	83.3 ± 16.7	100.0 ± 0.0	100.0 ± 0.0	81.6 ± 5.2
	Shoot > 1 cm, root < 1 cm		20.0 ± 20.0	6.7 ± 6.7	19.2 ± 3.6	33.3 ± 33.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	9.1 ± 4.4
	Shoot > 1 cm		11.1 ± 11.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11.1 ± 11.1	0 ± 0	0 ± 0	0 ± 0	2.6 ± 1.7
	Total regeneration (%)		18.3 ± 3.3	18.3 ± 4.4	28.3 ± 6.0	11.7 ± 1.7	20.0 ± 5.0	13.3 ± 1.7	11.7 ± 1.7	10.0 ± 2.9	8.3 ± 4.4	15.6 ± 1.6
Ceratophyllum demersum	Root > 1 cm		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Root > 1 cm, shoot < 1 cm		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Shoot > 1 cm, root < 1 cm		0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Shoot > 1 cm		100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Total regeneration (%)		81.7 ± 3.3	85.0 ± 5.8	75.0 ± 2.9	83.3 ± 1.7	71.7 ± 1.7	76.7 ± 6.0	66.7 ± 1.7	53.3 ± 10.9	65.0 ± 7.6	73.1 ± 2.4

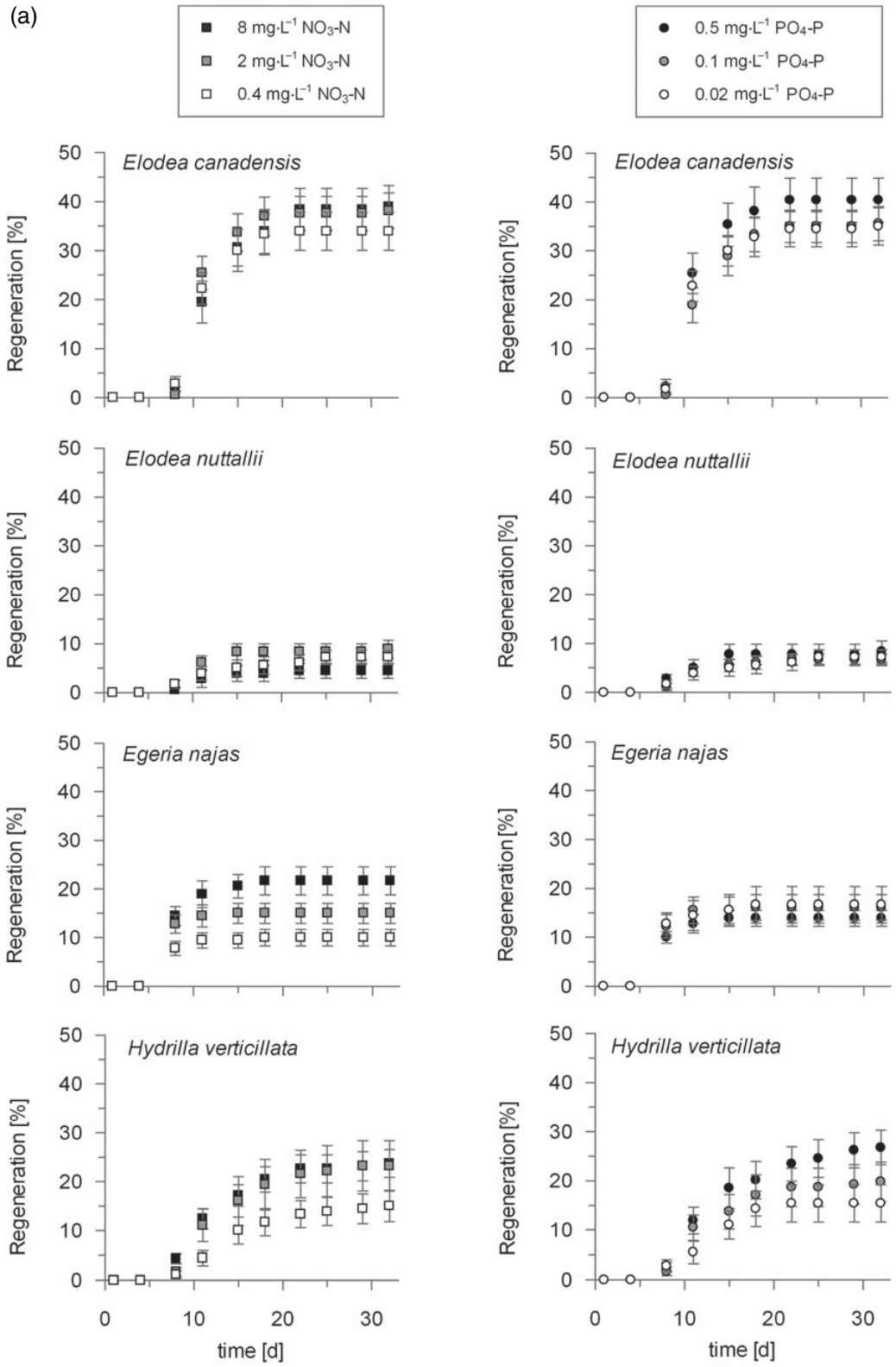
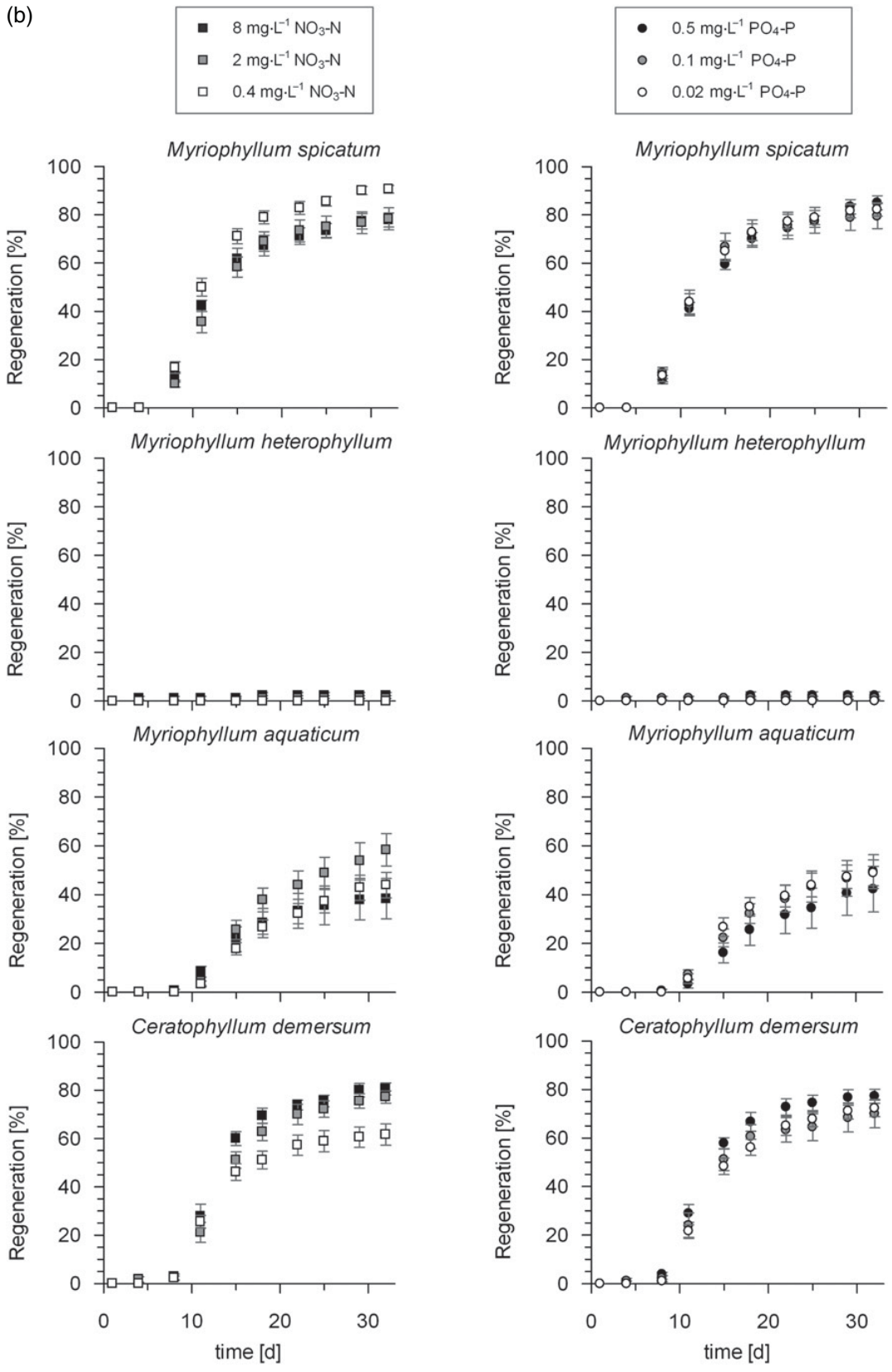


Fig. 2. (a) Effect of different nitrate and phosphate concentrations on the regeneration of *Elodea canadensis*, *Elodea nuttallii*, *Egeria najas* and *Hydrilla verticillata* with five nodes per plant fragment. (b) Effect of different nitrate and phosphate concentration on the regeneration of *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, *Myriophyllum aquaticum* and *Ceratophyllum demersum* with one node per plant fragment.


(b)
8 mg·L⁻¹ NO3-N
2 mg·L⁻¹ NO3-N
0.4 mg·L⁻¹ NO3-N
0.5 mg·L⁻¹ PO4-P
0.1 mg·L⁻¹ PO4-P
0.02 mg·L⁻¹ PO4-P
Myriophyllum spicatum
Myriophyllum heterophyllum
Myriophyllum aquaticum
Ceratophyllum demersum
Regeneration [%]
time [d]

(Fig. 2). (b) (*Contd*)

predominantly regenerated by the production of new shoots, the mostly free-floating *C. demersum* regenerated by new shoots only, while the production of roots seems unnecessary in contrast to the other studied species. The combination of the mostly free-floating growth form and the high regeneration capacity under high nutrients seems one of the key parameters for the weed potential of this species, particularly in eutrophic waters, in both its native and introduced range (Wells *et al.*, 1997; Hilt *et al.*, 2006).

Myriophyllum heterophyllum, which was documented to have a much higher regeneration capacity in a study with sediment (Hussner, 2008), regenerated mostly by roots. Thus, it seems likely that the species predominantly use their roots for nutrient uptake during regeneration, which might explain the strong differences between both studies.

Submerged *M. aquaticum* did not reach the high regeneration capacities of emerged plants (Hussner, 2009), which might be caused by the fact, that the emerged growth generally perform much better and is consequently the dominant growth form in the field (Hussner and Lösch, 2005; Eusebio *et al.*, 2013).

Generally, obligate submerged growing aquatic plant species can use both their root and shoot system for nutrient uptake (Carignan and Kalff, 1980; Eugelink, 1998), but here we found general differences in the regeneration type of the species, which regenerated either by roots, by shoots or the concurrent growth. The differences might be caused by general differences in the nutrient uptake of the studied species (Chambers *et al.*, 1989; Eugelink, 1998).

Regeneration from plant fragments is the first step in future colonization of submerged plants within a freshwater habitat. For successful colonization, the production of new roots is a key factor (Vari, 2013). Interestingly, in four of the tested species here (*M. spicatum*, *M. aquaticum*, *H. verticillata* and *C. demersum*), regeneration started mostly by the production of new shoots, whereas in *E. canadensis*, *E. nuttallii*, *E. najas* and *M. heterophyllum*, predominantly a concurrent growth of new shoots and roots was documented right from the beginning of plant regeneration. This might cause higher colonization respectively uprooting rates in the field, but Barrat-Segretain *et al.* (1998) and Barrat-Segretain and Bornette (2000) reported only a low percentage of colonization of regenerated fragments in *E. canadensis*. Fragments of other species were not found to root in the sediment (Barrat-Segretain and Bornette, 2000), but developed turions in autumn.

To summarize, the present study documented differences in the regeneration capacity and regeneration types within the species. At least for nitrate, a significant effect on the regeneration capacity of some submerged species was found, which might explain differences in the colonization time of freshwater habitats with different trophic levels by submerged plant species.

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