

Effect of density and neighbours on interactions between invasive plants of similar growth form

Marcio Jose Silveira, Gabrielle Thiébaut

▶ **To cite this version:**

Marcio Jose Silveira, Gabrielle Thiébaut. Effect of density and neighbours on interactions between invasive plants of similar growth form. Aquatic Ecology, 2020, 54 (2), pp.463-474. 10.1007/s10452-020-09753-1 . hal-02530842

HAL Id: hal-02530842

https://univ-rennes.hal.science/hal-02530842

Submitted on 12 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Effect of density and neighbours on interactions between invasive plants of similar growth form.

[1,2]Márcio José Silveira* and [2]Gabrielle Thiébaut

[1]State University of Maringá – UEM Research group in Limnology, Ictiology and Aquaculture – Nupelia Laboratory of Limnology and Aquatic Macrophytes

[2]Laboratoire Ecosystemes, Biodiversite´, Evolution (ECOBIO), UMR 6553 CNRS, University of Rennes 1, 35042 Rennes, France

*Author correspondence: E-mail address: s.marciojs@gmail.com ORCID: 0000-0002-6490-176X

Accepted Manuscript

Abstract

Invasive aquatic plants constitute a great problem in many freshwater systems. Although many studies have addressed the potential threats of invasion by exotic species in aquatic ecosystems, few studies have considered the interactions (competition, facilitation, coexistence) between exotic aquatic plants of similar growth form in invaded communities. Our aim was to investigate experimentally the effect of density in monocultures and the effect of neighbour identity and plant density on a focal species in mixed cultures. We used *Elodea canadensis*, *Egeria densa* and *Lagarosiphon major* as model species and conducted two experiments, one in a monoculture and another in a mixed culture following an additive design. Individuals were planted in pots with several treatments based on the identity and density of neighbour species. Our results demonstrated that the development of an invasive submerged plant could be influenced either positively (facilitation) or negatively (competition or inhibition) by the presence of more than one neighbour species of different densities. An increase in density significantly stimulated competition intensity, although the effect of density on the performance of the invader also depended on neighbour identity. A facilitative effect of *E. canadensis* on the apical growth of *E. densa* was established. However, despite these results, the competitive outcome also depended on other environmental factors (e.g. underwater radiation) and not on plant density and neighbour identity alone.

Keywords: non-native species, clonal plants, morphological traits, Hydrocharitaceae

Accepted Manuscript

Introduction

Invasive aquatic plants constitute a major problem in many freshwater systems (Dugdale et al. 2012), being able to affect both biodiversity and ecosystem functioning (Clayton and Edwards 2006). Although many studies have addressed the potential threats of invasion of aquatic ecosystems by exotic species (Spencer and Rejmanek 1989; Rahel and Olden 2008), few studies have considered the interactions (competition, facilitation, coexistence) between exotic aquatic plants with a similar growth form in invaded communities (Barrat-Segretain and Elger 2004; Mony et al. 2007; Silveira et al. 2018). There is some evidence that positive interactions (facilitation) can occur (Thomaz et al. 2012). Exotic species may facilitate the successful establishment of other exotic species by creating suitable environmental conditions (e.g. increasing nitrogen availability). For example, Santos et al. (2011) found that invasion by *Myriophyllum spicatum* facilitated the spread of *Egeria densa* in California.

Conversely, competition is widely regarded as one of the most important mechanisms of species invasion and has been shown to play a large role in macrophyte communities (Moen and Cohen 1989; Mc Creary 1991). This negative interaction is expected to occur mostly between species with a similar growth form and between closely related species, assuming that closely related species share similar niches and traits (Violle et al. 2011). However, only a few studies have focused on the competition between aquatic plant species with such characteristics (James et al. 1999; Barrat-Segretain and Elger 2004; Mony et al. 2007; Sousa et al. 2010; Gérard et al. 2014). The occurrence of invasive plant species in high densities may generate competition for resources (Ren and Zhang 2009), which can inhibit the growth of other plants (Wang et al. 2008). Thus, plant density manipulation is one of the main tools to study competition between plant species, as it can modify plant growth by influencing plant nutrient uptake (Creed et al. 1997; Xie et al. 2006).

Elodea canadensis Michaux, *Egeria densa* Planchon, and *Lagarosiphon major* Rid. Moss ex Wager are considered to be the most troublesome submersed species in the world. They belong to the Hydrocharitaceae family and have an elodeid life form. The two submerged species *E. densa* and *L. major* form dense monospecific mats in their introduced range, whereas *E. canadensis* can co-exist with native submerged plants. However, *E. densa* and *L. major* can co-occur in freshwater ecosystems, for example, they co-exist in Lake Lacanau, a shallow lake located in the southwest of France (Ribaudo et al. 2018). The three species are dioecious clonal plants that mainly disperse vegetatively in their natural habitats, and even small fragments of all three species can establish and develop into new

Accepted Manuscript

macrophyte beds (Howard-Williams 1994; Riis et al. 2012). Native from North America, only female plants of *E. canadensis* were introduced outside their native range (Cook and Urmi–König 1985). Similarly to *E. canadensis, E densa* and *L. major*, coming from South-America and South-Africa respectively, were introduced into France, from where they have become widely distributed in many European countries (James et al. 1999; Cook and Urmi-König 1984; McKee et al. 2002; Silveira at al. 2018). Despite their similarities, the distinctive nature of each genus is demonstrated by the fact that *Elodea* is unable to hybridize with *Egeria* (Cook and Urmi-Konig 1985). Les et al. (2006) showed that the genus *Lagarosiphon* is not closely related to the genera *Elodea* and *Egeria*, but they recognized a clade containing *Egeria, Elodea* and *Lagarosiphon* at the rank of subfamily. Our aim was to investigate experimentally the effect of density in monospecific cultures and the effect of neighbour identity and plant density on the growth of focal species in mixed cultures during the early growth stage of these invaders. We used *E. canadensis*, *E. densa* and *L. major* as model species to test the following hypotheses: i) the performance of the invaders is reduced at high density both in monocultures and in mixtures; and ii) the presence of plants with a similar growth form may increase competition in mixed cultures.

Materials and methods

Material sampling

More than 280 fragments of each species (*E. canadensis, E. densa* and *L. major*) were collected randomly in the spring from three different ponds located in Brittany in western France (Table 1). Ramets, hereafter called "Plants", were derived from a single clone either of *E. canadensis, E.densa* or *L. major*.

Water was sampled close to the macrophyte beds where the plants were collected. Samples were kept refrigerated during transport to the laboratory and chemical analyses were carried out within 24h after sampling. Plants were collected from sites with alkaline water and a mean temperature of 15.3°C, with low oxygen content (mean value O_2 = 31.5 %) and moderate conductivity (mean value conductivity = 308µS cm^{-1}). All species grew in eutrophic waters (see Supplementary Material S1). In the laboratory, the apical shoots of *E. canadensis* (*Ec*), *E. densa* (*Ed*) and *L. major* (*Lm*) were washed to remove invertebrates, algae and debris. Plants were acclimatized for one week at ambient temperature (close to 20°C) in tap water which was slightly basic with a moderate nutrient

Accepted Manuscript

concentration (mean annual values according to French Water Agency data: conductivity = 400 µS cm^{-1}; pH= 8.10; [NO_3^- N] = 1.44 mg L^{-1}; [NH_4^+ N] = 0.03 mg L^{-1}; [PO_4^{3-}P] = 0.05 mg L^{-1}).

Experimental design

Two experiments were conducted. In the first experiment, plants were cultivated as monocultures with two density levels. In the second experiment, the effects of both neighbour identity and density were tested in mixed cultures. The experiments were performed simultaneously.

Monoculture experiment

Each pot (L x W x H): 8cm x 8cm x 15cm was filled with 2 cm of substrate (loam) and 3cm of sand and tap water. Fertile fine-grained sediments, such as loam, contain high levels of organic matter with anaerobic conditions and are suitable for the growth of *L. major* (Martin and Coetzee 2014) and other macrophyte species. Individuals of each species were prepared by taking an apical shoot of 7 cm length, without buds or lateral stems. These ramets were planted 2 cm apart in the centre of each pot. The distance between ramets was the same for high (4 individuals) and low (2 individuals) densities. Pots were randomly distributed in the growth chamber (photoperiod: 12 h of light/12 h of darkness; light intensity 50% corresponding to 300-500 µmol m^{-2} s^{-1}) and at a temperature of 19°C. Light intensity and temperature were chosen according to the growth optimum of the three invasive species in spring (Riis et al. 2012; Silveira and Thiébaut 2017).

After four weeks, four morphological traits were measured: length of the stem, leaf area, number of lateral shoots and number of roots. To measure leaf area, the surface of one leaf taken at 3 cm from each plant apex was scanned and thereafter measured using Scion Image software. Relative Growth Rate (RGR) and Leaf Area Growth Rate (LAGR) of the macrophytes were calculated using the following equation: RGR = (ln *L2*-ln *L1*)/(*T2*-*T1*), where *L1* and *L2* refer to plant length at times T1 and T2 (Barrat-Segretain and Elger 2004). LAGR was calculated using the same equation, where L1 and L2 refer to leaf area surface. LAGR was considered as a proxy of plant Photosynthetic ability (Weraduwage et al. 2015). This experiment also served as the control for calculating Relative Neighbour Effect (RNE) in the mixed culture experiment.

Mixed culture experiment

The same experimental design was applied to the mixed culture experiment. The effects of the experimental conditions were tested by combining different ratios of *E. densa*, *E. canadensis* and *L. major* individuals for a total density of two or four individuals per pot. Individuals were planted 2 cm

Accepted Manuscript

apart in the centre of each pot. The distance between ramets was the same for high and low densities. Experimental conditions consisted of planting individuals of *E. canadensis, E. densa* and *L. major* to give two plant densities (2 or 4 individuals) and using different species ratios (i.e. Mid-ratio = 1/1 or 2/2; High-ratio = 3/1 and Low-ratio = 1/3). The first number is the focal species and the second number the neighbour. The treatments consisted of pots (containing two or four individuals), with the following ratios: Mid-ratio pots: pots including two individuals, one focal species and one neighbour species - 1/1, or pots including four individuals, two focal species and two neighbour species - 2/2 .These treatments are shown in Figures 2 and 3 as: Mid-ratio (1) = 1/1; and Mid-ratio (2) = 2/2. High-ratio pots: including four individuals with a ratio of three individuals of the focal species to one neighbour species - 3/1. Low-ratio pots: including four individuals with a ratio of one individual of the focal species to three neighbour species - 1/3. The combinations of focal species and neighbours were: focal *E. canadensis* with *L. major* and *E. densa* as neighbour species; focal *E. densa* with *L. major* and *E. canadensis* as neighbour species; and focal species *L. major* with *E. densa* and *E. canadensis* as neighbour species. Each treatment had five replicates. The pots were placed randomly in the growth chamber under the same conditions as the monoculture experiment.

At the end of the experiment, the same four morphological traits were measured: Relative Growth Rate (RGR), Leaf Area Growth Rate (LAGR), number of lateral shoots and number of roots. We also measured competition intensity. The relative competitive index (RCI) which compares the performance of a target plant grown in monoculture or mixed with neighbours, is one of the most widely used indices to quantify competition between plant species (Wilson 2007), whereas the Relative Neighbour Effect (RNE, Markham and Chanway 1996) is a a calculation of the neighbour effect relative to the plant with the greatest performance. The RNE is an improvement of the RCI index as it compares the performance of plants growing in monoculture and in the presence of neighbour species (Weigelt and Jolliffe 2003). The RNE index is symmetric around zero and constrained between +1 (competition) and –1 (facilitation), so it can be used to estimate facilitation interactions. We calculated the RNE index for each trait as follows:

$$RNE = (P_{control} - P_{mixture})/x$$

where $P_{control}$ is the trait of the target species planted in monoculture (= control) and $P_{mixture}$ is the trait of the same target species in the presence of a neighbour species. The term x depends on which value of P is greater. If $P_{control} > P_{mixture}$, then $x = P_{control}$; however, if $P_{mixture} > P_{control}$, then $x = P_{mixture}$. The

Accepted Manuscript

traits used were: RGR, number of shoots, number of roots and leaf area of each individual planted in monoculture or mixed culture.

Statistical analyses

To test for the effects of density and species identity on the morphological traits of the plant species in the monoculture experiment, we performed a two-way ANOVA. We applied Tukey's HSD test to test for pairwise differences between treatments. In the mixed culture experiment, to test the effect of neighbour identity, plant density and its interactions on the RNE of each trait and on each morphological trait, we used a two-way ANOVA. Permutation tests avoid the assumption of normality (Anderson 2001). For this reason, the two-way ANOVA of both treatments (e.g. monoculture and mixed culture) was performed by permutation ($\alpha = 0.05$; 9999 permutations). These analyses were done using software R Development Core Team (R Core Team 2016). The effects of neighbour identity, plant density and their interactions on the RNE of each trait and on each morphological trait were tested using a "post hoc" Tukey test. These analyses and graphics were performed using Statistica TM 10.0.

Results

Effect of plant density in monoculture

We found greater RGR and a higher number of shoots in *E. canadensis* than in *E. densa* and *L. major* (Table 1; Fig. 1 a and c). The LAGR did not differ between species (Table 1, Fig.1b). Our test indicated a significant difference in the production of roots among species: *E. densa* produced a significantly lower number of roots compared to the other species at both low and high densities (Table 1; Fig. 1 d). The LAGR was lower for *L. major* at high density and for *E. canadensis* at low density (Table 1, Fig.1b).

Accepted Manuscript

Table 1. Results of a two–way ANOVA for each morphological trait of monoculture treatments of *E. canadensis, E. densa* and *L. major* at two plant densities (low= 2:0 and high=4:0). Significant results are in bold type.

Morphological traits	*df*	*F*	*p*
Relative growth rate (cm d^{-1})			
Plant density	1	2.06	0.163
Species	2	67.58	**0.000**
Plant density X Species	2	2.10	1.44
Number of lateral shoots			
Plant density	1	0.62	0.437
Species	2	99.08	**0.000**
Plant densities X Species	2	2.36	1.15
Number of roots			
Plant density	1	7.63	**0.010**
Species	2	49.70	**0.000**
Plant density X Species	2	1.05	0.364
Leaf area growth rate (cm^2 d^{-1})			
Plant density	1	0.19	0.662
Species	2	0.40	0.673
Plant density X Species	2	3.57	**0.043**

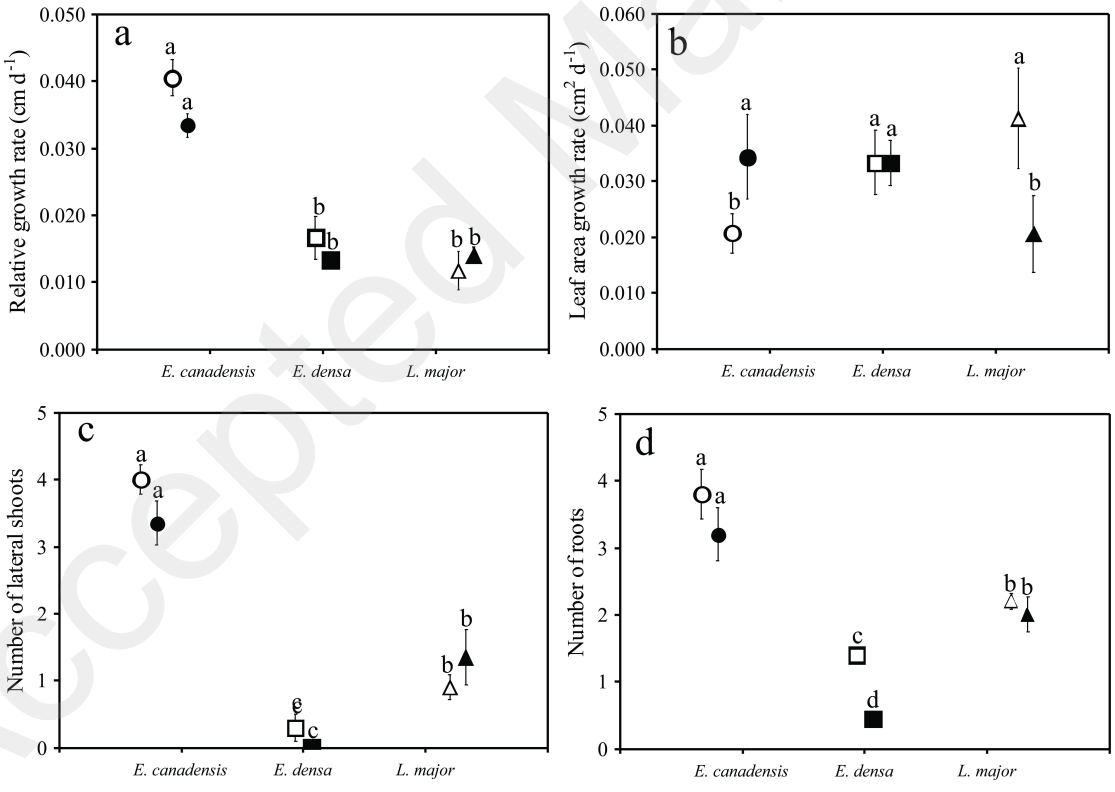


Figure 1: Means and standard errors of each morphological trait (Relative growth rate = RGR, lateral shoot number, root number and Leaf area growth rate = LAGR) of *E. canadensis, E. densa* and *L. major* in monocultures at two plant densities. Empty symbols = low density; Black symbols = high density. Circle: *E. canadensis*, Square: *E. densa*, Triangle: *L. major*. Different small letters above columns are used to indicate a significant difference between species and densities.

Accepted Manuscript

Effect of density and neighbour identity on RNE and morphological traits

The calculation of the RNE index for each morphological trait indicated that there was competition or facilitation between the three species depending on the different treatments (Fig. 2.A-M). However, at Mid-ratio (1), length of *E. densa* was slightly facilitated by the presence of *E. canadensis* (Fig. 2.B - treatment Mid-ratio (1)). Strong competition was established at high density for the production of lateral shoots of *E. canadensis* when it was outnumbered by *L. major* (Table 2, Fig. 2.D - treatment Low-ratio). Higher competition intensities were shown for the formation of the number of roots of *E. canadensis* when the neighbour species was mainly *L. major* and *E. densa* (Fig. 3.G - treatment Mid-ratio (2), Low-ratio and High-ratio), however root formation was stimulated in the presence *L. major* in the Mid-ratio (1) treatment. Leaf area growth of all species, RGR of *E. canadensis* and *L. major*, and the production of lateral shoots by *E. densa* and *L. major* were not impacted by the interactions between neighbour identity and plant density (Table 2; Fig. 2).

Table 2: Results of a two–way ANOVA on the Relative Neighbour Effect (RNE) index calculated for each morphological traits of *E. canadensis, E. densa* and *L. major*. Significant results are in bold type.

	E. canadensis			*E. densa*			*L. major*		
Morphological traits	***df***	***F***	***p***	***df***	***F***	***p***	***df***	***F***	***p***
RNE of length									
Plant density	3	1.87	0.164	3	4.35	**0.050**	3	0.86	0.469
Neighbour species	1	3.62	**0.051**	1	0.85	0.361	1	0.19	0.660
Plant density X Neighbour species	3	0.24	0.865	3	5.51	**0.003**	3	1.09	0.365
RNE of lateral shoots									
Plant density	3	8.76	**0.000**	2	0.46	0.711	3	0.95	0.424
Neighbour species	1	0.06	0.806	2	1.01	0.320	1	0.005	0.941
Plant density X Neighbour species	3	3.11	**0.039**	4	0.56	0.639	3	1.58	0.211
RNE of roots									
Plant density	3	8.93	**0.000**	3	2.68	**0.051**	3	5.88	**0.002**
Neighbour species	1	1.31	0.260	1	0.001	1.000	1	0.004	0.983
Plant density X Neighbour species	3	2.13	0.114	3	1.16	0.337	3	0.56	0.640
RNE of Leaf area									
Plant density	3	1.02	0.392	3	0.56	0.644	3	0.65	0.577
Neighbour species	1	0.05	0.810	1	0.67	0.417	1	0.001	1.000
Plant density X Neighbour species	3	0.39	0.758	3	0.43	0.730	3	0.51	0.687

Accepted Manuscript

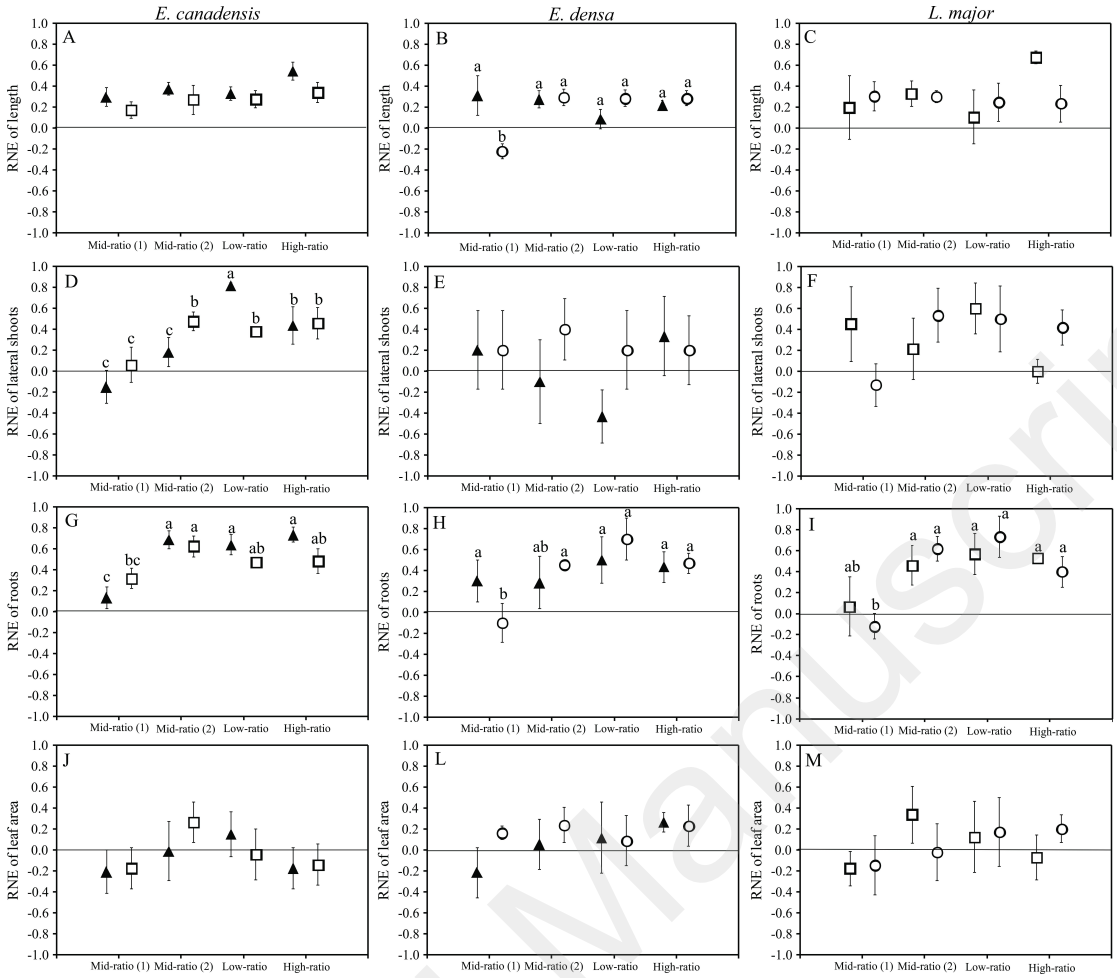


Figure 2: Means and standard errors of the RNE of each morphological trait (Relative Growth Rate = RGR), lateral shoot number, root number and leaf area growth rate = LAGR) of *E. canadensis, E. densa and L. major* at two plant densities and different ratios (Mid-ratio (1) = 1/1; Mid-ratio (2) = 2/2; Low-ratio =1/3 and High-ratio = 3/1). The first number is the focal species and the second number the neighour. Empty Circle: *E. canadensis*, Empty Square: *E. densa*, Black Triangle: *L. major*. Different small letters above columns are used to indicate a significant difference between species and densities.

Our results showed no effect of plant density and neighbour species on the RGR of *L. major* (Table 3, Fig. 3C), on the number of lateral shoots of *E. densa* and *L. major* (Table 3, Fig. 3, E, F) or on the LAGR of *E. canadensis, E. densa* and *L. major* (Table 3, Fig. 3 J, L, M).

However, they demonstrated significant effects of both plant density and neighbour species on the RGR and the number of lateral shoots of *E. canadensis* (Table 3, Fig. 3A, D). *E. canadensis* showed a lower RGR in the presence of *L. major* than in the presence of *E. densa* in the High-ratio treatment, whereas the presence of *E. densa* induced a reduction of the production of lateral shoots in the Mid-ratio (2) and Low-ratio (Fig. 3A, D) treatments. *E. canadensis* exhibited lower root production in the presence of *L. major* at high density (Fig. 3G - treatment High-ratio). The RGR of *E. densa*

Accepted Manuscript

depended on plant density and neighbour identity (Table 3, Fig. 3B). *E. canadensis* stimulated the RGR of *E. densa* (Treatment Mid-ratio (1), Fig. 3B). *L. major* had high root production whether the neighbour was *E. canadensis* or *E. densa* in the Mid-ratio (1) treatment (Fig. 3I).

Table 3: Results of a two–way ANOVA for each morphological trait of *E. canadensis, E. densa* and *L. major* in mixed cultures. Significant results are in bold type.

	E. canadensis			*E. densa*			*L. major*		
Morphological traits	***df***	***F***	***p***	***df***	***F***	***p***	***df***	***F***	***p***
Relative growth rate (cm d^{-1})									
Plant density	3	3.66	**0.022**	3	3.36	**0.030**	3	0.79	0.507
Neighbour species	1	6.78	**0.013**	1	1.40	0.244	1	0.00	0.976
Plant density X Neighbour species	3	0.46	0.706	3	6.77	**0.001**	3	1.39	0.263
Number of lateral shoots									
Plant density	3	9.92	**0.000**	3	0.37	0.768	3	1.54	0.222
Neighbour species	1	11.77	**0.001**	1	0.06	0.803	1	0.10	0.744
Plant density X Neighbour species	3	1.98	0.135	3	0.44	0.724	3	0.75	0.524
Number of roots									
Plant density	3	10.48	**0.000**	3	2.92	**0.048**	3	6.57	**0.001**
Neighbour species	1	1.23	0.274	1	0.000	1.000	1	0.07	0.783
Plant density X Neighbour species	3	3.77	**0.019**	3	1.36	0.271	3	0.43	0.729
Leaf area growth rate (cm^2 d^{-1})									
Plant density	3	0.58	0.624	3	1.55	0.218	3	1.40	0.259
Neighbour species	1	0.54	0.464	1	1.30	0.262	1	0.00	0.954
Plant density X Neighbour species	3	1.31	0.285	3	1.62	0.202	3	0.40	0.753

Accepted Manuscript

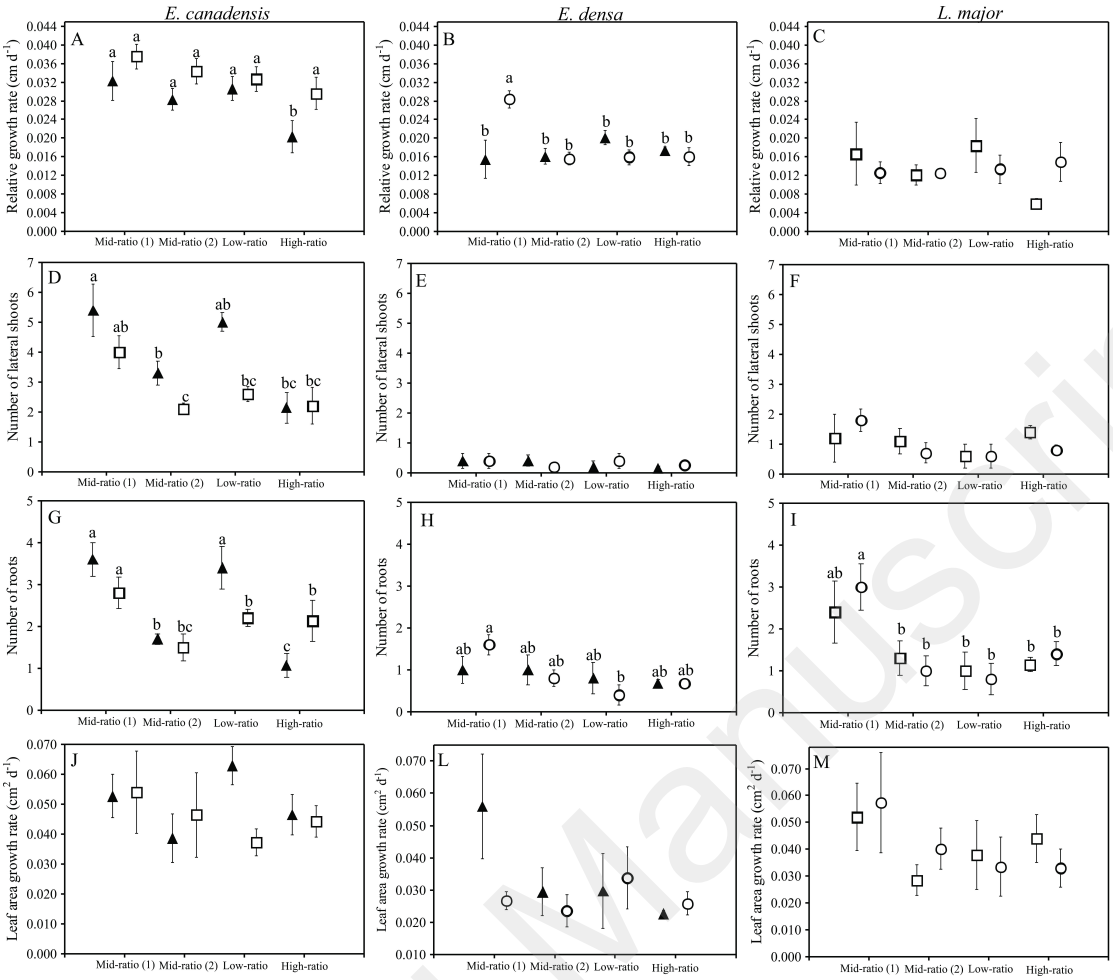


Figure 3: Means and standard errors of each morphological trait (relative Growth Rate = RGR), lateral shoot number, root number and leaf area growth rate = LAGR) of *E. canadensis, E. densa and L. major* at two plant densities and different ratios (Mid-ratio (1) = 1/1; Mid-ratio (2) = 2/2; Low-ratio =1/3 and High-ratio = 3/1). The first number is the focal species and the second number the neighour. Empty Circle: *E. canadensis*, Empty Square: *E. densa*, Black Triangle: *L. major*. Different small letters above columns are used to indicate a significant difference between species and densities.

Discussion

Effect of density on morphological traits of invaders in monoculture and in mixtures.

Our results demonstrated that the performance of invasive submerged plants was density-dependent and that it could be influenced positively (facilitation) or negatively (competition) by the identity of neighbour species. Indeed, in high density, the LAGR of *L. major* and the root number of *E. densa* were reduced. A high density increased competition for resources by limiting light interception and nutrient uptake, and consequently reduced leaf growth and root production. However,

Accepted Manuscript

leaf area growth of *E. canadensis* was stimulated at high density in monoculture, suggesting an investment in photosynthetic capacity as a strategy to overcompensate for stress. No intraspecific competition for initial growth (apical and lateral growth) was seen in monocultures independently of treatments with low or high densities. The latter results were surprising, because two individuals of the same species have exactly the same ecological requirements, and competition may occur rapidly when one factor is limited in the environment (Barrat-Segretain and Elger 2004). The short period of the experiment may not have been long enough to induce nutrient stress and this could explain the weak competitiveness between individuals, as longer experiments have shown strong intraspecific competition effects and niche differentiation (Barrat-Segretain and Elger 2004; Stiers et al. 2011). However, other studies have reported equivalent or stronger interspecific effects (Aguiar et al. 2001; Mony et al. 2007; Spencer and Rejmánek 2010, Yu et al. 2018). For example, Yu et al. (2018) found higher interspecific competition than intraspecific competition for *E. densa*. Our results also showed an interspecific competition for root production between the three invaders. Moreover, an increase in density significantly stimulated competition intensity, although the effect of density on the performance of the invader also depended on neighbour identity. Our results in mixed culture were broadly similar to the findings in monoculture, demonstrating that a high density had a strong impact on the interspecific competition for all three species. From this, we can infer that the results obtained in monoculture and mixed cultures are probably due to the fact that the plants are morphologically similar, that the mechanisms driving intraspecific and interspecific competition are very similar for these submerged plants and that the performance of invaders was density-dependent. Thus, our results partially corroborate our first hypothesis for *E. canadensis*, *E. densa* and *L. major*.

The presence of plants with similar growth form may increase competition in mixed cultures.

Although the morphological development of *E. canadensis* was mainly influenced by plant density, the numbers of lateral shoots (= lateral growth) and roots of this species were slightly related to the identity of the neighbour species. The lowest apical growth and root and lateral shoot production of *E. canadensis* when this species grew with *L. major* could be consequences of competition for nutrient uptake and space between *E. canadensis* and *L. major*. It is well known that the presence of plants with similar growth form and architecture can negatively affect nutrient concentrations in the sediment and solar radiation available for the plant community which may increase competition, thus

Accepted Manuscript

inhibiting plant growth. Other authors have demonstrated similar results, for example, Riis et al. (2012) also showed that *L. major* and *E. canadensis* differ in their branching and stem length under similar experimental conditions (i.e. at temperatures close to 20°C and 50% light). Under these conditions, *E. canadensis* is the most competitive species regarding morphological characteristics at 20 °C especially due to its high degree of branching (Riis et al. 2012), on the other hand, *L. major* has a highest stem length. Our results showed low competition intensity for branching and root production at high intensities. Surprisingly, the response of *E. canadensis* differed in the presence of *E. densa* and *L. major*. Furthermore, a facilitative effect of *E. canadensis* on the RGR (apical growth) of *E. densa* was established. The presence of *E. canadensis* stimulated the RGR of *E. densa* (RGR in the presence of Ec = 0.028 ± 0.004 cm per day, in presence of Lm = 0.015 ± 0.009 cm per day). Conversely, the presence of *E. densa* did not influence the RGR of *E. canadensis*. Thus, interactions between *E. canadensis* and *E. densa* were distinctly asymmetrical. Plant density did not influence *L. major* traits, except for root production in mixtures, indicating that species coexistence is favoured instead of competitive exclusion (Stiers et al. 2011). Thus our hypothesis, that the presence of plants with similar growth form may increase competition in mixed cultures was partially validated.

Neighbour identity had no impact on *L. major*. The RGR of *L. major* at high density was on average 0.014 cm per day in the presence of *E. canadensis,* whereas the RGR of *E. canadensis* was around 0.030 cm per day at high density in mixtures. Moreover, the leaf area growth of *L. major*, a proxy for photosynthetic ability, was not affected by density or neighbour identity. The apical growth ability of *E. canadensis* may enable it to shade out neighbouring species and outpace the establishment of other species such as *L. major*. These results are not consistent with the literature. For example *L. major* may be successful in out-competing *Elodea* spp. as a result of its ability to photosynthesize and consequently grow, particularly under very stressful conditions of high pH and low free CO_2, perhaps through more efficient bicarbonate utilization than the other species (James et al. 1999). Competition intensity for root production of *L. major* increased slightly with density, whatever the neighbour species. Therefore the outcome of competition between species of the same family, having similar architecture and competition ability, is not the same. For example, Mony et al. (2007) showed that *H. verticillata* may out-compete *E. densa* in many situations and Hofstra et al. (1999) established that the growth of *H. verticillata* appeared to be impeded by the dense *E. densa* canopy and that under particular environmental conditions *E. canadensis* may still coexist with *H. verticillata* or even

Accepted Manuscript

dominate some habitats. Our results are original because they also suggested that the interactions between invaders depended on the identity of the neighbour in mixed cultures.

Our experimental study indicated that *E. canadensis* was characterized by the highest RGR and number of roots and lateral shoots in both treatments (monoculture and mixed culture), suggesting that *E. canadensis* is a stronger competitor than *E. densa* and *L. major* (Riis et al. 2012; Silveira and Thiébaut 2017). Furthermore, Redekop et al (2016) demonstrated that *E. canadensis* shows a higher dispersal capacity via fragmentation than *E. densa* and *L. major*. This capacity increases its potential invasiveness. However, the highest dispersal ability of *E. canadensis* does not explain its low abundance in the field, where it has been shown that *E. canadensis* was displaced by *Elodea nuttallii* and *E. nuttallii* by *L. major* (James et al. 2006). Furthermore, *E. canadensis* was not considered as an aggressive species in Polish lakes (Kolada and Kutyla 2016). Similarly, a clear difference was obtained between experimental conditions and field observations in the study of Hofstra et al. (1999). These authors established that *H. verticillata*, a world-wide invasive species belonging to the Hydrocharitaceae family, had little apparent impact on the competitor species biomass (*E. densa, L. major, E. canadensis*), whereas *H. verticillata* out-competed these species in Lakes in New Zealand.

This study experimentally demonstrated that despite the fact that the three invasive species were morphologically similar, the positive (facilitation) or negative (competition) effects of density on performance of each species depend on the identity of the neighbour species and on the degree of density. This finding implies that the presence of one invasive species (focal species) can be inhibited or facilitated by the introduction of other exotic species (neighbour species). Our study demonstrated that the species least affected by neighbour species was the invasive *L. major*, whereas *E. canadensis* and *E. densa*, were affected by the presence of other neighbour species. These results suggest that *L. major* can be considered as the most aggressive species.

However, we emphasize that these observations were recorded in the early stages of fragment regeneration, and whether they also pertain to later life stages is a matter for further investigation.

Acknowledgements

The authors thank Dra. Vanessa C. Harthman (Universidade Estadual de Maringá) for fruitful discussion, Msc. Dieson André by help with quality of the figures and the two anonymous reviewers for suggestions that have improved our manuscript a lot. We also thank Bertrand Coupé and Marc Hervé for their help in the field and Nathalie Josselin-Le Bris for assistance with water analyses. M.J.

Accepted Manuscript

Silveira is grateful to the National Council for Scientific and Technological Development (CNPq), for providing a Post Doctorate scholarship and Rennes Metropole.

References

Anderson, MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci., 58: 626-639.

Aguiar MR, Lauenroth WK, Peters DP (2001) Intensity of intra- and interspecific competition in coexisting shortgrass species. J Ecol 89: 40–44.

Barrat-segretain MH, Elger A (2004) Experiments on growth interactions between two invasive macrophytes species. J Veg Sci 15: 109–114.

Clayton J, Edwards T (2006) Aquatic plants as environmental indicators of ecological condition in New Zealand lakes. Hydrobiologia 570:147-151.

Cook CDK, Urmi-könig K (1984) A revision of the genus *Egeria* (Hydrocharitaceae). Aquat Bot 19: 73-96.

Cook CDK, Urmi-könig K (1985) A revision of the genus Elodea (Hydrocharitaceae). Aquat Bot 21: 111–156.

Creed JC, Norton TA, Kain JM (1997) Intraspecific competition in *Fucus serratus* germlings: the interaction of light, nutrient and density. J Exp Mar Biol Ecol 212: 211–223.

Dugdale TM, Clements D, Hunt TD, Butler KL (2012) Survival of a submerged aquatic weed (*Egeria densa*) during lake drawdown within mounds of stranded vegetation. Lake Reser Manag 28:153-157.

Gérard J, Natacha B, Ludwig T (2014) Effect of water column phosphorus reduction on competitive outcome and traits of *Ludwigia grandiflora* and *L. peploides*, invasive species in Europe. Aquat Inv 9: 157–166.

Hofstra DE, Clayton J, Green JD, Auger M (1999) Competitive performance of *Hydrilla verticillata* in New Zealand. Aquat Bot 63: 305–324.

Howard-Williams C, Clayton JS, Coffey BT, Johnstone I M (1987) Macrophyte invasions In: VINER AB Ed. Inland Waters of New Zealand DSIR Bulletin 241. Department of Scientific and Industrial Research Science Information Publishing Centre, Wellington, New Zealand, pp. 307–331.

Markham JH, Chanway CP (1996) Measuring plant neighbour effects. Fun Ecol 10: 548–549

Accepted Manuscript

James CS, Eaton JW, Hardwick K (1999). Competition between three submerged macrophytes, *Elodea canadensis* Michx, *Elodea nuttallii* (Planch) St John and *Lagarosiphon major* (Ridl) Moss. Hydrobiologia 415: 35–40.

James CS, Eaton JW, Hardwick K (2006) Responses of three invasive aquatic macrophytes to nutrient enrichment do not explain their observed field displacements. Aquat Bot 84: 347–353.

Kolada A, Kutyta S (2016) *Elodea canadensis* (Michx.) in Polish lakes: a non-aggressive addition to native flora. Biol Inv 18: 3251-3264.

Les DH, Moody ML, Soros CL (2006) A reappraisal of phylogenetic relationships in the monocotyledon family Hydrocharitaceae (Alismatidae). Aliso 22: 211-230.

Martin GD, Coetzee, JA (2014) Competition between two aquatic macrophytes, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and *Myriophyllum spicatum* Linnaeus (Haloragaceae) as influenced by substrate sediment and nutrients. Aquat Bot 114: 1-11.

McCreary JN (1991) Competition as a mechanism of submersed macrophyte community structure. Aquat Bot 4:177-193

Mckee D, Hatton K, Eaton JW, Moss B (2002). Effects of simulated climate warming on macrophytes in freshwater microcosm communities. Aquat Bot 74: 71–83

Mony C, Koschnick TJ, Haller WT, Muller S (2007) Competition between two invasive Hydrocharitaceae (*Hydrilla verticillata* (L.f.) Royle and *Egeria densa* Planch) as influenced by sediment fertility and season. Aquat Bot 86: 236–242

Moen RA, Cohen Y (1989) Growth and competition between *Potamogeton pectinatus* L. and *Myriophyllum exalbescens* Fern. in experimental ecosystems. Aquat Bot 33: 257-270

Redekop P, Hofstra D, Husser A (2016) *Elodea canadensi*s shows a higher dispersal capacity via fragmentation than *Egeria densa* and *Lagarasiphon major*. Aquat Bot 130: 45-49.

Ren MX, Zhang QG (2009) The relative generality of plant invasion mechanisms and predicting future invasive plants. W Res 49: 449-460.

Ribaudo C, Tison-Rosebery J, Buquet D, Jan G, Jamoneau A, Abril G, Anschutz P and Bertrin V (2018) Invasive Aquatic Plants as Ecosystem Engineers in an Oligo-Mesotrophic Shallow Lake. Front Plant Sci 9:1781

Accepted Manuscript

Riis T, Olesen B, Clayton JS, Lambertini C, Brix H, Sorrell B (2012) Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. Aquat Bot 102: 56–60.

Santos MJ, Anderson LW, Ustin SL (2011) Effects of invasive species on plant communities: an example using submersed aquatic plants at the regional scale. Biol Inv 13: 443–457.

Silveira MJ, Thiébaut G (2017) Impact of climate warming on plant growth varied according to the season. Limnologica 65: 4-9

Silveira MJ, Chollet S, Thiébaut G, Thomaz SM (2018) Abiotic factors, not herbivorous pressure, are primarily responsible for the performance of an invasive aquatic plant. Ann. Limnol. - Int J Lim 54: 12

Sousa WTZ, Thomaz SM, Murphy KJ (2010) Response of native *Egeria najas* Planch. and invasive *Hydrilla verticillata* (L.f.) Royle to altered hydroecological regime in a subtropical river. Aquat Bot 92: 40–48.

Spencer DF, Rejmanek M (2010). Competition between two submersed aquatic macrophytes, *Potamogeton pectinatus* and *Potamogeton gramineus*, across a light gradient. Aquat Bot 92: 239–244.

Stiers I, Njambuya J, Triest L (2011) Competitive abilities of invasive *Lagarasiphon majo*r and native *Ceratophyllum dermersum* in monocultures and mixed cultures in relation to experimental sediment dredging. Aquat Bot 95: 161-166.

Thomaz SM, Agostinho AA, Gomes LC, Silveira MJ, Rejmánek M, Aslan CE, Chow E (2012) Using space-for-time substitution and time sequence approaches in invasion ecology. Fresh Biol 13: 2401–2410.

Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. Ecol Lett 14:782–787.

Wang JW, Yu D, Xiong W, Han YQ (2008) Above-and belowground competition between two submersed macrophytes. Hydrobiologia 607: 113-122.

Weigelt A, Jolliffe P (2003) Indices of plant competition. J Ecol 91: 707– 720.

Accepted Manuscript

Weraduwage SM, Chen J, Anozie FC, Morales A, Weise SE, Sharkey TD (2015) The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana.* Frontiers P Sc 6: 167.

Wilson MV (2007) Measuring the components of competition along productivity gradients. J Ecol 95: 301–308.

Xie YH, An SQ, Wu BF, Wang WW (2006) Density-dependent root morphology and root distribution in the submerged plant *Vallisneria natans*. Environ Exp Bot 57: 195-200.

Yu Hongwei Shen, Yu Nan Siqi, Yu Dan, Chunhua, Liu (2018) Responses of the native species *Sparganium angustifolium* and the invasive species *Egeria densa* to warming and interspecific competition. PLOS ONE. 13. e0199478.

Accepted Manuscript

Supplementary material

Table S1: Chemical composition of the water from sampling sites in the spring.

Table S1: Chemical composition of the water from sampling sites in the spring.

Sites, species	**Coordinates**	**Temperature (°C)**	**Conductivity (µS cm^{-1})**	**Oxygen (%)**	**pH**	**NNO_3^- (mg L)**	**NNH_4^+ (mg L)**	**PO_4^{3-} (mg L)**
Guéméné-Penfao *E. densa*	(47°37'58"N, 01°53'23"W	16.6 ± 0.28	311±1	27.2±4.9	7.7± 0.2	4.1±0.18	1.04±0.30	0.190±0.06
Tréverien *E. canadensis*	(48°22'00"N, 01°56'00"W)	14.1± 3.48	303±8	35.8 ±40.3	7.2 ±0.8	0.89±4.5	0.13±0.01	0.63±0.95
Sainte Marie *L. major*	(47°69'04"N, 02°00'03"W)	15.1 ±2.21	310±19	31.5±5.8	8.4±1.5	3.8±1.60	0.25±1.07	0.35±1.45

Accepted Manuscript

Figure captions

Figure 1: Means and standard errors of each morphological trait (Relative growth rate = RGR, lateral shoot number, root number and Leaf area growth rate = LAGR) of *E. canadensis, E. densa* and *L. major* in monocultures at two plant densities. Empty symbols = low density; Black symbols = high density. Circle: *E. canadensis*, Square: *E. densa*, Triangle: *L. major*. Different small letters above columns are used to indicate a significant difference between species and densities.

Figure 2: Means and standard errors of the RNE of each morphological trait (Relative Growth Rate = RGR), lateral shoot number, root number and leaf area growth rate = LAGR) of *E. canadensis, E. densa and L. major* at two plant densities and different ratios (Mid-ratio (1) = 1/1; Mid-ratio (2) = 2/2; Low-ratio =1/3 and High-ratio = 3/1). The first number is the focal species and the second number the neighour. Empty Circle: *E. canadensis*, Empty Square: *E. densa*, Black Triangle: *L. major*. Different small letters above columns are used to indicate a significant difference between species and densities.

Figure 3: Means and standard errors of each morphological trait (relative Growth Rate = RGR), lateral shoot number, root number and leaf area growth rate = LAGR) of *E. canadensis, E. densa and L. major* at two plant densities and different ratios (Mid-ratio (1) = 1/1; Mid-ratio (2) = 2/2; Low-ratio =1/3 and High-ratio = 3/1). The first number is the focal species and the second number the neighour. Empty Circle: *E. canadensis*, Empty Square: *E. densa*, Black Triangle: *L. major*. Different small letters above columns are used to indicate a significant difference between species and densities.

Accepted Manuscript

Table 1. Results of a two–way ANOVA for each morphological trait of monoculture treatments of *E. canadensis, E. densa* and *L. major* at two plant densities (low= 2:0 and high=4:0). Significant results are in bold type.

Table 2: Results of a two–way ANOVA on the Relative Neighbour Effect (RNE) index calculated for each morphological trait of *E. canadensis, E. densa* and *L. major*. Significant results are in bold type.

Table 3: Results of a two–way ANOVA for each morphological trait of *E. canadensis, E. densa* and *L. major* in mixed cultures. Significant results are in bold type.

Table S1: Chemical composition of the water from sampling sites in the spring.

Accepted Manuscript