



HAL
open science

Responses of three invasive alien aquatic plant species to climate warming and plant density

Márcio José Silveira, Fernanda Moreira Florencio, Vanessa de Carvalho Harthman, Gabrielle Thiébaud

► To cite this version:

Márcio José Silveira, Fernanda Moreira Florencio, Vanessa de Carvalho Harthman, Gabrielle Thiébaud. Responses of three invasive alien aquatic plant species to climate warming and plant density. *Journal of Plant Research*, 2023, 136 (6), pp.817-826. 10.1007/s10265-023-01482-4 . hal-04192320

HAL Id: hal-04192320

<https://hal.science/hal-04192320>

Submitted on 11 Dec 2023

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.

Responses of three invasive alien aquatic plant species to climate warming and plant density

^{1,2}Márcio José Silveira*, ⁴Fernanda Moreira Florêncio, ³Vanessa de Carvalho Harthman, ²Gabrielle Thiébaud

¹Universidade Estadual de Minas Gerais, Unidade Ubá. Av. Olegário Maciel, 1427 CEP 36500-000-Ubá, MG- Brazil. ORCID - <https://orcid.org/0000-0002-6490-176X>

² University Rennes, CNRS, ECOBIO - UMR 6553, F-35000 Rennes, France. ORCID: <https://orcid.org/0000-0002-1530-052X>

³Federal University of Mato Grosso do Sul – UFMS/Campus Pantanal. Av Rio Branco, Bairro Universitário, CEP 79304-902, Corumbá-MS, Brazil. ORCID - <https://orcid.org/0000-0002-6237-5866>

⁴Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais – PEA, Universidade Estadual de Maringá – UEM. Av Colombo, 5790, Bloco G90, Jardim Universitário, CEP 87020-900, Maringá-PR, Brazil. ORCID: <https://orcid.org/0000-0003-2536-4313>

*Author correspondence: E-mail address: s.marcoijs@gmail.com

Abstract

Climate warming may impact plant invasion success directly, as well as indirectly through changes among interactions within plant communities. However, the responses of invasive alien aquatic species to plant density and rising temperatures remain largely unknown. We tested the effects of plant density and neighbour plant identity at different temperatures to better understand the performance of a community of invasive species exposed to climate warming. A microcosm experiment was conducted with three invasive aquatic plants species — *Elodea canadensis*, *Egeria densa* and *Lagarosiphon major* —, at mono and polycultures with low and high plant density, at 16 °C, 19 °C and 23 °C. The results clearly demonstrated that rising temperature influenced, either as a single parameter or as a combined factor, at least one of the measured traits of the three invasive species. Leaf area of *E. densa*, root number of *L. major* and growth of *E. densa* and *L. major* were influenced by temperature, plant density and neighbour identity. Plant density influenced all traits with the exception of leaf area of *E. canadensis* and lateral branch production of *E. densa*. Neighbour identity had no effect on growth rate and leaf area of *E. canadensis*, on lateral branch and roots production of *E. densa* and on leaf area of *L. major*. These findings establish that rising temperature could enhance competition or facilitation among *E. canadensis*, *L. major* and *E. densa* and could cancel the beneficial effects of the presence of a neighbour species; however, the magnitude of this effect was strongly dependent on plant density. Rising temperature due to climate change will likely play a crucial role in interactions between invasive species within plant communities and in the further spread of these invasive aquatic plants.

Keywords: Competitive intensity, *Egeria densa*, *Elodea canadensis*, facilitation, *Lagarosiphon major*, plant density, rising temperature

Introduction

Climate warming can profoundly alter ecosystem processes, such as carbon cycling, nitrogen remineralization, primary and secondary production, food web stability, community resistance to invasions, and species extinction rates (Bellard et al. 2013; Dukes and Mooney 1999; Melillo et al. 2002; Petchey et al. 1999). For example, temperature increase will directly stimulate metabolic rates and increase soil microbial activity. These changes would promote fast-growing plants, which may benefit from positive plant-soil feedback by enhanced decomposition and nutrient mineralization. Consequently, climate changes impose considerable shifts on interactions within communities (Hegland et al. 2008; Lemoine 2015). Adverse living conditions caused by climate changes might force negative or positive interactions among species (He et al. 2013). Predicting the effects of climate changes on plant-pollinator interactions (Hegland et al. 2008) and on plant-herbivore interactions (Hegland et al. 2008) has become an active and important area of research. Unfortunately, despite a considerable surge in climate-related research over the past decade, a clear understanding of the role of rising temperature in determining plant-plant interactions within aquatic macrophyte communities is still lacking.

Aquatic macrophytes can show different thermal tolerances (Cook and Urmi-König 1984; Madsen and Brix 1997; Silveira and Thiébaud 2017). Slight changes in temperature can have substantial impacts on plant growth and physiology such as carbohydrate content (Gillard et al. 2017) and tissue stoichiometry (Velthuis et al. 2017; Zhang et al. 2016, 2020). Variation in above- or belowground systems traits have been the focus of concern and they are observed to change with increasing temperature (Riis et al. 2012; Thiébaud et al. 2021; Zhang et al. 2019a). Thus, climate changes could alter above- and belowground resource allocation by plants (Velthuis et al. 2017). However, as the traits responses to increased temperature are species-specific (Silveira and Thiébaud 2017), variation in trait response among species can also change the direction and magnitude of interactions within plant communities (Weltzin et al. 2003).

Studies focusing on competition between different invasive alien species with the same growth form are highly suitable for revealing which species will have a better competitive ability and which traits promote greater invasiveness (Feng and Fu 2008; Pysek and Richardson 2007). Relatively few investigations have described interactions between invasive aquatic species of similar growth forms (Riis et al. 2012) and/or that are phylogenetically close (Mony et al. 2007). Furthermore, the response of a plant to competition also depends on coexisting species, and whether or not they share a similar niche, also known as the Similarity Theory (Abrams 1983; Gruntman and Novoplansky 2004; MacArthur and Levins 1967; Milla et al. 2009; Silveira and Thiébaud 2020). Interspecific competition has been shown to be stronger between functionally similar species (Petruzzella et al. 2018). Indeed, shoot biomass and relative growth rate of *Lagarosiphon major* Rid. Moss ex Wager was found to be lower both in rooted plant communities and in mixtures that were dominated by rooted plants (Petruzzella et al. 2018). Thus, examining plant responses to different neighbour species is vital to understanding the direction of plant-plant interactions. In addition, rising temperature and plant neighbour identity may also affect growth and survival of juvenile plants (Creed et al. 1997; Michelan et al. 2013). Furthermore, the occurrence of invasive aquatic plant species at high densities may

generate competition for resources (Hess et al. 2019; Ren and Zhang 2009), which can lead to growth inhibition of other plants (Silveira et al. 2018; Wang et al. 2005).

Thus, future climate modifications may affect community composition and structure not only directly through abiotic changes, but also indirectly by modifying the direction and intensity of species interactions, including competition (Lord et al. 2017; Netten et al. 2010; Zhang et al. 2019b) and facilitation (Brooker 2006). Growth responses to climate warming may therefore occur at parallel magnitudes in plant communities, although considerable variation in responses among species should be expected. As studies have been focusing mainly on the comparison of native and alien species, there is, to our knowledge, no study based on the combined effects of rising temperature, plant density and neighbour species identity on invasive aquatic plants.

Some species are close phylogenetically (Chen et al. 2012) and have similar life forms as rooted submerged plants, for example, *Elodea canadensis* Michaux, *Egeria densa* Planch and *L. major*. They are all widespread species in European ponds, streams and reservoirs (Cook and Urmi-König 1984, 1985; Mckee et al. 2002). *E. canadensis*, native to North America, was first recorded in the British Isles early in the 19th century and is now naturalized and widespread in Europe (Thiébaud, 2007). This species is considered to be stenothermic, with optimum temperatures ranging between 10 °C and 25 °C (Madsen and Brix, 1997). Native to South America, *E. densa* is already widely distributed in many European countries, such as Great Britain, Netherlands, Germany (Yarrow et al. 2009), and France (Thiébaud et al. 1997). The species appears to be confined to warm-temperate and cool subtropical conditions (Cook and Urmi-König, 1984). *L. major* is another alien species, native to Southern Africa, that has invaded several countries in Europe (James et al. 1999; Mckee et al. 2002). Its optimum growth is between 20°C and 23 °C, and it can be absent below temperatures of 10 °C (Natural Heritage Trust, 2003). *Lagarosiphon major* and *E. densa* colonize only the western part of France, with the introduced populations having adapted to an oceanic climate, whereas *E. canadensis* is widespread in French freshwaters from sub-continental to Mediterranean climates.

We previously established that the invasive species *E. canadensis* will perform better than the invasives *E. densa* and *L. major* under climate warming (Silveira and Thiébaud 2017). Since *E. canadensis* has a wider thermal tolerance than the other two species, it is able to grow under a wider range of temperatures. It can also adjust its responses to climate changes more quickly than the other two species, which come from a different climate (Silveira and Thiébaud 2017). We subsequently found 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 (Silveira and Thiébaud, 2020). Previous studies have suggested that *E. canadensis* is a stronger competitor than *E. densa* and *L. major* (Riis et al. 2012; Silveira and Thiébaud 2017). Thus, the question we are addressing now is how the interactions between these three invasive alien species (*E. canadensis*, *E. densa* and *L. major*) can be modified by increasing water temperatures and how plant density and neighbour identity influence the growth of these invasive plants. Our hypotheses were that (i) rising temperature will enhance competition among *E. canadensis*, *L. major* and *E. densa*, and (ii) rising temperature will favor the growth of *E. canadensis*, since this species has a wider thermal tolerance. The premise of hypothesis (i) is that morphological similarity between species means similar

traits involved in resource acquisition and use, which leads to niche overlap and competition (Tavares et al. 2022), and of hypothesis (ii) is that *E. canadensis* can potentially adjust its response to climate changes (Riis et al. 2012).

Materials and methods

Three invasive alien submerged aquatic plants, *E. canadensis*, *E. densa* and *L. major*, were chosen as models. Small shoots of *E. canadensis*, *E. densa* and *L. major* were collected randomly in spring from three small, shallow natural ponds in Brittany in western France (Table 1). Shoots, called hereafter “plants”, were derived from a single clone either of *E. canadensis*, *E. densa* or *L. major*. In parallel, a water sample was collected at each site and taken to the laboratory for chemical composition analysis. The plants were collected from sites characterized by: mean temperature of 15.3 °C (range 12.8 – 17 °C), low mean oxygen content of 34.4 % (range 23 – 45%), moderate mean conductivity of 274 μ S cm⁻¹ (range 105 – 397 μ S cm⁻¹), moderate phosphate concentration and high ammonium level. *Elodea canadensis* and *E. densa* were found in alkaline waters, whereas *L. major* was sampled in acidic to slightly acidic waters with higher ammonium concentrations. Plants were acclimatized for one week in tap water at room temperature (close to 20 °C).

Table 1: Sites where each species was collected with their respective latitude and longitude coordinates.

Sites	Species	Latitude	Longitude
Chapelle de Brain	<i>E. densa</i>	47°41'20"N	01°55'37"W
Guéméné-Penfao	<i>E. densa</i>	47°37'58"N	01°53'23"W
Saint-Martin-sur-Oust	<i>E. densa</i>	47°44'44"N	02°15'05"W
Sainte Marie	<i>L. major</i>	47°69'04"N	02°00'03"W
Guïgnen	<i>L. major</i>	47°55'16"N	01°51'38"W
Tréverien	<i>E. canadensis</i>	48°22'00"N	01°56'00"W
Bruz	<i>E. canadensis</i>	48°01'32"N	01°44'41"W
LeRheu	<i>E. canadensis</i>	48°06'10"N	01°47'39"W

Experimental design

In the laboratory, apical shoots of *E. canadensis*, *E. densa* and *L. major* were washed to remove invertebrates, algae and debris and then prepared for planting in pots [dimensions (L x W x H): 8cm x 8cm x 15cm], containing 2 cm of substrate (loam) and 3 cm of sand. Fertile finely-grained sediments, such as loam, contain high organic matter contents with anaerobic conditions, which are suitable for the growth of *L. major* (Martin and Coetzee 2014) and other macrophyte species. Apical shoots of 7-cm, without roots, buds or lateral branches, were selected for planting the species in pots. The species were planted in monoculture (control treatments) or in mixed conditions. Two densities were tested in monoculture and in mixed cultures: low density (two plants) and high density (four plants). As the distance between ramets was the same, four individuals per pot corresponded to high density whereas two individuals per pot corresponded to low density. The experimental design followed an addition

series model (Martin and Coetzee 2014; Spitters 1983) consisting of factorial combinations of different densities with different neighbour species. In mixed conditions, each species was planted with a different neighbour species at two densities (focal:neighbor at 1:1 = low density and at 1:3 = high density). For this treatment the combinations of focal and neighbour species in low or high density were: focal *E. canadensis* with *L. major* or *E. densa* as neighbour species; focal *E. densa* with *L. major* or *E. canadensis* as neighbour species; and focal *L. major* with *E. densa* or *E. canadensis* as neighbour species.

Three temperatures were tested: 16 °C, 19 °C and 23 °C. The temperature of 16 °C is the annual mean temperature in Brittany, so this temperature was considered the control treatment. Moreover, this was the average temperature recorded *in situ* at the moment of sampling the plants. The temperatures of 19 °C and 23 °C were based on climate predictions for Northern latitudes (McKee et al. 2002) that water temperature will increase from 3 °C to 7 °C above the mean annual water temperature in Brittany. The pots with different plant densities were placed separately in growth chambers with temperatures of 16 °C, 19 °C or 23 °C. Each treatment (combinations of plant density, neighbour species and temperature) had five replicates, totalizing 630 shoots.

All experimental pots were filled with tap water, which was slightly basic with moderate nutrient concentration (mean annual values, according to French Water Agency data: conductivity = 400 $\mu\text{S cm}^{-1}$; pH = 8.10; $[\text{NO}_3^- \text{N}] = 1.44 \text{ mg L}^{-1}$; $[\text{NH}_4^+ \text{N}] = 0.03 \text{ mg L}^{-1}$; $[\text{PO}_4^{3-} \text{P}] = 0.05 \text{ mg L}^{-1}$). The pots were randomly distributed in the growth chamber, where they remained for four weeks (photoperiod 12 h light/12 h dark; light intensity 50% corresponding to 300–500 $\mu\text{mol m}^{-2} \text{ s}^{-1}$). A 50% light intensity was chosen because these three non-native species may have a growth optimum under these light conditions (Riis et al. 2012).

Morphological responses of focal species to treatments

Four morphological traits related to growth were measured for each focal species at the end of the experiment: relative growth rate (RGR), lateral branch number (LBN), root number and leaf area growth rate (LAGR). RGR, or the rate of accumulation of new dry mass per unit of existing dry mass, is a major determinant of plant competitiveness (Lowry et al. 2018). It is an indirect measurement of the rate of resource acquisition, and numerous studies have found that increased crop RGR increases weed suppression (Didon 2002). LBN indicates potential propagule production (Xie et al. 2010) and lateral growth. A higher number of lateral branches induces greater lateral growth and higher spatial competition. It also suggests a greater potential to produce propagules as a result of fragmentation, which potentially increases propagule pressure and plant dispersal (an important trait of invasive species). Leaf area is a very important parameter for understanding the growth and physiological responses of invasive plant species under different environmental factors (Azeem et al. 2020). Root number represents the plant investment in root formation, which could favor nutrient uptake, and also demonstrate competitive ability (Lopes-Zamora et al. 2004). RGR and LAGR were calculated for each plant according to the following equation:

$$\text{RGR or LAGR} = (\ln L_2 - \ln L_1) / (T_2 - T_1)$$

where $L1$ and $L2$ refer to plant length or plant leaf area, respectively, at times 1 and 2 ($T1$ and $T2$, respectively) (Barrat-Segretain and Elger 2004).

The impact of neighbour species on focal species was quantified for each treatment using the Relative Interaction Index (RII). This index was calculated as the difference between focal species performance (i.e., RGR, LBN, root number and LAGR) in the presence and absence of a given neighbour species. The equation used to calculate the index follows, according to Yu et al. (2018):

$$\text{Relative Interaction Index: RII} = (P_{\text{mono}} - P_{\text{mix}}) / (P_{\text{mono}} + P_{\text{mix}})$$

where P_{mono} is the focal species performance (see above) in the absence of competition (i.e., monoculture treatment), and P_{mix} is the average of the traits of the focal species performance in each pot in the presence of competition (i.e., mixed culture treatments) (Yu et al. 2018), and it was calculated for both high- and low-density treatments. RII has defined limits (-1 and +1), is symmetrical around zero and is positive for competition and negative for facilitation (Armas et al. 2004; Hagiwara et al. 2010; Yu et al. 2018).

Statistical analyses

All analyses were carried out with R software (R Core Team 2020). We used a three-way permutational analysis of variance (ANOVA) with Tukey's HSD test for post hoc comparisons to test the effects of temperature, plant density and neighbour identity on RGR, LBN, root number and LAGR of *E. canadensis*, *E. densa* and *L. major*. The permutational ANOVA was carried out using the *aovp* function of the 'lmPerm' package (Bob and Torchiano 2016). Tukey's HSD test was carried out with the *PostHocTest* function of the 'DescTools' package (Signorell 2021), with the "method" argument of the function set to "hsd".

Results

Response of *Elodea canadensis*

Lateral branch number (LBN) of *E. canadensis* depended both on the interaction between temperature and plant density and the interaction between plant density and neighbour species (Table 2; Table S1). At low densities of both neighbour species, temperature had no effect over the interaction between them and *E. canadensis* (Fig. 1c). However, at high density of *E. densa*, there was a strong competition with *E. canadensis* LBN at 23 °C (Fig. 1d), significantly higher than at lower temperatures. Also, the presence of *L. major* at high density facilitated *E. canadensis* LBN at 16 °C and 19 °C (Fig. 1d), while a slight competition was observed at 23 °C.

Table 2. Summary of the results of a Permutative Anova testing the main effect of temperature (T), density of macrophytes (D), neighbour species (NS) and their interactions on the RII index of the relative growth rate (RGR), of lateral branch number (LBN), of root number and of Leaf Area Growth Rate (LAGR) of *Elodea canadensis*, *Egeria densa*, *Lagarosiphon major*.

RII	<i>Elodea canadensis</i>			<i>Egeria densa</i>			<i>Lagarosiphon major</i>		
	df	SS	P	df	SS	P	df	SS	P
RGR									
T	2	0.03	0.358	2	0.89	0.058	2	18.58	0.001
D	1	0.01	0.495	1	0.49	0.030	1	2.52	0.902
NS	1	0.03	0.521	1	0.17	1.000	1	2.25	0.213
T x D	2	0.08	0.101	2	0.51	0.142	2	9.42	0.092
T x NS	2	0.03	0.349	2	0.90	0.039	2	3.59	0.571
D x NS	1	0.00	0.706	1	1.32	0.000	1	6.30	0.118
T x D x NS	2	0.04	0.230	2	0.32	0.490	2	13.75	0.012
LBN									
T	2	0.76	0.003	2	0.58	0.332	2	2.70	0.016
D	1	0.53	0.012	1	0.36	0.233	1	1.43	0.035
NS	1	1.12	0.000	1	0.03	0.824	1	1.32	0.036
T x D	2	1.24	0.000	2	0.49	0.305	2	0.28	0.572
T x NS	2	0.05	0.646	2	0.63	0.260	2	3.51	0.043
D x NS	1	0.41	0.031	1	0.19	0.554	1	0.17	0.296
T x D x NS	2	0.08	0.703	2	0.00	1.000	2	1.87	0.127
Root number									
T	2	0.10	0.675	2	0.85	0.334	2	0.18	0.786
D	1	0.11	0.390	1	0.93	0.136	1	3.32	0.000
NS	1	0.36	0.007	1	0.56	0.200	1	0.75	0.055
T x D	2	1.17	0.000	2	1.29	0.202	2	0.15	0.532
T x NS	2	0.01	0.983	2	0.46	0.814	2	0.37	0.641
D x NS	1	0.06	0.248	1	0.22	0.528	1	1.70	0.005
T x D x NS	2	0.14	0.381	2	0.70	0.276	2	2.02	0.016
LAGR									
T	2	0.02	0.941	2	0.71	1.000	2	0.43	0.168
D	1	0.07	0.784	1	1.52	0.320	1	0.02	0.804
NS	1	0.01	0.638	1	0.19	0.284	1	0.03	0.491
T x D	2	0.50	0.447	2	0.67	0.522	2	1.00	0.064
T x NS	2	0.42	0.196	2	0.01	0.146	2	0.01	1.000
D x NS	1	0.01	0.980	1	0.10	0.961	1	0.14	0.745
T x D x NS	2	0.64	0.118	2	0.07	0.065	2	0.30	0.393

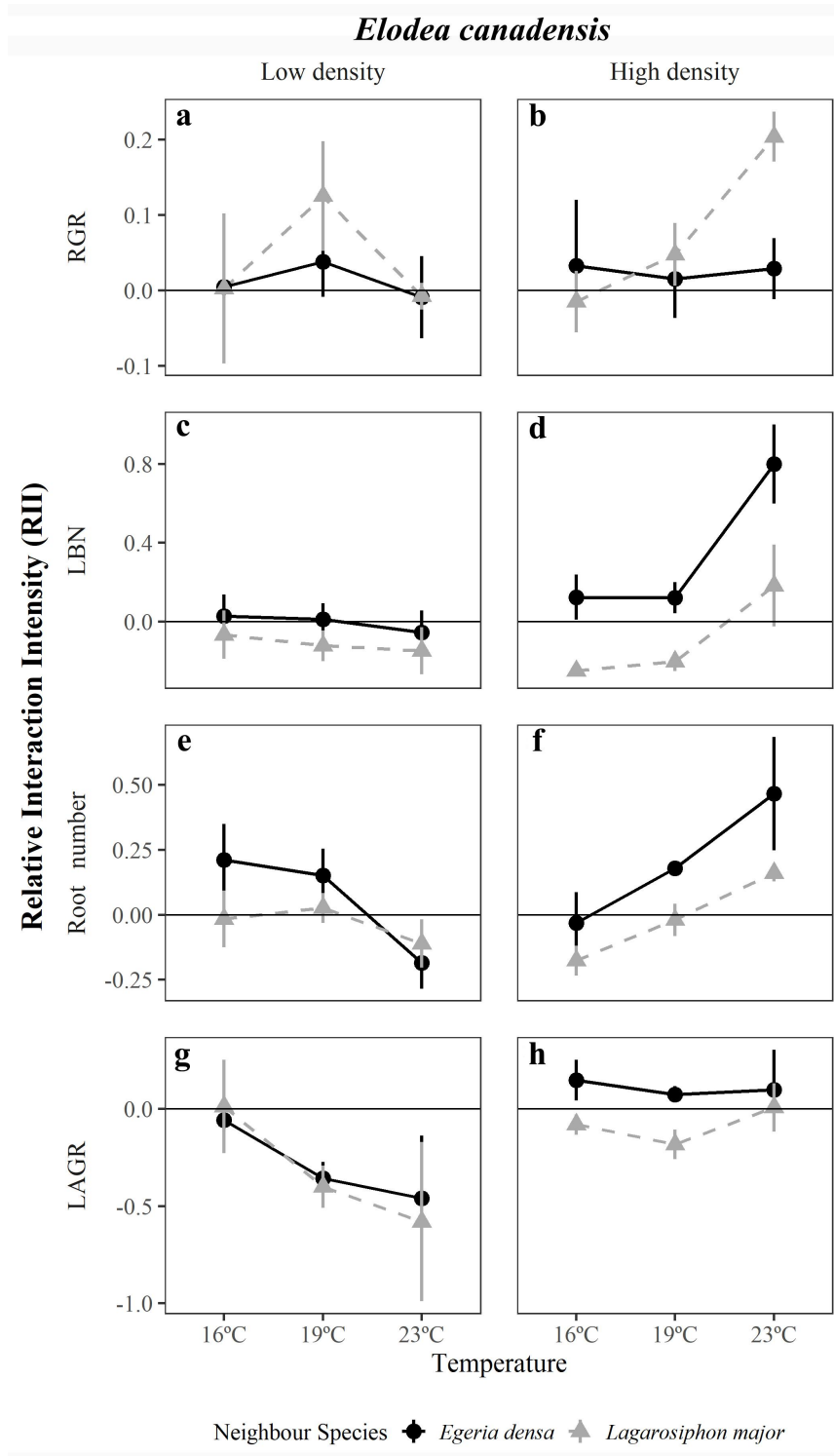


Figure 1. Relative Interaction Intensity (RII) of relative growth rate (RGR), lateral branch number (LBN), root number and Leaf Area Growth Rate (LAGR) of *Elodea canadensis*.

Root number was affected by neighbour species identity and by the interaction between temperature and density (Table 2; Table S1). Generally, *E. densa* exerted a stronger relationship with *E. canadensis* root number. At high density, the rise in temperature increased root competition between

both neighbour species (Fig. 1f). Also, root number of *E. canadensis* was slightly facilitated by both *E. densa* and *L. major* at low density at 23 °C (Fig. 1e), while at lower temperatures the interaction between *E. canadensis* and *E. densa* had a competitive nature (Fig. e).

For both RGR and LAGR of *E. canadensis*, no significant relationship was observed.

Response of *Egeria densa*

RGR competition was affected by the interaction between temperature and neighbour species identity, and the interaction between plant density and neighbour species identity (Table 2; Table S2). The presence of *L. major* in low density at 23 °C was responsible for a strong competition with RGR of *E. densa* (Fig. 2a), while at high density, *L. major* had a facilitating role at lower temperatures (Fig. 2b).

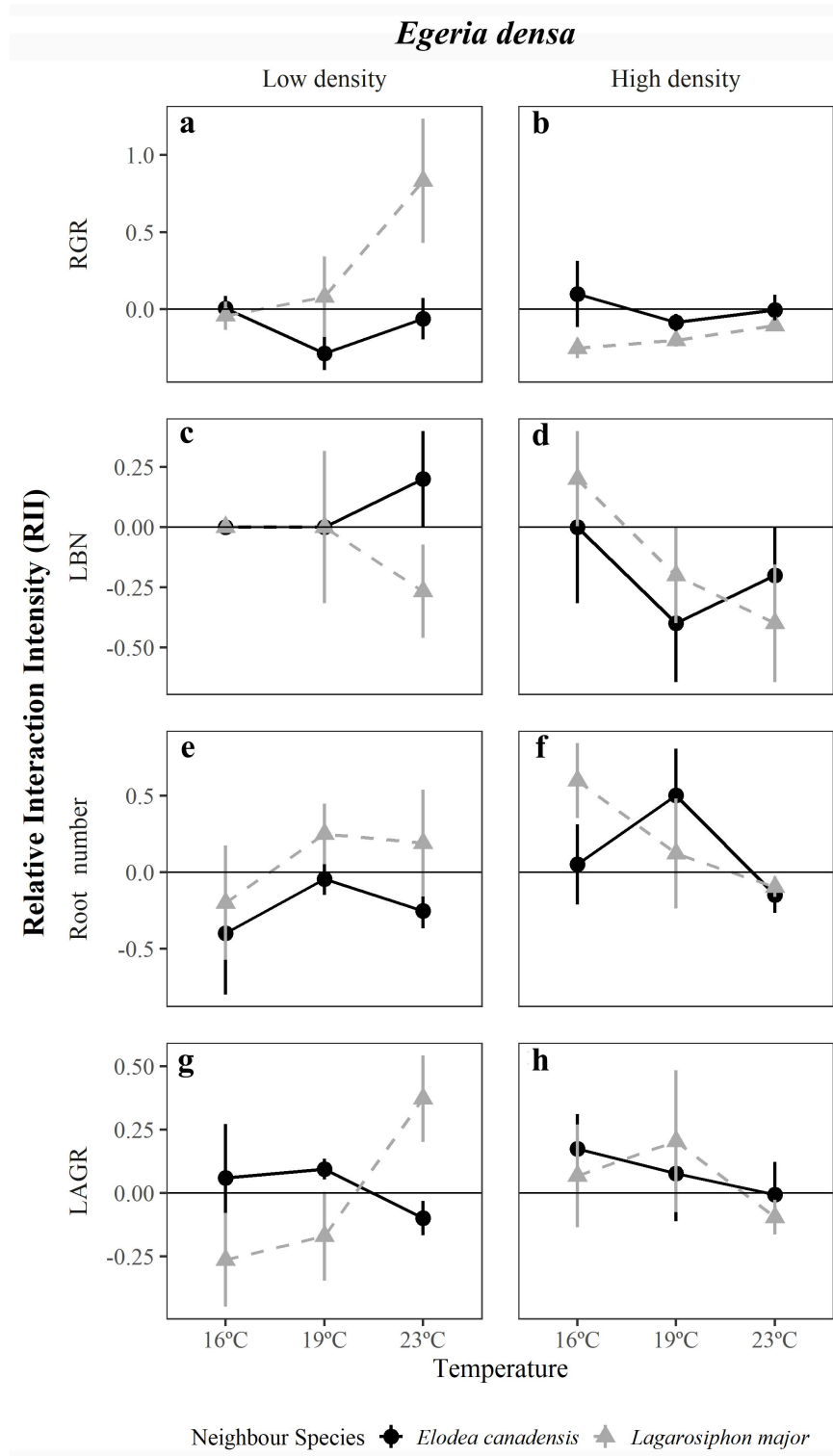


Figure 2. Relative Interaction Intensity (RII) of relative growth rate (RGR), lateral branch number (LBN), root number and Leaf Area Growth Rate (LAGR) of *Egeria densa*.

There was a marginally significant interaction among the effects of temperature, plant density and neighbour species over LAGR ($p = 0.065$; Table 2; Table S2). At low density of *L. major*, temperature

increase caused a shift in the interaction direction with *E. densa* LAGR, going from facilitation (at 16 °C) to competition (at 23 °C) (Fig. 2g). At low density, there was also a shift in interaction direction between *E. canadensis* and *E. densa*, going from competition (at 19 °C) to facilitation (at 23 °C) (Fig. 2g). However, at high density of both neighbour species, temperature did not have an effect on the interaction intensity of *E. densa* LAGR (Fig. 2h).

For both LBN and root number of *E. densa*, no significant relationship was observed.

Response of *Lagarasiphon major*

RGR was affected by an interaction among temperature, density of macrophytes and neighbour species (Table 2; Table S3). At 23 °C, the presence of *E. densa* at low density induced a strong competition with RGR of *L. major* (Fig. 3a), whereas RGR was not affected at high density (Fig. 3b).

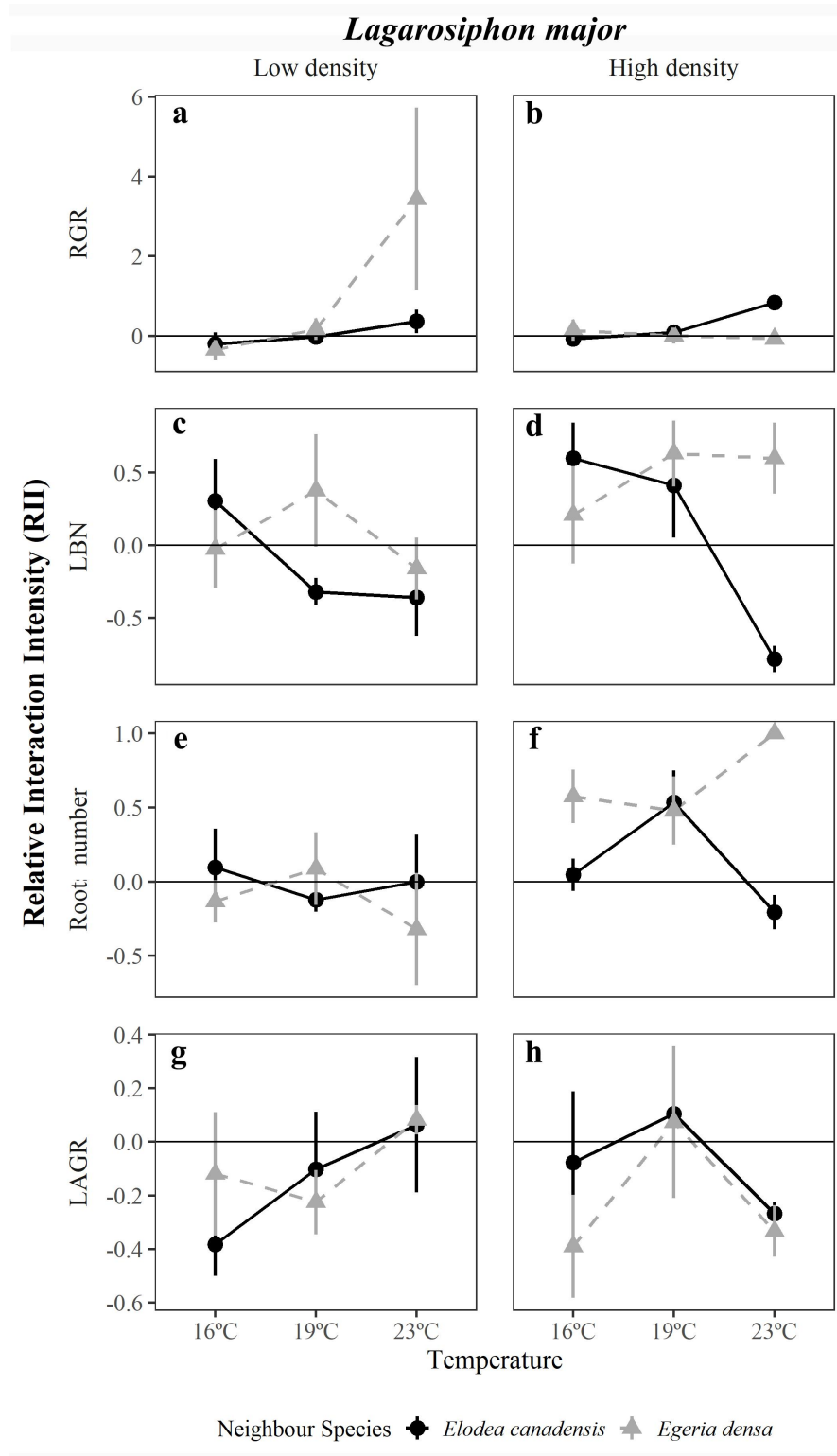


Figure 3. Relative Interaction Intensity (RII) of relative growth rate (RGR), lateral branch number (LBN), root number and Leaf Area Growth Rate (LAGR) of *Lagarosiphon major*.

LBN depended on plant density and on the interaction between temperature and neighbour species (Table 2; Table S3). Generally, high plant density had a stronger interaction with *L. major* LBN. In low density, *E. densa* had a competition interaction with *L. major* LBN at 19 °C (Fig. 3c), while in high density, the competition occurred also at 19 °C and 23 °C (Fig. 3c, d). *E. canadensis*, in low and high density, shifted its competitive interaction with *L. major* LBN at lowest temperatures to facilitation at highest temperatures (Fig. 3c, d).

Root number, as observed for RGR, suffered the effect of the interaction among temperature, plant density and neighbour species identity (Table 2; Table S3). In high density, a strong competitive interaction between *L. major* root number and *E. densa* was observed throughout all temperatures (Fig. 3f). However, at the presence of *E. canadensis*, the competition observed at 19 °C shifted to facilitation at 23 °C (Fig. 3f). In low density, both neighbour species exerted minimal interaction with *L. major* root number at all temperatures.

Again, there was a marginally significant interaction among the effects of temperature and plant density over LAGR ($p = 0.064$; Table 2; Table S3). While *E. densa* in low density had a facilitative interaction with *L. major* LAGR at 19 °C, this interaction shifted to competition at 23 °C (Fig. 3g). However, at high density of *E. densa*, the interaction was strongly facilitative at 23 °C (Fig. 3h). The facilitation at 23 °C was also observed for *E. canadensis* at high density (Fig. 3h), as at low density a facilitation was observed at 16 °C (Fig. 3g).

Discussion

The results of the present experimental study clearly demonstrated that competition or facilitation between the studied species depend on temperature, neighbour species identity and macrophyte density. It also demonstrated that the possible growth advantage of *Elodea canadensis* with rising temperature, as proposed in our hypotheses, may not be true, once its development can be impacted by competition with *Egeria densa* in high density. We can also observe that temperature had the potential to shift interaction direction between different species, turning previously positive interactions (e.g., facilitation) into negative ones (e.g., competition), and vice versa, and several times this effect was greatly dependent on species density.

We found that root and lateral branch numbers of *E. canadensis* suffered competition with *E. densa* at increasing temperature when *E. densa* was present in high density. On the other hand, *E. canadensis* root growth was facilitated by both neighbour species at high temperature when they were present in low density. Also, a high density of *Lagarosiphon major* facilitated the growth of lateral branches at lower temperatures. Ergo, the effects of increased temperature on *E. canadensis* growth can be positive or negative, depending on macrophyte composition and density around it. Studies had demonstrated that the invasive *E. densa* is very competitive and could form dense monospecific stands in the context of climate warming. Possibly, the treatments with high density of *E. densa* caused a higher competition for resources and space, and it is likely that these factors were responsible for the inhibition of *E. canadensis*.

Other studies have demonstrated that the greater density of *E. densa* can impact the growth of *E. canadensis* (Silveira and Thiébaud, 2020), but also of other aquatic plants species (Hosfra et al. 1999).

For this reason, we believe that the “invasiveness” of *E. densa* could explain the failure of *E. canadensis* and *L. major* to invade certain aquatic environments in South America, such as in Brazil and Argentina, where *E. densa* is native and very abundant (Cabrera et al. 2013). Moreover, other species of submerged macrophytes can also inhibit the growth of *E. canadensis* (e.g., *E. canadensis* was found to be displaced by *Elodea nuttallii* in Rhone River floodplain - France) (Barrat-Segretain 2001; Larson 2007; Thiébaud et al. 1997). *E. canadensis* was also found not to be an aggressive species in Polish lakes, and that climate warming may contribute to reducing its spread in several habitats (Kolada and Kutyla 2016), since other species can be better adapted to rising temperature (e.g., *E. densa*) and, as a consequence, can outperform *E. canadensis*.

Although increasing temperature is a well-known driver of *E. densa* growth (Riis et al. 2012; Silveira and Thiébaud, 2017), density of macrophytes and neighbour species identity also affected its performance. For example, at low density and high temperature, the presence of *E. canadensis* facilitated the leaf area growth of *E. densa*, whereas the presence of *L. major* induced severe competition for this trait, and the opposite was observed at lower temperatures, where both interactions changed directions (see result section). Some studies found that rising temperature could significantly affect the growth of submerged macrophytes by altering individual ecological stoichiometry (Zhang et al. 2016, 2019), but our results demonstrated that the neighbour species identity is an important factor that can determine whether there is a positive effect on growth by facilitation, or even its inhibition by competition increase.

The aquatic plant *L. major* possesses traits that classify it as a ‘good invader’, such as vegetative growth habit, broad environmental tolerance and high relative growth rate (Hofstra et al. 1999; Rattray et al. 1994; Sutherland 2004). This species may successfully out-compete other submerged macrophytes due to its ability to photosynthesize and, consequently, to outgrow other submerged plants, particularly under very stressful conditions (James et al. 1999). On the other hand, positive interactions (facilitation) can occur between species with similar morphology (Thomaz et al. 2012), as the species used in this study. Exotic species may facilitate the establishment of other exotic species by creating suitable environmental conditions (e.g., increasing nitrogen availability). For example, the presence of other exotic submerged macrophytes facilitated the occurrence and spread of *E. densa* in certain aquatic environments (Petruzzella et al. 2018; Santos et al. 2011).

All measured traits of *L. major* suffered some intensity of interaction with the analyzed species. Despite its high competitive ability, our findings showed strong competition for root and lateral branch production and growth rate at the warmest temperature in the presence of *E. densa*, however this interaction was highly dependent on density. Other studies demonstrated that the presence of *E. canadensis* can also exert strong competition with branch production of *L. major* (Riis et al. 2012). Possibly this strong competition may be related with the higher growth rate of *E. canadensis* and *E. densa* in relation to *L. major* (Riis et al. 2012; Silveira and Thiébaud 2020; Silveira et al. 2017). Besides, *E. densa* is more competitive than *E. canadensis* and *L. major* in warmer waters (Riis et al. 2012), which meets our results, showing that the presence of this species can increase competition with *L. major* at high temperature and high macrophyte density. Furthermore, *L. major* have previously shown strong negative response at high temperature, which would suggest less phenotypic plasticity (Riis et al.

2012), but we have seen here that this condition depends on the community composition surrounding it, since the presence of *E. canadensis* in high density facilitated the development of *L. major* in high temperature. Thus, it is likely that the competitive ability of *L. major* depends upon a combination of factors, including the neighbour species identity and temperature (Silveira and Thiébaud 2017).

Indeed, the interactions between the three species were often asymmetric at the highest temperature. For example, facilitation by *L. major* occurred in the production of lateral branches of *E. densa*, whereas the number of lateral branches of *L. major* was strongly reduced in presence of *E. densa*. Furthermore, this study demonstrates that rising temperature due to climate change may play a crucial role in positive or negative interactions between these invasive species within plant communities, where species previously inhibited by others can be facilitated with rising temperature, or species previously facilitated can turn to be inhibited in the new scenarios. However, when considering competitive strength among species, neighbour species can decrease or increase biotic pressure. Consequently, these effects should be more or less exacerbated by the presence of a neighbour species. In other words, neighbour species identity is also fundamental to plant-plant interactions (Lang et al. 2012; Mölder et al. 2014), as well as the density of the co-occurring species.

Conclusions

The present experimental study demonstrated that climate warming may change the direction and intensity of interactions among invasive alien plant species and, consequently, influence freshwater ecosystem functioning. These results are of great relevance because both climate warming and invasive species have been identified as increasing threats to biodiversity (Bellard et al. 2013). Our findings indicate that alien invasive aquatic macrophytes respond differently to temperature and neighbour plants density and identity, and even to interactions among these factors, showing that the performance and interaction between these invasive species can be modified by biotic factors and climate changes. Although it is difficult to predict the effect of climate warming on the invasiveness of aquatic macrophyte species within a given community, our results suggest that, in some situations, rising temperature could cancel the beneficial effects of the presence of neighbour species, shifting it into growth inhibition, and could also turn a negative effect into establishment facilitation. This could greatly alter community structural dynamics, making previously well-established environments susceptible to invasions and other disturbances arising from this imbalance. Therefore, the establishment of further general patterns dealing with the impacts of a global warming on biological interactions is needed.

Acknowledgements

We acknowledge with appreciation the comments provided by reviewers and the handling editor, which improved the quality of our manuscript. 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. Silveira is grateful to the National Council for Scientific and Technological Development (CNPq), for providing a Post-Doctorate scholarship and Rennes Metropole by grant for study periods at France.

References

- Abrams P (1983) The Theory of Limiting Similarity . *Annu Rev Ecol Sys* 14:359 - 376.
- Armas C, Ordiales R, Pugnaire FI (2004). Measuring plant interactions: a new comparative index. *Ecology*. 85:2682–2686.
- Azeem A, Javed Q, Sun J, Du D (2020). Artificial neural networking to estimate the leaf area for invasive plant *Wedelia trilobata*. *N J Bot* 38: 1-8.
- Barrat-segretain MH, Elger A (2004). Experiments on growth interactions between two invasive macrophytes species. *J Veg Sci* 15: 109–114.
- Barrat-Segretain M-H (2001) Invasive species in the Rhone River floodplain (France): replacement of *Elodea canadensis* Michaux by *E. nuttallii* St. John in two former river channels. *Archiv Hydrobiologie* 152: 237-251.
- Bellard C, Thuiller W, Leroy B, Genovesi P, Bakkenes M, Courchamp F (2013). Will climate change promote future invasions? *Glob Change Biol* 19: 3740–3748.
- Brooker RW (2006) Plant–plant interactions and environmental change. *New Phytol* 171:271–284
- Bob W, Torchiano M. (2016). *lmPerm*: permutation tests for linear models. R package version 2.1.0. <https://CRAN.R-project.org/package=lmPerm>
- Cabrera WG, Dalto YM, Mattioli FM, Carruthers RI, Anderson LW (2013). Biology and ecology of Brazilian elodea (*Egeria densa*) and its specific herbivore, *Hydrellia* sp, in Argentina. *Biol Cont* 58: 133–147.
- 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.
- Chen LY, Chen JM, Gituru RW, Wang QF, (2012). Generic phylogeny, historical biogeography and character evolution of the cosmopolitan aquatic plant family Hydrocharitaceae. *BMC Evol Biol* 12: 30.
- 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.
- Didon UME (2002) Variation between barley cultivars in early response to weed competition. *J A Crop Sci* 188:176-184.
- Dukes JS, Mooney HA. (1999) Does global change increase the success of biological invaders? *Trends Ecol Evol* 14: 135– 139.
- Feng YL, Fu GL (2008) Nitrogen allocation, partitioning and use efficiency in three invasive plant species in comparison to their native congeners. *Biol Inv* 10:891–902
- Gillard M, Thiébaud G, Deleu C, Leroy B. (2017) Present and future distribution of three aquatic plants taxa across the world: decrease in native and increase in invasive ranges. *Biol Inv* 19: 2159–2170.

- Gruntman M, Novoplansky A (2004). Physiologically mediated self/non-self discrimination in roots. *Proc Natl Acad Sci* 101:3863–3867.
- Hagiwara Y, Kachi N, Suzuki J (2010). Effects of temporal heterogeneity of water supply on the growth of *Perilla frutescens* depend on plant density. *Ann Bot.* 106:173.
- He Q, Bertness MD, Altieri AH (2013) Global shifts towards positive species interactions with increasing environmental stress. *Ecol Lett* 16: 695-706
- Hess MCM, Mesléard F, Buisson E (2019). Priority effects: emerging principles for invasive plant species management. *Ecol Eng* 127: 48–57. doi: 10.1016/j.ecoleng.2018.11.011
- Hegland SJ, Nielsen A, Lázaro A, Bjerknæs AL, Totland Ø (2009). How does climate warming affect plant-pollinator interactions? *Ecol Lett* 12, 184–195.
- Hostfra DE, Clayton J, Green JD, Auger M (1999). Competitive performance of *Hydrilla verticillata* in New Zealand. *Aquat Bot* 63:305–324.
- 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.
- Kolada A, Kutytka S (2016). *Elodea canadensis* (Michx.) in Polish lakes: a non-aggressive addition to native flora. *Biol Inv* 18: 3251-3264.
- Lang AC, Härdtle W, Baruffol M, Böhnke M, Bruehlheide H, Schmid B, et al. (2012). Mechanisms promoting tree species co-existence: experimental evidence with saplings of subtropical forest ecosystems of China. *J Veg Sci* 23:837–846. doi: 10.1111/j.1654-1103.2012.01403.x
- Larson D (2007) Non-indigenous freshwaters plants. Patterns, processes and risk evaluation. Dissertation, Swedish University of Agricultural Sciences, Uppsala
- Lemoine NP (2015) Climate change may alter breeding ground distributions of eastern migratory monarchs (*Danaus plexippus*) via range expansion of *Asclepias* host plants. *PLoS ONE* 10: e0118614
- Lopes-Zamora I, Comerford NB, Muchovej, RM (2004) Root development and competitive ability of the invasive species *Melaleuca quinquenervia* (Cav.) S.T. Baker in the South Florida Flatwoods. *Plant Soil* 263: 239-247.
- Lord JP, Graves BD (2017). Impact of climate change on direct and indirect species interactions. *Mar Ecol Prog Ser* 571: 1–11.
- Lowry CJ, Smith RJ (2018) Weed control through crop plant manipulations in non-chemical weed control.
- MacArthur RH, Levins R. (1967) The limiting similarity, convergence and divergence of coexisting species. *Am Nat* 101: 377–385.
- McKee D, Hatton K, Eaton JW, Atkinson D, Atherton A, Harvey et al. (2002). Effects of simulated climate warming on macrophytes in Freshwater microcosm communities. *Aquat Bot* 74: 71–83.

- Madsen TV, Brix H (1997) Growth, photosynthesis and acclimation by two submerged macrophytes in relation to temperature. *Oecologia* 110: 320–327
- 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.
- Michelan TS, Thomaz SM, Bini LM (2013). Native macrophyte density and richness affect the invasiveness of a tropical poaceae species. *Plos one* 8: 1–8.
- Milla RN, Forero DM, Escudero AN, Iriando JM (2009) Growing with siblings: a common ground for cooperation or for fiercer competition among plants? *P R Soc Biol Sci* 276:2531–2540.
- Melillo JM, Steudler PA, Aber JD, Newkirk KM, Lux H, Bowles FP, Catrical C, Magill AH, Ahrens T, Morrisseau S (2002) Soil warming and carbon-cycle feedbacks to the climate system *Science* 298: 2173–2176
- Mölder I, Leuschner C (2014). European beech grows better and is less drought sensitive in mixed than in pure stands: tree neighbourhood effects on radial increment. *Trees* 28: 777–792. doi: 10.1007/s00468-014-0991-4
- 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.
- Natural Heritage Trust (2003) *Lagarosiphon - Lagarosiphon major*. In: *Weed management guide*, Australia: Natural Heritage Trust.
- Netten JJC, Arts GHP, Gylstar R, Vannes EH, Scheffer M, Roijackers RMM (2010). Effect of temperature and nutrients on the competition between free-floating *Salvinia natans* and submerged *Elodea nuttallii* in mesocosms. *F App Limn* 177: 125–132.
- Petruzzella A, Manschot J, van Leeuwen CHA, Grutters BMC, Bakker ES (2018) Mechanisms of invasion resistance of aquatic plant communities. *Front Plant Sci* 9:134.
- Pyšek P, Richardson DM. (2007) Traits associated with invasiveness in alien plants: where do we stand? In: Nentwig W (ed) *Biological invasions. Ecological Studies*, Vol. 193. Springer-Verlag, Berlin & Heidelberg, pp 97– 125.
- Petchey OL, McPhearson PT, Casey TM, Morin PJ (1999). Environmental warming alters food-web structure and ecosystem function. *Nature* 402: 69–72.
- Ratray MR, Howard-Williams C, Brown JMA (1994). Rates of early growth of propagules of *Lagarosiphon major* and *Myriophyllum triphyllum* in lakes of differing trophic status. *Marine Fresh Res* 28: 235–241.
- Ren MX, Zhang QG (2009). The relative generality of plant invasion mechanisms and predicting future invasive plants. *Weed Res* 49:449–460.
- 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.

- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- 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 Invasion* 13:443–457
- Silveira MJ, Thiébaud G (2017) Impact of climate warming on plant growth varied according to the season. *Limnologica* 65: 4-9.
- Silveira MJ, Alves DC, Thomaz SM (2018) Effects of the density of the invasive macrophyte *Hydrilla verticillata* and root competition on growth of one native macrophyte in different sediment fertilities. *Ecol Res.* <https://doi.org/10.1007/s11284-018-1602-4>
- Silveira MJ, Thiébaud G (2020) Effect of density and neighbours on interactions between invasive plants of similar growth form. *Aquatic Ecol* 54:463–474 - [https://doi.org/10.1007/s10452-020-09753-1\(0123456789](https://doi.org/10.1007/s10452-020-09753-1(0123456789).
- Signorell, Andri et al. (2021). DescTools: Tools for descriptive statistics. R package version 0.99.40. <https://cran.r-project.org/package=DescTools>
- Spitters CJT (1983). An alternative approach to the analysis of mixed cropping experiments. Estimation of competition effects. *J Agr Sci* 31: 1-11.
- Sutherland WJ, Pullin AS, Dolman PM, Knight TM (2004) Mismatches between conservation science and practice. *Trends Ecol Evol* 19: 565–566.
- Tavares MT, De Souza BC, Menezes BS, Araújo FS, Sfair J, Zandavalli RB (2022) Phylogenetic relatedness and competition: a pot experiment with semiarid tree species. *CERNE*, v.28, e-102819.
- Thiébaud G (2007) Invasion success of non-indigenous aquatic and semi-aquatic plants in their native and introduced ranges: A comparison between their invasiveness in North America and in France. *Biol Inv* 9: 1–12.
- Thiébaud G, Tarayre M, Jambon O, Le Bris N, Colinet H, Renault D (2021) Variation of thermal plasticity for functional traits between populations of an invasive aquatic plant from two climatic regions. *Hydrobiologia* 848:2077–2091 [https://doi.org/10.1007/s10750-020-04452-2\(0123456](https://doi.org/10.1007/s10750-020-04452-2(0123456)
- Thiébaud G, Rolland T, Robach F, Tremolières M, Muller S (1997) Quelques conséquences de l'introduction de deux espèces de macrophytes, *Elodea canadensis* Michaux et *Elodea nuttallii* St. John, dans les écosystèmes aquatiques continentaux: exemple de la plaine d'Alsace et des Vosges du Nord (Nord-Est de la France). *Bulletin Français de la Pêche et de la Pisciculture* 344/345: 441-452 [doi:10.1051/kmae:1997040](https://doi.org/10.1051/kmae:1997040).
- 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
- Velthuis M, Van Deelen E, Van Donk E, Zhang P, Bakker ES (2017). Impact of temperature and nutrients on carbon: nutrient tissue stoichiometry of submerged aquatic plants: an experiment and meta-analysis. *Front. Plant Sci.* 8:655. [10.3389/fpls.2017.00655](https://doi.org/10.3389/fpls.2017.00655)

- Wang LW, Showalter AM, Ungar IA (2005). Effects of intraspecific competition on growth and photosynthesis of *Atriplex prostrata*. *Aquat Bot* 83: 187–192.
- Weltzin JF, Bridgham SD, Pastor J, Chen J, Harth . (2003). Potential effects of warming and drying on peatland plant community composition. *Glob Change Biol* 9: 141–151.
- Yarrow M, Marin VH, Finlayson M, Tironi A, Delgado LE, Fischer F (2009) The ecology of *Egeria densa* Planchon (Liliopsida: alismatales): a wetland ecosystem engineer? *Rev Chil Hist Nat* 82: 299–313.
- Yu H, Shen N, Yu S, Yu D, Liu C (2018) Responses of the native species *Sparganium angustifolium* and the invasive species *Egeria densa* to warming and interspecific competition. *PLoS ONE* 13: e0199478. <https://doi.org/10.1371/journal.pone.0199478>
- Xie YH, An SQ, Wu BF, Wang WW (2006). Density-dependent root morphology and root distribution in the submerged plant *Vallisneria spiralis*. *Environ Exp Bot* 57: 195-200.
- Zhang R, Tielbörger K (2019) Facilitation from an intraspecific perspective stress tolerance determines facilitative effect and response in plants. *New Phytol* 221: 2203–2212.
- Zhang P, Grutters BMC, van Leeuwen CHA, Xu J, Petruzzella A, van den Berg RF and Bakker ES (2019) Effects of rising temperature on the growth, stoichiometry, and palatability of aquatic plants. *Front Plant Sci* 9:1947. doi: 10.3389/fpls.2018.01947
- Zhang X, Odgaard R, Olesen B, Lauridsen TL, Liboriussen L, Sondergaard M, Liu Z, Jeppesen E (2016) Warming shows differential effects on late-season growth and competitive capacity of *Elodea canadensis* and *Potamogeton crispus* in shallow lakes. *Inland Waters* 5: 421-432.
- Zhang P, Kuramae A, van Leeuwen CHA, Velthuis M, van Donk E, Xu J and Bakker ES (2020) Interactive effects of rising temperature and nutrient enrichment on aquatic plant growth, stoichiometry, and palatability. *Front Plant Sci* 11:58. doi: 10.3389/fpls.2020.00058

Supplementary material

Table S1: Summary of Tukey's HSD post hoc test for *Elodea canadensis*. For treatments: Temperature (T); Densities (D) and Neighbours species (NS).

<i>Elodea canadensis</i>	Dif.	Lower CI	Uper CI	<i>p</i>
Root number				
NS				
<i>L. major-E. densa</i>	-0.15	-0.28	-0.03	0.015 *
T x D				
16°C:High-19°C:High	-0.18	-0.50	0.13	0.513
23°C:High-19°C:High	0.23	-0.08	0.55	0.248
19°C:Low-19°C:High	0.01	-0.30	0.32	1.000
16°C:Low-19°C:High	0.02	-0.30	0.33	1.000
23°C:Low-19°C:High	-0.23	-0.54	0.09	0.277
23°C:High-16°C:High	0.42	0.10	0.73	0.003 **
19°C:Low-16°C:High	0.19	-0.12	0.51	0.459
16°C:Low-16°C:High	0.20	-0.11	0.51	0.409
23°C:Low-16°C:High	-0.04	-0.36	0.27	0.998
19°C:Low-23°C:High	-0.23	-0.54	0.09	0.289
16°C:Low-23°C:High	-0.22	-0.53	0.10	0.331
23°C:Low-23°C:High	-0.46	-0.78	-0.15	0.001 ***
16°C:Low-19°C:Low	0.01	-0.30	0.32	1.000
23°C:Low-19°C:Low	-0.24	-0.55	0.08	0.238
23°C:Low-16°C:Low	-0.25	-0.56	0.07	0.204
LBN				
T				
16°C-19°C	0.01	-0.20	0.21	0.996
23°C-19°C	0.24	0.04	0.45	0.016 *
23°C-16°C	0.24	0.03	0.44	0.020 *
D				
Low-High	-0.19	-0.33	-0.05	0.009 **
NS				
c.sp.2-c.sp.1	-0.27	-0.41	-0.13	0.000 ***
T x D				
16°C:High-19°C:High	-0.02	-0.38	0.33	1.000

23°C:High-19°C:High	0.53	0.18	0.89	0.001	***
19°C:Low-19°C:High	-0.01	-0.37	0.34	1.000	
16°C:Low-19°C:High	0.02	-0.33	0.38	1.000	
23°C:Low-19°C:High	-0.06	-0.42	0.29	0.995	
23°C:High-16°C:High	0.55	0.20	0.91	0.000	***
19°C:Low-16°C:High	0.01	-0.35	0.36	1.000	
16°C:Low-16°C:High	0.04	-0.31	0.40	0.999	
23°C:Low-16°C:High	-0.04	-0.39	0.32	1.000	
19°C:Low-23°C:High	-0.55	-0.90	-0.19	0.000	***
16°C:Low-23°C:High	-0.51	-0.86	-0.16	0.001	**
23°C:Low-23°C:High	-0.59	-0.95	-0.24	0.000	***
16°C:Low-19°C:Low	0.04	-0.32	0.39	1.000	
23°C:Low-19°C:Low	-0.05	-0.40	0.31	0.999	
23°C:Low-16°C:Low	-0.08	-0.44	0.27	0.981	
D x NS					
Low:<i>E. densa</i>-High:<i>E. densa</i>	-0.35	-0.61	-0.09	0.004	**
High:<i>L. major</i>-High:<i>E. densa</i>	-0.44	-0.70	-0.18	0.000	***
Low:<i>L. major</i>-High:<i>E. densa</i>	-0.46	-0.72	-0.20	0.000	***
High: <i>L. major</i> -Low: <i>E. densa</i>	-0.09	-0.34	0.17	0.816	
Low: <i>L. major</i> -Low: <i>E. densa</i>	-0.11	-0.37	0.15	0.688	
Low: <i>L. major</i> -High: <i>L. major</i>	-0.02	-0.28	0.24	0.996	

Table S2: Summary of Tukey's HSD post hoc test for *Egeria densa*. For treatments: Temperature (T); Densities (D) and Neighbours species (NS).

<i>Egeria densa</i>	Dif.	Lower CI	Uper CI	<i>p</i>
RGR				
T				
16°C-19°C	0.08	-0.21	0.36	0.801
23°C-19°C	0.29	0.00	0.58	0.050
23°C-16°C	0.21	-0.08	0.50	0.187
LAGR				
T x D x NS				
16°C:High:E. canadensis-19°C:High:E. canadensis	0.10	-0.72	0.91	1.000
23°C:High:E. canadensis-19°C:High:E. canadensis	-0.08	-0.90	0.73	1.000
19°C:Low:E. canadensis-19°C:High:E. canadensis	0.02	-0.80	0.83	1.000
16°C:Low:E. canadensis-19°C:High:E. canadensis	-0.02	-0.83	0.80	1.000
23°C:Low:E. canadensis-19°C:High:E. canadensis	-0.18	-0.99	0.64	1.000
19°C:High:L. major-19°C:High:E. canadensis	0.13	-0.69	0.94	1.000
16°C:High:L. major-19°C:High:E. canadensis	-0.01	-0.83	0.81	1.000
23°C:High:L. major-19°C:High:E. canadensis	-0.17	-0.99	0.64	1.000
19°C:Low:L. major-19°C:High:E. canadensis	-0.25	-1.06	0.57	0.996
16°C:Low:L. major-19°C:High:E. canadensis	-0.34	-1.16	0.48	0.951
23°C:Low:L. major-19°C:High:E. canadensis	0.30	-0.52	1.11	0.982
23°C:High:E. canadensis-16°C:High:E. canadensis	-0.18	-1.00	0.64	1.000
19°C:Low:E. canadensis-16°C:High:E. canadensis	-0.08	-0.90	0.74	1.000
16°C:Low:E. canadensis-16°C:High:E. canadensis	-0.11	-0.93	0.70	1.000
23°C:Low:E. canadensis-16°C:High:E. canadensis	-0.27	-1.09	0.54	0.990
19°C:High:L. major-16°C:High:E. canadensis	0.03	-0.79	0.85	1.000
16°C:High:L. major-16°C:High:E. canadensis	-0.11	-0.92	0.71	1.000
23°C:High:L. major-16°C:High:E. canadensis	-0.27	-1.09	0.55	0.991
19°C:Low:L. major-16°C:High:E. canadensis	-0.34	-1.16	0.47	0.947
16°C:Low:L. major-16°C:High:E. canadensis	-0.44	-1.25	0.38	0.786
23°C:Low:L. major-16°C:High:E. canadensis	0.20	-0.62	1.01	0.999
19°C:Low:E. canadensis-23°C:High:E. canadensis	0.10	-0.72	0.92	1.000
16°C:Low:E. canadensis-23°C:High:E. canadensis	0.07	-0.75	0.88	1.000
23°C:Low:E. canadensis-23°C:High:E. canadensis	-0.09	-0.91	0.72	1.000
19°C:High:L. major-23°C:High:E. canadensis	0.21	-0.61	1.03	0.999
16°C:High:L. major-23°C:High:E. canadensis	0.07	-0.74	0.89	1.000
23°C:High:L. major-23°C:High:E. canadensis	-0.09	-0.91	0.73	1.000
19°C:Low:L. major-23°C:High:E. canadensis	-0.16	-0.98	0.65	1.000
16°C:Low:L. major-23°C:High:E. canadensis	-0.26	-1.07	0.56	0.994
23°C:Low:L. major-23°C:High:E. canadensis	0.38	-0.44	1.19	0.904
16°C:Low:E. canadensis-19°C:Low:E. canadensis	-0.03	-0.85	0.78	1.000
23°C:Low:E. canadensis-19°C:Low:E. canadensis	-0.19	-1.01	0.62	1.000
19°C:High:L. major-19°C:Low:E. canadensis	0.11	-0.71	0.93	1.000

16°C:High:L. major-19°C:Low:E. canadensis	-0.03	-0.84	0.79	1.000
23°C:High:L. major-19°C:Low:E. canadensis	-0.19	-1.01	0.63	1.000
19°C:Low:L. major-19°C:Low:E. canadensis	-0.26	-1.08	0.55	0.993
16°C:Low:L. major-19°C:Low:E. canadensis	-0.36	-1.17	0.46	0.932
23°C:Low:L. major-19°C:Low:E. canadensis	0.28	-0.54	1.09	0.989
23°C:Low:E. canadensis-16°C:Low:E. canadensis	-0.16	-0.98	0.66	1.000
19°C:High:L. major-16°C:Low:E. canadensis	0.14	-0.67	0.96	1.000
16°C:High:L. major-16°C:Low:E. canadensis	0.01	-0.81	0.82	1.000
23°C:High:L. major-16°C:Low:E. canadensis	-0.16	-0.97	0.66	1.000
19°C:Low:L. major-16°C:Low:E. canadensis	-0.23	-1.05	0.59	0.998
16°C:Low:L. major-16°C:Low:E. canadensis	-0.32	-1.14	0.49	0.965
23°C:Low:L. major-16°C:Low:E. canadensis	0.31	-0.50	1.13	0.974
19°C:High:L. major-23°C:Low:E. canadensis	0.30	-0.51	1.12	0.978
16°C:High:L. major-23°C:Low:E. canadensis	0.17	-0.65	0.98	1.000
23°C:High:L. major-23°C:Low:E. canadensis	0.00	-0.81	0.82	1.000
19°C:Low:L. major-23°C:Low:E. canadensis	-0.07	-0.89	0.75	1.000
16°C:Low:L. major-23°C:Low:E. canadensis	-0.16	-0.98	0.65	1.000
23°C:Low:L. major-23°C:Low:E. canadensis	0.47	-0.34	1.29	0.702
16°C:High:L. major-19°C:High:L. major	-0.14	-0.95	0.68	1.000
23°C:High:L. major-19°C:High:L. major	-0.30	-1.12	0.52	0.980
19°C:Low:L. major-19°C:High:L. major	-0.37	-1.19	0.44	0.909
16°C:Low:L. major-19°C:High:L. major	-0.47	-1.28	0.35	0.710
23°C:Low:L. major-19°C:High:L. major	0.17	-0.65	0.98	1.000
23°C:High:L. major-16°C:High:L. major	-0.16	-0.98	0.65	1.000
19°C:Low:L. major-16°C:High:L. major	-0.24	-1.05	0.58	0.997
16°C:Low:L. major-16°C:High:L. major	-0.33	-1.15	0.48	0.959
23°C:Low:L. major-16°C:High:L. major	0.30	-0.51	1.12	0.978
19°C:Low:L. major-23°C:High:L. major	-0.07	-0.89	0.74	1.000
16°C:Low:L. major-23°C:High:L. major	-0.17	-0.98	0.65	1.000
23°C:Low:L. major-23°C:High:L. major	0.47	-0.35	1.28	0.712
16°C:Low:L. major-19°C:Low:L. major	-0.09	-0.91	0.72	1.000
23°C:Low:L. major-19°C:Low:L. major	0.54	-0.27	1.36	0.503
23°C:Low:L. major-16°C:Low:L. major	0.64	-0.18	1.45	0.270

Table S3: Summary of Tukey's HSD post hoc test for *Lagarosiphon major*. Temperature (T); Densities (D) and Neighbours species (NS).

<i>Lagarosiphon major</i>	Dif.	Lower CI	Uper CI	<i>p</i>
LBN				
T				
16°C-19°C	0.00	-0.46	0.45	1.000
23°C-19°C	-0.45	-0.91	0.00	0.053 .
23°C-16°C	-0.45	-0.90	0.01	0.055 .
D				
Low-High	-0.31	-0.62	0.00	0.050 .
NS				
<i>E. densa</i> - <i>E. canadensis</i>	0.30	-0.01	0.61	0.060 .
T x NS				
16°C: <i>E. canadensis</i> -19°C: <i>E. canadensis</i>	0.41	-0.38	1.20	0.652
23°C: <i>E. canadensis</i> -19°C: <i>E. canadensis</i>	-0.62	-1.41	0.17	0.206
19°C: <i>E. densa</i> -19°C: <i>E. canadensis</i>	0.46	-0.33	1.25	0.526
16°C: <i>E. densa</i> -19°C: <i>E. canadensis</i>	0.05	-0.74	0.84	1.000
23°C: <i>E. densa</i> -19°C: <i>E. canadensis</i>	0.17	-0.62	0.96	0.986
23°C:<i>E. canadensis</i>-16°C:<i>E. canadensis</i>	-1.02	-1.81	-0.23	0.005 **
19°C: <i>E. densa</i> -16°C: <i>E. canadensis</i>	0.05	-0.74	0.84	1.000
16°C: <i>E. densa</i> -16°C: <i>E. canadensis</i>	-0.36	-1.15	0.43	0.755
23°C: <i>E. densa</i> -16°C: <i>E. canadensis</i>	-0.23	-1.02	0.56	0.951
19°C:<i>E. densa</i>-23°C:<i>E. canadensis</i>	1.08	0.29	1.87	0.003 **
16°C: <i>E. densa</i> -23°C: <i>E. canadensis</i>	0.66	-0.13	1.45	0.147
23°C:<i>E. densa</i>-23°C:<i>E. canadensis</i>	0.79	0.00	1.58	0.050 *
16°C: <i>E. densa</i> -19°C: <i>E. densa</i>	-0.41	-1.20	0.38	0.636
23°C: <i>E. densa</i> -19°C: <i>E. densa</i>	-0.28	-1.08	0.51	0.891
23°C: <i>E. densa</i> -16°C: <i>E. densa</i>	0.13	-0.66	0.92	0.997
Root number				
D				
Low-High	-0.47	-0.72	-0.22	0.000 *
D x NS				
Low: <i>E. canadensis</i> -High: <i>E. canadensis</i>	-0.13	-0.60	0.33	0.871
High:<i>E. densa</i>-High:<i>E. canadensis</i>	0.56	0.09	1.03	0.013 *
Low: <i>E. densa</i> -High: <i>E. canadensis</i>	-0.25	-0.71	0.22	0.503
High:<i>E. densa</i>-Low:<i>E. canadensis</i>	0.69	0.23	1.16	0.001 **
Low: <i>E. densa</i> -Low: <i>E. canadensis</i>	-0.11	-0.58	0.35	0.918
Low:<i>E. densa</i>-High:<i>E. densa</i>	-0.81	-1.27	-0.34	0.000 *
T x D x NS				
16°C:High: <i>E. canadensis</i> -19°C:High: <i>E. canadensis</i>	-0.49	-1.53	0.56	0.899
23°C:High: <i>E. canadensis</i> -19°C:High: <i>E. canadensis</i>	-0.74	-1.78	0.30	0.405
19°C:Low: <i>E. canadensis</i> -19°C:High: <i>E. canadensis</i>	-0.66	-1.70	0.39	0.586

16°C:Low: <i>E. canadensis</i> -19°C:High: <i>E. canadensis</i>	-0.44	-1.48	0.61	0.948
23°C:Low: <i>E. canadensis</i> -19°C:High: <i>E. canadensis</i>	-0.53	-1.58	0.51	0.832
19°C:High: <i>E. densa</i> -19°C:High: <i>E. canadensis</i>	-0.05	-1.10	0.99	1.000
16°C:High: <i>E. densa</i> -19°C:High: <i>E. canadensis</i>	0.04	-1.00	1.09	1.000
23°C:High: <i>E. densa</i> -19°C:High: <i>E. canadensis</i>	0.47	-0.58	1.51	0.924
19°C:Low: <i>E. densa</i> -19°C:High: <i>E. canadensis</i>	-0.45	-1.49	0.60	0.943
16°C:Low: <i>E. densa</i> -19°C:High: <i>E. canadensis</i>	-0.67	-1.71	0.38	0.562
23°C:Low: <i>E. densa</i> -19°C:High: <i>E. canadensis</i>	-0.85	-1.90	0.19	0.209
23°C:High: <i>E. canadensis</i> -16°C:High: <i>E. canadensis</i>	-0.25	-1.30	0.79	0.999
19°C:Low: <i>E. canadensis</i> -16°C:High: <i>E. canadensis</i>	-0.17	-1.21	0.88	1.000
16°C:Low: <i>E. canadensis</i> -16°C:High: <i>E. canadensis</i>	0.05	-0.99	1.09	1.000
23°C:Low: <i>E. canadensis</i> -16°C:High: <i>E. canadensis</i>	-0.05	-1.09	1.00	1.000
19°C:High: <i>E. densa</i> -16°C:High: <i>E. canadensis</i>	0.43	-0.61	1.48	0.953
16°C:High: <i>E. densa</i> -16°C:High: <i>E. canadensis</i>	0.53	-0.51	1.57	0.840
23°C:High: <i>E. densa</i> -16°C:High: <i>E. canadensis</i>	0.95	-0.09	2.00	0.103
19°C:Low: <i>E. densa</i> -16°C:High: <i>E. canadensis</i>	0.04	-1.00	1.09	1.000
16°C:Low: <i>E. densa</i> -16°C:High: <i>E. canadensis</i>	-0.18	-1.22	0.86	1.000
23°C:Low: <i>E. densa</i> -16°C:High: <i>E. canadensis</i>	-0.37	-1.41	0.68	0.986
19°C:Low: <i>E. canadensis</i> -23°C:High: <i>E. canadensis</i>	0.08	-0.96	1.13	1.000
16°C:Low: <i>E. canadensis</i> -23°C:High: <i>E. canadensis</i>	0.30	-0.74	1.35	0.997
23°C:Low: <i>E. canadensis</i> -23°C:High: <i>E. canadensis</i>	0.21	-0.84	1.25	1.000
19°C:High: <i>E. densa</i> -23°C:High: <i>E. canadensis</i>	0.69	-0.36	1.73	0.521
16°C:High: <i>E. densa</i> -23°C:High: <i>E. canadensis</i>	0.78	-0.26	1.83	0.323
23°C:High:<i>E. densa</i>-23°C:High:<i>E. canadensis</i>	1.21	0.16	2.25	0.012 *
19°C:Low: <i>E. densa</i> -23°C:High: <i>E. canadensis</i>	0.29	-0.75	1.34	0.998
16°C:Low: <i>E. densa</i> -23°C:High: <i>E. canadensis</i>	0.07	-0.97	1.12	1.000
23°C:Low: <i>E. densa</i> -23°C:High: <i>E. canadensis</i>	-0.11	-1.16	0.93	1.000
16°C:Low: <i>E. canadensis</i> -19°C:Low: <i>E. canadensis</i>	0.22	-0.83	1.26	1.000
23°C:Low: <i>E. canadensis</i> -19°C:Low: <i>E. canadensis</i>	0.12	-0.92	1.17	1.000
19°C:High: <i>E. densa</i> -19°C:Low: <i>E. canadensis</i>	0.60	-0.44	1.65	0.705
16°C:High: <i>E. densa</i> -19°C:Low: <i>E. canadensis</i>	0.70	-0.35	1.74	0.493
23°C:High:<i>E. densa</i>-19°C:Low:<i>E. canadensis</i>	1.12	0.08	2.17	0.025 *
19°C:Low: <i>E. densa</i> -19°C:Low: <i>E. canadensis</i>	0.21	-0.83	1.25	1.000
16°C:Low: <i>E. densa</i> -19°C:Low: <i>E. canadensis</i>	-0.01	-1.05	1.03	1.000
23°C:Low: <i>E. densa</i> -19°C:Low: <i>E. canadensis</i>	-0.20	-1.24	0.85	1.000
23°C:Low: <i>E. canadensis</i> -16°C:Low: <i>E. canadensis</i>	-0.10	-1.14	0.95	1.000
19°C:High: <i>E. densa</i> -16°C:Low: <i>E. canadensis</i>	0.38	-0.66	1.43	0.980
16°C:High: <i>E. densa</i> -16°C:Low: <i>E. canadensis</i>	0.48	-0.56	1.52	0.908
23°C:High: <i>E. densa</i> -16°C:Low: <i>E. canadensis</i>	0.90	-0.14	1.95	0.148
19°C:Low: <i>E. densa</i> -16°C:Low: <i>E. canadensis</i>	-0.01	-1.05	1.04	1.000
16°C:Low: <i>E. densa</i> -16°C:Low: <i>E. canadensis</i>	-0.23	-1.27	0.82	1.000
23°C:Low: <i>E. densa</i> -16°C:Low: <i>E. canadensis</i>	-0.42	-1.46	0.63	0.964
19°C:High: <i>E. densa</i> -23°C:Low: <i>E. canadensis</i>	0.48	-0.56	1.52	0.909
16°C:High: <i>E. densa</i> -23°C:Low: <i>E. canadensis</i>	0.58	-0.47	1.62	0.756
23°C:High: <i>E. densa</i> -23°C:Low: <i>E. canadensis</i>	1.00	-0.04	2.04	0.072
19°C:Low: <i>E. densa</i> -23°C:Low: <i>E. canadensis</i>	0.09	-0.95	1.13	1.000
16°C:Low: <i>E. densa</i> -23°C:Low: <i>E. canadensis</i>	-0.13	-1.18	0.91	1.000

23°C:Low: <i>E. densa</i> -23°C:Low: <i>E. canadensis</i>	-0.32	-1.36	0.72	0.995	
16°C:High: <i>E. densa</i> -19°C:High: <i>E. densa</i>	0.10	-0.95	1.14	1.000	
23°C:High: <i>E. densa</i> -19°C:High: <i>E. densa</i>	0.52	-0.52	1.56	0.854	
19°C:Low: <i>E. densa</i> -19°C:High: <i>E. densa</i>	-0.39	-1.43	0.65	0.977	
16°C:Low: <i>E. densa</i> -19°C:High: <i>E. densa</i>	-0.61	-1.66	0.43	0.682	
23°C:Low: <i>E. densa</i> -19°C:High: <i>E. densa</i>	-0.80	-1.84	0.24	0.293	
23°C:High: <i>E. densa</i> -16°C:High: <i>E. densa</i>	0.42	-0.62	1.47	0.959	
19°C:Low: <i>E. densa</i> -16°C:High: <i>E. densa</i>	-0.49	-1.53	0.56	0.900	
16°C:Low: <i>E. densa</i> -16°C:High: <i>E. densa</i>	-0.71	-1.75	0.33	0.469	
23°C:Low: <i>E. densa</i> -16°C:High: <i>E. densa</i>	-0.90	-1.94	0.15	0.157	
19°C:Low: <i>E. densa</i> -23°C:High: <i>E. densa</i>	-0.91	-1.95	0.13	0.141	
16°C:Low:<i>E. densa</i>-23°C:High:<i>E. densa</i>	-1.13	-2.18	-0.09	0.023	*
23°C:Low:<i>E. densa</i>-23°C:High:<i>E. densa</i>	-1.32	-2.36	-0.28	0.004	**
16°C:Low: <i>E. densa</i> -19°C:Low: <i>E. densa</i>	-0.22	-1.27	0.82	1.000	
23°C:Low: <i>E. densa</i> -19°C:Low: <i>E. densa</i>	-0.41	-1.45	0.63	0.968	
23°C:Low: <i>E. densa</i> -16°C:Low: <i>E. densa</i>	-0.19	-1.23	0.86	1.000	
