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Response capacity to CO_2 depletion rather than temperature and light effects explain the growth success of three alien Hydrocharitaceae compared with native *Myriophyllum triphyllum* in New Zealand

Andreas Hussner[a,b,*], Deborah Hofstra[b], Peter Jahns[a], John Clayton[b]

[a] *Institute of Plant Biochemistry, Heinrich-Heine-Universität Düsseldorf, Universitätsstraße 1, D-40225 Düsseldorf, Germany*
[b] *National Institute of Water and Atmospheric Research, Gate 10 Silverdale Road Hillcrest, Hamilton 3216, PO Box 11115, Hamilton, New Zealand*

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ABSTRACT

Light, temperature and the availability of carbon and nutrients are major factors affecting aquatic plant growth. A three factorial experimental design in a large outdoor mesocosm experiment was used to evaluate the effects of light, temperature and carbon on the growth parameters of RGR (relative growth rate), LDMC (leaf dry matter content) and root:shoot ratio as well as pigment characteristics of invasive *Hydrilla verticillata, Egeria densa* and *Lagarosiphon major* and a native New Zealand milfoil, *Myriophyllum triphyllum*. The RGR of *H. verticillata, E. densa* and *L. major* was mainly affected by temperature, while RGR in *M. triphyllum* was predominantly affected by carbon. In *H. verticillata* light had a significant effect on the LDMC, whereas the LDMC of the other species was predominantly affected by carbon.

The pigment contents of all species were significantly higher in the low light treatments, and for *H. verticillata*, *E. densa* and *M. triphyllum* a significant effect of carbon was found. The $Chl_{a:b}$ ratio tended to increase with decreasing temperature (except for *M. triphyllum*) and decreasing light availability (except for *E. densa*). Carbon only had a significant effect on $Chl_{a:b}$ ratio of *M. triphyllum*. Generally, the growth and pigments of the three Hydrocharitaceae were mostly affected by temperature and light, while in *M. triphyllum* a strong effect of carbon was found. This could in part explain the relative success of the three exotic species within New Zealand waters, where *M. triphyllum* has been displaced by dense populations of these species in numerous waters.

1. Introduction

The growth of submerged aquatic plants is mainly affected by the light availability, nutrients, temperature, dissolved inorganic carbon (DIC) and the portion of the different carbon forms, which are determined by water pH (Barko and Smart, 1981; Barko et al., 1982; Sand-Jensen, 1989; Bowes and Salvucci, 1989). Even though most aquatic plants occur over a wide geographic range and are thus adapted to different habitat conditions (Cook, 1985; Santamaria, 2002), changes in light and temperature causes significant acclimation within submerged aquatic plants, which have been shown in several studies (e.g. Sculthorpe, 1967; Barko and Smart, 1981; Barko et al., 1982; Middelboe and Markager, 1997; Hussner et al., 2010, 2011). Even small differences, in for example water temperature, can affect the competitive strength of species, which has implications for assessing and predicting invasion by exotic plants (Sorte et al., 2013).

In addition to the effects of light and temperature, the availability of DIC resp. gaseous CO_2 might explain the growth performance and the presence or absence of some submerged plant species in aquatic habitats. Low CO_2 conditions occur either in habitats with naturally low DIC conditions or due to the carbon uptake by primary producers which increases the pH in dense macrophyte stands (Sand-Jensen, 1989; Santamaria, 2002). The ability to use both CO_2 and HCO_3^- can be regarded as a major factor influencing the distribution of submerged species in aquatic habitats (Sand-Jensen, 1989; Maberly and Madsen, 1998, 2002). While some species, like *Hygrophila polysperma* Roxb. T. Anderson, are restricted to CO_2 usage, other species (e.g. *Hydrilla verticillata* L.f. Royle) can grow well under high pH values >9 using HCO_3^- as a carbon source (Bowes and Salvucci, 1989; Pagano and Titus, 2007).

Submerged aquatic plants have developed different strategies to cope with CO_2 depletion. About 50% of all species show a type of a carbon concentrating mechanisms (CCM) and are thus able to use

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[*] Corresponding author at: Institute of Plant Biochemistry, Heinrich-Heine-Universität Düsseldorf, Universitätsstraße 1, D-40225 Düsseldorf, Germany. Tel.: +49 211 8114290.
E-mail addresses: hussnera@uni-duesseldorf.de, hussnera@hhu.de (A. Hussner).

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HCO_3^- as an additional carbon source (Madsen and Sand-Jensen, 1991; Maberly and Madsen, 2002). The most well documented CCM in submerged plants is the single cell C_4 mechanism in *H. verticillata* and *Egeria densa* Planch., which showed increased PEPC activity under CO_2 depletion (Bowes, 2011), while most other CCMs are still largely unknown (Bowes, 2011). However, there is some evidence, that the capacity to use HCO_3^- as a carbon source under CO_2 depletion is a common trait in invasive submerged aquatic plants (Spencer and Bowes, 1990; Hussner, 2012).

Based on these physiological acclimations, differences in some general growth parameters (relative growth rate, RGR, e.g. Madsen, 1993; Hussner and Jahns, in press; root to shoot ration, Hussner and Jahns, in press) and plant traits (e.g. specific leaf area, SLA, Madsen et al., 1996; leaf dry matter content, LDMC, Hussner and Jahns, in press; Eusebio Malheiro et al., 2013) have been demonstrated amongst submerged aquatic plants under different DIC concentrations.

Submerged aquatic plants are known for their generally high phenotypic plasticity, which allows the species to cover a wide geographic range (Santamaria, 2002; Riis et al., 2010). The driving forces of the phenotypic plasticity are often highly diverse (e.g. Strand and Weisner, 2001; Santamaria, 2002), but light and temperature have been shown as drivers for morphological changes (Barko and Smart, 1981; Barko et al., 1982; Riis et al., 2010). Recently a strong phenotypic response was reported in submerged *Myriophyllum aquaticum* (Vell.) Verdc. under limiting CO_2 conditions (Eusebio Malheiro et al., 2013). In this example, leaf surface was maximized to increase the uptake of CO_2 (Eusebio Malheiro et al., 2013). Winkel and Borum (2009) described improved connections to high concentrations of sedimentary CO_2 via the roots, which serve as an important carbon source for at least some submerged species.

In the present study, the effects of temperature, light and CO_2 availability on the growth and pigment content of three invasive alien submerged plant species and one New Zealand native plant were investigated. *H. verticillata*, *E. densa* and *Lagarosiphon major* (Ridl.) Moss are three elodeid species from the family hydrocharitaceae that were introduced into New Zealand and have become nuisance weeds in rivers and lakes (Clayton, 1996). As well as their occurrences in New Zealand, these three species are also known as successful weeds in other parts of the world (Chambers et al., 2008; Hussner, 2012). *Myriophyllum triphyllum* Orchard is a New Zealand native tall growing (<3 m) species of still and flowing waters (Webb et al., 1988; Coffey and Clayton, 1988). It is recognized that *H. verticillata, L. major* and *E. densa* have a competitive advantage against New Zealand macrophytes, as these hydrocharitaceaen species form light excluding canopies over the top of the native vascular plants. Thus, in addition to the recognized patterns of growth amongst these species, it was hypothesized in the present study, that aside from the effects of light and temperature, the carbon availability will have a species specific effect on plant growth, as native *M. triphyllum* may have (like most other *Myriophyllum* species, Maberly and Madsen, 2002) a lower capacity to use HCO_3^- than exotic *H. verticillata, E. densa* and *L. major* and its growth would be more negatively affected under low CO_2 conditions. This would lead to lower growth rates and potentially competitive strength of *M. triphyllum* in waters with high macrophyte densities, which usually show high pH values during the day due to the photosynthetic carbon uptake.

2. Material and methods

2.1. Plant cultivation

Myriophyllum triphyllum was collected from the Waihou River, North Island, New Zealand, where the species forms dense beds in the spring fed fast flowing river. *H. verticillata*, *E. densa* and *L. major* were collected from cultivated plants within the secure plant compound of the NIWA (National Institute of Water and Atmospheric Research) research facilities at Ruakura. All plants were pre-cultivated prior to the experiment, planted in the same substrate that was also used for the subsequent experiment, under 80% shade cloth in a large flow-through tank at the NIWA research facility.

For the experiments, similar sized shoots of *H. verticillata*, *E. densa*, *L. major* and *M. triphyllum* were planted in small pots (diameter of 8 cm and a volume of approx. 300 ml), that were filled with soil (containing 0.36% particulate nitrogen and 770 mg total recoverable phosphorus per kg soil) that was covered with a 0.5 cm thick layer of sand. Representative shoots of each species were used to determine initial dry weights (DW) after drying shoots at 90 °C to a constant weight. Initial dry weights were (Mean ± SE): 0.051 ± 0.017 g for *H. verticillata*, 0.119 ± 0.043 g for *E. densa*, 0.143 ± 0.027 g for *L. major* and 0.158 ± 0.055 g for *M. triphyllum*.

2.2. Experimental setup

The experimental design was a three factorial design (light × temperature × carbon). Large temperature controlled outdoor mesocosm tanks at the NIWA Ruakura research facility were used (Burnett et al., 2007) for the experiment. Six tanks in total were used as large water baths, with two tanks adjusted to one of the three temperatures (15, 20 and 25 °C). One tank at each temperature was shaded by a 50% shade cloth (high light, HL), the other by 80% shade cloth (low light, LL), resulting in two different light availabilities for each different temperature treatment. The irradiance was measured 5 cm below the water surface and logged twice per minute by an underwater PAR sensor (Li192SA, LiCor, Lincoln, USA). The maximum irradiances during the days were 1237 μmol photons $m^{-2} s^{-1}$ (HL) and 494 μmol photons $m^{-2} s^{-1}$ (LL) (95% percentiles: HL: 618 μmol photons $m^{-2} s^{-1}$; LL: 247 μmol photons $m^{-2} s^{-1}$), with means of 172 μmol photons $m^{-2} s^{-1}$ (HL) and 69 μmol photons $m^{-2} s^{-1}$ (LL). Daily sum of irradiance in mol m^{-2} under the 80% shade cloth was: Min 0.62, Mean 2.79, Max 6.85 and under the 50% shade cloth was: Min 1.56, Mean 6.97, Max 17.12.

Twelve 30 L buckets were placed in each of the six tanks to provide independent experimental units, with each bucket containing one pot of each plant species. The buckets were filled with water from a storage tank, with a conductivity of 202 $\mu S\,cm^{-1}$ and an initial pH of 7.0 ± 0.1. Six of the buckets per tank were continuously bubbled with ambient air resulting in a pH of 8.0 ± 0.2 and low amounts of free CO_2 (LC treatments). The remaining six buckets had CO_2 added continuously from commercial gas bottles with

Table 1

The availability of CO_2 and HCO_3^- within the different treatments. Data shown are means ± SE. (15, 20, 25: temperature in °C; LL: low light, HL: high light; LC: low CO_2, HC: high CO_2).

Treatment	CO_2 [mmol/l]	HCO_3^- [mmol/l]
15, HL, LC	0.02 ± 0.00	0.39 ± 0.06
15, LL, LC	0.02 ± 0.00	0.44 ± 0.01
15, HL, HC	0.52 ± 0.06	0.61 ± 0.02
15, LL, HC	0.56 ± 0.14	0.59 ± 0.07
20, HL, LC	0.01 ± 0.01	0.40 ± 0.04
20, LL, LC	0.01 ± 0.01	0.41 ± 0.03
20, HL, HC	0.46 ± 0.09	0.57 ± 0.03
20, LL, HC	0.48 ± 0.04	0.59 ± 0.04
25, HL, LC	0.01 ± 0.00	0.41 ± 0.02
25, LL, LC	0.02 ± 0.00	0.43 ± 0.05
25, HL, HC	0.43 ± 0.06	0.56 ± 0.05
25, LL, HC	0.45 ± 0.08	0.62 ± 0.03