Carbon assimilation through a vertical light gradient in the canopy of invasive herbs grown under different temperature regimes is determined by leaf- and whole plant-architecture

Andreas Jorgensen¹*, Brian K. Sorrell¹, Franziska Eller¹

¹Aarhus University, Department of Bioscience, Ole Worms Alle 1, 8000 Aarhus C, Denmark

*Corresponding author: andreas.andreasjorgensen@gmail.com
• **Background and Aims** This study examined the acclimation to temperature of two globally invasive species *Iris pseudacorus* and *Lythrum salicaria*, which share the same habitat type but differ in morphology. *Iris pseudacorus* has long vertical leaves, allowing light penetration through the canopy, while *L. salicaria* has stems with small horizontal leaves, creating significant self-shading. We aimed to build a physiological understanding of how these two species respond to different growth temperatures with regard to growth and gas exchange-related traits over the canopy.

• **Methods** Growth and gas exchange-related traits in response to low (15 °C) and high (25 °C) growth temperature regimes were compared. Plants were grown in growth chambers, and light response curves were measured with infrared gas-analyzers after 23-33 days at three leaf positions on each plant, following the vertical light gradient through the canopy. After 37 days of growth, above-ground biomass, photosynthetic pigments and leaf N concentration were determined.

• **Key results** The maximum photosynthesis rate was lower in lower leaf positions but did not differ significantly between temperatures. *Iris pseudacorus* photosynthesis decreased with decreasing leaf position, more so than *L. salicaria*. This was explained by decreasing N and chlorophyll concentrations towards the leaf base in *I. pseudacorus*, while pigment concentrations increased towards the lower canopy in *L. salicaria*. Biomass, shoot height and specific leaf area increased with temperature, more so in *I. pseudacorus* than in *L. salicaria*. Light response curves revealed that *L. salicaria* had a higher degree of shade-acclimation than *I. pseudacorus*, probably due to self-shading in *L. salicaria*.

• **Conclusions** High temperature decreased C-assimilation at the bottom of the canopy in *L. salicaria*, while C-assimilation in *I. pseudacorus* was less affected by temperature. As vegetative growth and flowering was stimulated by temperature, the invasive potential of these species is predicted to increase under global warming.
Key words: horizontal leaves, *Iris pseudacorus* L., lanceolate leaves, light-response curves, *Lythrum salicaria* L., maximum light-saturated photosynthesis rate, N allocation, photosynthetic pigments
INTRODUCTION

Invasive plant species negatively affect native species diversity and ecosystem functioning (Mack et al., 2005; Gritti et al., 2006). After novel species are introduced to new habitats by human activity, natural species distribution patterns can be altered, causing potentially severe changes to ecosystem dynamics. As climatic conditions are a major constraint on the distribution of plant species, climate change can also be a powerful driver of both native and invasive species distributions (Fernández de Castro et al., 2018; Moran and Alexander, 2014; Walther et al., 2009). Climate warming is likely to shift habitats to higher latitudes and elevations (IPCC, 2014), which could promote invasions by species already adapted to higher temperatures, ultimately shifting species distributions globally.

Invasive plants are often characterized by high productivity and effective dispersal, enabling them to out-compete less productive native species and dominate ecosystems where they are introduced. Invasive strategies for maintaining rapid dispersal include, but are not limited to, large numbers of seeds produced, high seed viability, dispersal by fragmentation and spread by rhizomes or, as is often the case, combinations of these (Thompson et al., 1987; Gaskin et al., 2016). Furthermore, productivity is generally higher at high temperatures since metabolic activity increases with temperature (Berry and Bjorkman, 1980). The positive relationship between temperature and productivity favours already highly productive species (Dachler, 2003), enhancing their capacity for rapid growth and resource competition. Morphology can also be important in determining the invasive success of a species, since morphological traits such as leaf or root system architecture greatly affect the ability to utilize resources such as nutrients and light energy (Comas et al., 2013; Wells et al., 1986).

With temperatures rising globally and invasions likely becoming more frequent, it is increasingly important to understand physiological responses of invasive plants to warmer temperatures. Our study focuses on purple loosestrife (*Lythrum salicaria* L.) and yellow flag
iris (*Iris pseudacorus* L.), two herbaceous wetland species native to Europe that are highly invasive on other continents. *Lythrum salicaria* behaves invasively in USA and Canada (Blossey et al., 2001; Hovick et al., 2011), while *I. pseudacorus* has been formally classified as a weed in the USA (USDA, 2013), Canada (Forest Operations, Lands & Natural Resource, 2019), Japan (Ministry of the Environment, 2005), New Zealand (Ministry for Primary Industries, 2017) and South Africa (Department of Environmental Affairs, 2016). Both species are common in water-saturated soils in wetlands and adjacent to lakes, ponds, rivers or streams in temperate and subtropical regions (Mal et al., 1992; Jacobs et al., 2011). In Europe, both species have natural ranges from Norway to southern Spain (Barkley et al., 1980; Thompson et al., 1987). This large latitudinal gradient means that both species can persist across a relatively wide thermal range, which is indicative of some degree of thermal plasticity in both species. These two species provide an interesting subject for invasion biology, because they grow under similar environmental conditions, yet they have distinctly different morphologies, providing two seemingly successful adaptive strategies for colonizing freshwater wetland habitats.

The aim of this study was therefore to understand how the two globally invasive species *I. pseudacorus* and *L. salicaria* respond to different growth temperatures in a comparative analysis of growth and gas exchange-related traits, as well as pigment, nitrogen and carbon content. *Lythrum salicaria*, a perennial eudicot, has multiple woody stems with numerous side-branches. Both stems and side-branches have large numbers of relatively small horizontal leaves (Mullin, 1998). This growth form creates a significant amount of self-shading, especially in the lower parts of the canopy. Self-shading does not merely attenuate the incoming light quantity, it also results in light scattering or diffuse light. Diffuse light can result in lowered photosynthesis rates even at similar total irradiance (Earles et al., 2017; Urban et al., 2014; Brodersen et al., 2008). The shading effects may therefore be amplified by
diffuse lighting, although the response seems to be species-specific and driven by leaf biochemical traits (Berry and Goldsmith, 2019). Although assimilation rates are expressed on an area-basis, total leaf area does not necessarily equal plant growth, as C partitioning between organs can differ and lead to significant changes in plant growth (Weraduwage et al., 2015). While a large leaf area index (LAI) allows the plant to harvest large amounts of light energy per unit ground area, respiration may increase in certain plant species due to the larger amount of shaded leaf area, lowering net C assimilation (Bunce, 1989). As respiration generally increases faster with temperature than photosynthesis (Berry and Bjorkman, 1980), we hypothesized that L. salicaria is better adapted to cooler growth temperatures where C loss due to respiration is limited. Furthermore, since L. salicaria is a C3 plant, both photosynthesis and photorespiration are expected to increase with temperature, which means that even though the photosynthesis rate (A) is likely to increase with temperature, a smaller increase in C-assimilation is expected due to photorespiratory C-loss. These conditions favour photosynthesis at low temperatures.

*Iris pseudacorus*, a perennial monocot, has long vertical leaves (up to 1 meter) produced directly from the base of the plant. This leaf architecture minimizes self-shading and thereby reduces daytime respiration from shaded leaves (Jespersen et al., 2017). A high maximum light-saturated photosynthesis rate ($A_{\text{max}}$) is expected throughout the plant since most of the leaf area is exposed to high light intensities. However, prolonged exposure to very high light intensities can cause photoinhibition, primarily because of damage to photosystem II (PSII), which decreases photosynthesis rate (Aro et al., 1993; Powles, 1984). Photoinhibition is often more severe at low temperatures (Long and Humphries, 1994; Takahashi and Murata, 2008) because of a slower repair of PSII due to lower activity of repair enzymes at low temperatures (Takahashi and Murata, 2008). We therefore hypothesize
that *I. pseudacorus* is best adapted to warm growing temperatures where photoinhibition is limited.

With light intensity decreasing through the canopy, a decreasing $A_{\text{max}}$ from the top to the bottom of the plant is expected for both species. As light attenuation through the canopy is likely to be significantly greater in *L. salicaria* than in *I. pseudacorus* due to greater self-shading, a greater decrease in assimilation rates is expected in *L. salicaria*.

**MATERIALS AND METHODS**

*Plant material and experimental setup*

Seeds of *Lythrum salicaria* and *Iris pseudacorus* were germinated in a greenhouse in early August 2018. *Lythrum salicaria* seeds were commercial seeds from Denmark. *Iris pseudacorus* seeds were taken from a Danish population which had been grown in a greenhouse for at least 2 years (originally collected in the wild). On 27 August seedlings were brought to the growth facilities at Aarhus University. Each seedling was placed in a 1.5 L pot and peat soil was used as the growth substrate. Eight replicates of each species were placed on the floor in 4.9 m$^2$ growth cambers (Karl Weis, Berlin, Germany) at 15 °C (low temperature treatment) and 25 °C (high temperature treatment) during the light period. The temperature regimes were chosen to differ greatly to cause detectable acclimation responses, but also to lie within the natural range of the species’ experienced growth temperatures. Seedlings from each species were of similar height at the beginning of the experiment. Mean
height of *I. pseudacorus* seedlings was 57.2 ± 5.3 cm while *L. salicaria* seedlings measured 50.3 ± 17.4 cm.

The growth chamber settings were as follows: Light intensity at 1 meter above the floor was 400 μmol m⁻² s⁻¹ PAR (photosynthetic active radiation) using Phillips GP-E 600W 400V light sources. Relative humidity was ~75%. The day-night cycle was 16 hours of light and 8 hours of darkness and night temperatures were 2 °C lower than the day temperatures. Twice weekly each plant was given 100 mL of a nutrient solution consisting of 500 mg L⁻¹ “Pioner NPK Makro 19-2-15 + Mg (Green)” and 0.05 mL L⁻¹ “Pioner Mikro Plus with Iron” (Horticoop Scandinavia). Nutrient concentrations are listed in Table 1. The pH of the nutrient solution was adjusted with sulfuric acid to ~6.5 in order to maximize nutrient uptake by the plants. The plants were watered approx. daily as needed. Twice weekly the plants were rotated within the chamber in order to avoid effects of environmental gradients inside the chamber. The species were not mixed but grouped separately within the chamber to avoid shading effects, especially in the *I. pseudoacorus* canopy. The plants were grown in the chambers for 37 days until they were harvested on 2 October.

**Growth parameters**

For each plant, shoot height and above-ground biomass were determined on the final day of the treatment. For *I. pseudacorus*, height was measured from the soil surface to the tip of the longest leaf. For *L. salicaria*, height was measured from the soil surface to highest point of the plant, either a leaf or inflorescence. The plants were then harvested and the above-ground biomass determined after drying in an oven at 80 °C for 48 hours, followed by drying at 60 °C for 72 hours.
Photosynthetic parameters

Three leaf positions on each plant were marked. On *L. salicaria*, leaf positions represented distinct leaves positioned along the vertical light gradient through the canopy. Leaves were clustered partly within each other in *I. pseudacorus*, hence leaf positions were chosen along the same, outer leaf to obtain measurements from the entire vertical light gradient. The leaf positions were denoted as upper (top of plant), middle (middle of plant) and lower (bottom of plant) position.

Photosynthetic light response curves were obtained after 23-33 days of temperature treatment. *Lythrum salicaria* was measured between 18-21 September and *I. pseudacorus* was measured between 24-28 September. Measurements were made between 08:00 and 18:00 hours. Light response curves were obtained using two LI-6800 portable photosynthesis systems with 6800-02 LED light sources (Li-COR Biosciences, Lincoln, Nebraska, USA). Of these, one was used in the low temperature chamber and the other in the high temperature chamber. Pilot measurements under similar environmental conditions showed that measuring differences between the two machines were negligible. A built-in automatic program for light response curves was used with a minimum waiting time of 90 s and a maximum waiting time of 180 s between each measurement. Before the curve was started, gas exchange rates were allowed to stabilize at the highest light intensity. The photosynthesis rate was then measured sequentially at 2000, 1500, 1000, 500, 250, 120, 60, 30, 15 and 0 μmol m⁻² s⁻¹ PAR. Relative air humidity was set to 55% and air flow was set to 500 μmol s⁻¹. The leaf chamber CO₂ concentration was set at 400 μmol mol⁻¹ with carbon dioxide provided from an external CO₂ cartridge. The leaf temperature was set to 16 °C in the low temperature treatment and 26 °C in the high temperature treatment. The set leaf temperatures were chosen to resemble the actual leaf temperatures measured with the LI-6800 portable photosynthesis system right before conducting the light response curves. The built-in LI-190 PAR light sensor was used to
determine the ambient light intensity at all leaf positions upon measurement of the light response curves. It was ensured that the upper position was similar for all replicates, and that the lower positions were 50-100 μmol m\(^{-2}\) s\(^{-1}\) PAR lower than the upper position. The relatively large difference in light intensities between positions (Table 2) resulted from large differences between plant stature and growth of leaves at different heights between temperature treatments and species. Relative differences in light intensity between leaf positions as well as at upper leaf positions are shown in Table 2.

For *I. pseudacorus*, which had parallel leaves, the measured leaf area was determined using a ruler and entered into the gas analyser prior to measuring light response curves for each leaf position. For *L. salicaria*, which has lanceolate leaves, the leaf area was set at a constant value (3 cm\(^2\)) on the gas analyser and corrected afterwards by harvesting the leaf and determining the exact leaf area, which had been inside the chamber, with a LI-3100 area meter (Li-COR Biosciences, Lincoln, NE, USA). It was necessary to calculate the corrected photosynthetic rate for each leaf using this area. This was done by entering the corrected areas into the Excel spreadsheet generated by the gas analyser console at the end of each measuring day.

Light response curves were fitted with Excel macros provided as online material by Lobo et al. (2013). The model fitting the equation of Prioul and Chartier (1977) was chosen since it provided the lowest error sum of squares (SSE). The equation of the fit is given by equation (1), where \(A\) = photosynthesis rate, \(I\) = light intensity, \(\Phi\) = quantum yield, \(R_d\) = dark respiration and \(\theta\) = convexity constant:

\[
A = \frac{\Phi I + \sqrt{(\Phi I A_{\text{max}})^2 - 4\Phi I A_{\text{max}} R_d}}{2\theta} - R_d
\]

(1)
A fit was made for each curve and for each leaf position and the following parameters were determined: maximum light saturated photosynthesis rate ($A_{max}$), quantum yield ($\Phi$), light compensation point ($I_c$), light saturation point ($I_k$) and dark respiration rate ($R_d$).

**Specific leaf area (SLA)**

After completing each light response curve, the area of the leaf that had been inside the gas analyzer chamber was marked with yellow tape. The leaves were harvested and the marked areas were excised and measured with a leaf area meter (LI-3100, LiCor Biosciences, Lincoln, NE, USA). The leaf segments were then wrapped in aluminium foil and stored in a freezer at -18 °C. Throughout this procedure, a relatively large proportion of the total biomass of each *I. pseudacorus* replicate was harvested after completing the light response curves, due to the nature of the growth form of this species. The remainder of the harvested leaf was therefore dried in a drying oven for 48 hours at 60 °C and stored in a desiccator to later be added to the species’ aboveground biomass. All frozen leaf segments were freeze-dried for 24 hours and stored in a desiccator until weighing. The leaf segment area was divided by its dry weight to determine SLA as described by Perez-Hardguindeguy (2013).

**Photosynthetic pigments and carbon and nitrogen concentrations of leaves**

The leaf segments from which SLA was determined were ground in a ball mill (MM 400, Retsch, Haan, Germany). Approximately 5 mg of ground leaf was extracted in 96% ethanol, and the concentrations of total chlorophyll (Chl$_{a+b}$), Chl$_a$, Chl$_b$ and total carotenoids (C$_{x+c}$) in the extract were measured by spectrophotometry according to Lichtenthaler (1987). Carbon (C) and nitrogen (N) concentrations in the same ground leaf material were measured with an elemental analyser (Fisons Instruments, Model NA2000, Rodano, MI, Italy).
Statistical analyses

The experiment was a 2 x 2 fully factorial design, with the factors “Temperature” (low and high temperature treatment, respectively 15 °C and 25 °C) and “Species” (L. salicaria and I. pseudoacorus). For light response-related factors and SLA, an additional factor (leaf position; upper, middle and lower) was considered.

Four light response curves showed negative values or zero for $R_\text{d}$, probably due to very low rates of gas exchange. These were all curves for “lower leaf position” in L. salicaria (two replicates in low temperature and two replicates in high temperature). These curves and their related parameters were excluded in the statistical analysis of photosynthetic parameters as well as from the average values of these parameters. Hence, $n$ was six rather than eight for the photosynthetic parameters in those treatments.

A Levene’s test for homogeneity of variance was conducted for all growth, photosynthetic and leaf element-related parameters. Above-ground biomass, C/N and chlorophyll/carotenoid ratios were log$_2$ transformed in order to satisfy homoscedasticity.

For shoot height and above ground biomass a two-way analysis of variance (ANOVA) was conducted. Here, the main factors were temperature (high or low) and species (L. salicaria or I. pseudoacorus). The interaction term “temperature x species” was included in the ANOVA. It was ensured that all plants were initially of similar height by comparing the initial heights of the seedlings within species between temperature treatment groups by using an $F$-test and student’s $t$-test. The initial heights were measured from the top of the pot to the top of the plant. The tests showed no significant differences in initial height between within-species treatment groups. Thus, any differences in final shoot height (within species, between temperature treatments) were a consequence of the temperature treatment.
For all photosynthetic and leaf element-related parameters as well as SLA, a three-way ANOVA was conducted. Here, the fixed main factors were temperature (high or low), species (L. salicaria or I. pseudacorus) and leaf position (upper, middle or lower). All possible interaction terms were included. Type 3 Sum of Squares were used since, by the exclusion of 4 replicates, the three-way factorial design was unbalanced. Finally, a Tukey’s Honestly Significant Differences test was used to determine the differences between treatment groups for all parameters. All statistical analyses were performed with a 5% significance level using RStudio (RStudio Team 2016; RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA).

RESULTS

Growth parameters

Species and growth temperature had a significant effect on both above-ground biomass and shoot height. However, no significant interaction between species and growth temperature was found for these two growth parameters (Table 3). Lythrum salicaria had significantly higher above-ground biomass and shoot height than I. pseudacorus (Figure 1A, B). Both species produced the greatest biomass and tallest shoots in the high temperature treatment (Figure 1A, B). Iris pseudacorus showed a stronger response to increased temperature for both parameters compared to L. salicaria. Iris pseudacorus had 42.31% higher biomass and 39.20% taller shoots at 25 ºC compared to 15 ºC, while L. salicaria only had 22.02% higher biomass and 22.16% taller shoots. We observed that all individuals of L. salicaria at 25 ºC were flowering, compared to 15 ºC, where only 50% of the replicates were flowering, although phenological traits were not quantified. No flowers were produced in I. pseudacorus.
Photosynthetic parameters and SLA

No significant interactions between species and temperature, temperature and leaf position or a three-way interaction were found in any of the measured parameters (Table 3). A significant interaction between species and leaf position was found for 1c, 1k and SLA (Table 3). In L. salicaria, 1c was lowest in the lower and highest in the upper leaf positions, while it remained more similar across leaf positions in I. pseudacorus, although slightly increasing with decreasing leaf position (Figure 2A). The light saturation point was highest in the middle leaf position in I. pseudacorus while it remained similar, albeit somewhat decreasing from upper to lower leaf position, in L. salicaria (Figure 2B). The two species showed opposite responses in SLA across leaf positions. Here, I. pseudacorus had a lower SLA in the lower leaf positions while L. salicaria had a higher SLA in the lower leaf positions (Figure 2C). Temperature did not have a significant effect on any of the physiological parameters except SLA, which was generally higher in the high temperature treatment for both species (Table 3; Figure 2C).

Species and leaf position had a significant effect on A max and Φ but no significant interaction was found (Table 3). Both A max and Φ were significantly higher in I. pseudacorus than in L. salicaria. Overall, upper leaf positions had a higher A max and Φ than lower leaves in both species, although Φ in L. salicaria at low temperature was similar among leaf positions (Figure 2D, E).

Dark respiration rate was not significantly affected by any of the factors included in the ANOVA (Table 3). However, the interaction term “species x leaf position” had P = 0.0617 which was close to the significance threshold of 0.05, and Tukey’s Honestly Significant Differences test indicated a trend of similar R d among leaf positions in I. pseudacorus but decreasing R d with lower leaf position in L. salicaria (Figure 2F). Dark respiration rates were overall highest in I. pseudacorus.
Compared to each other, the leaf positions had similar light responses in both species, with upper leaves reaching higher assimilation rates ($A$) at high light intensity than middle leaves, which again reached higher $A$ at high light intensity than lower leaves (Figure 3). Upper and middle leaf positions generally had similar $A$ whereas lower leaves had overall lower $A$. The observed pattern was especially obvious in *L. salicaria* at high temperature. Standard deviations indicate that these observations might not be significant in most cases.

The shape of the curves was very different between the species. *Lythrum salicaria* leaves showed a steep increase in $A$ with increasing light intensity from 0-250 $\mu$mol PAR m$^{-2}$ s$^{-1}$ which was followed by saturation of the curve with fairly constant $A$ at light intensities above 500 $\mu$mol PAR m$^{-2}$ s$^{-1}$ (Figure 3A, B). *Iris pseudacorus* leaves showed the same initially steep increase in $A$ with increasing light intensity from 0-250 $\mu$mol PAR m$^{-2}$ s$^{-1}$. However, this was followed by a softer flattening of the curve than for *L. salicaria*, and the curve continued increasing at the higher light intensities without reaching saturation at 2000 $\mu$mol PAR m$^{-2}$ s$^{-1}$ (Figure 3C, D).

**Photosynthetic pigments and leaf C and N concentrations**

Total chlorophyll (Chl$\alpha$+$\beta$) and total carotenoid (C$\alpha$+$\epsilon$) concentrations were significantly affected by species, temperature and leaf position. Furthermore, significant interactions were found between species and leaf position and between temperature and leaf position (Table 3). In both species, total chlorophyll and carotenoid concentrations were highest at high temperature, but in some cases mainly at specific leaf positions (Figure 4A, B). In *L. salicaria*, chlorophyll and carotenoid concentrations increased with lower leaf position at both temperatures. In *I. pseudacorus*, the opposite pattern was observed, where “lower” leaf
position had the lowest pigment concentrations (Figure 4A, B). The chlorophyll/carotenoid (Chl\textsubscript{a+b}/C\textsubscript{x+c}) ratio was significantly affected by species and temperature but no significant interactions were found (Table 3). Chlorophyll/carotenoid ratios were higher in \textit{I. pseudacorus} than in \textit{L. salicaria}, and a higher ratio was observed at high temperature in both species. Although the factor “Leaf position” had no significant effect on Chl\textsubscript{a+b}/C\textsubscript{x+c} according to the ANOVA results, a high $F$-ratio with $P = 0.0517$ was found, very close to the significance threshold of $P < 0.05$. Furthermore, Tukey’s Honestly Significant Differences Test showed a trend that Chl\textsubscript{a+b}/C\textsubscript{x+c} decreased with lower leaf position in both species (Figure 4D). Chlorophyll a/b ratio was only affected significantly by temperature (Table 3), with higher Chl a/b at the low temperature.

Leaf N, C and C/N ratio were significantly affected by species, leaf position and their interaction (Table 3). Both C and especially N concentrations were highest in \textit{I. pseudacorus}, but decreased with lower leaf position, while remaining similar among leaf positions in \textit{L. salicaria} (Figure 5A, B). Leaf C/N ratio was 34-104\% higher in \textit{L. salicaria} than in \textit{I. pseudacorus} (Figure 5C). In \textit{I. pseudacorus}, the C/N ratio increased with lower leaf position, in particular at the high temperature, whereas the C/N ratio of \textit{L. salicaria} was similar between leaf positions at both temperatures. Photosynthetic nitrogen use efficiency was significantly affected by species, temperature, leaf position and “species x leaf position” (Table 3, Figure 5D). In general, PNUE was higher at high temperature than at the low temperature. While PNUE of \textit{I. pseudacorus} decreased with lower leaf position, in \textit{L. salicaria} PNUE was similar between leaf positions and only lower at the lowest leaf position in the high temperature.
DISCUSSION

This study aimed to understand gas exchange, growth, pigment and C/N-related responses in *L. salicaria* and *I. pseudacorus* to different temperature regimes in order to build an understanding of temperature acclimation in invasive plants with different morphology. *I. pseudacorus* has vertical leaves which provide minimal self-shading while *L. salicaria* has horizontal leaves and extensive self-shading. We conducted a comparative analysis of the two species exposed to high (25 °C) and low (15 °C) growth temperature treatments. Remarkably, temperature did not significantly affect any of the gas exchange-related traits (*A_{max}, \Phi, I_c, I_k, R_d*). With a temperature difference as large as 10 °C, an effects on these traits would have been expected, since gas exchange is often affected by temperature, even though this can vary among species (Niu et al., 2008; Turnbull et al., 2002). The absence of temperature effects on gas exchange-related traits suggests that the thermal range chosen for our experiment was well within the natural range in which both species can function normally, and that both species are relatively plastic, since they were able to maintain constantly high photosynthesis rates across the temperature range used (Yamori et al. 2005).

We found that leaf position in the canopy had a much stronger effect on gas exchange than temperature. Quantum yield and especially *A_{max}* decreased with lower leaf position in both species. The effect of leaf position was most pronounced in the high temperature treatment and in *L. salicaria*, where the lower leaf position had a very low *A_{max}* compared to middle and upper leaf positions. As light intensity is attenuated through the canopy, photosynthetic rates and *A_{max}* can be expected to decline (Bjorkman, 1981; Hirose and Werger, 1987). A typical response to low light availability is an increased investment in light-harvesting associated pigments (Lichtenthaler et al. 1981; Melis and Harvey, 1981). This strategy was indeed used by *L. salicaria*, which allocated more Chl$_{a+b}$ to lower leaves, as opposed to *I. pseudacorus*. Hence, while low *A_{max}* in lower leaf positions can partly be
attributed to less acclimation of light harvesting in *I. pseudoacorus*, this cannot fully explain the response in *L. salicaria*.

Another shade-acclimation strategy of *L. salicaria* was allocation of similar amounts of N to all leaf layers and maintenance of similar C/N ratios. Nitrogen concentration in leaves can serve as a proxy for investment in photosynthetic enzymes (Evans, 1989; Poorter and Evans, 1998; Sun et al. 2016) and, especially at low temperature, this investment lead to sustained PNUE in *L. salicaria*. However, PNUE of the lower leaf was reduced at 25 °C, which suggests either an overinvestment of N or N- allocation to other than assimilation-related structures, since it was not met by a similarly maintained photosynthesis (Onoda et al. 2004). In *I. pseudacorus*, N allocation as well as PNUE was reduced with decreasing leaf position. Also, higher chlorophyll and carotenoid concentrations were observed in upper and middle leaf positions, suggesting that *A*<sub>max</sub> was maximized in upper and middle leaf positions by increased concentrations of photosynthetic pigments and N allocation. *Lythrum salicaria* acclimated to reduced light availability by increasing photosynthetic pigment concentrations, the relative amount of Chls, which is mainly associated with photosynthetic light harvesting complexes (Hoober et al. 2007), and by maintaining high leaf N concentrations in the lower part of the canopy.

SLA also reflected the different light-acclimation strategies of the two species, as SLA of *L. salicaria* increased with lower leaf positions while decreasing in *I. pseudacorus*. Leaves with high SLA are generally thinner than leaves with low SLA, due to fewer structural components. Thin leaves are less costly to produce (Daehler, 2003), which can allow the plant to invest resources in other structures, such as flowers, enhancing dispersal. Thinner leaves also allow for a more efficient gas diffusion over the leaf surface, ultimately favouring higher rates of photosynthesis (Flexas et al., 2012). Moreover, the different growth forms of the two species can explain the differences in SLA. The lower parts of the leaves in *I. pseudacorus* are
likely to contain relatively large amounts of structural leaf components in order to support the long vertical leaf. This trend has been found in grasses which have similar leaf shape (Ocheltree et al., 2012). In *L. salicaria*, the same is not necessary since the leaf is attached to the stem providing structural support. Thinner leaves are a typical acclimation to shade, allowing for a higher light penetration (Blondeel et al., 2020). Shading was more pronounced for lower leaves of *L. salicaria* grown at 25 °C than at 15 °C, which was reflected in the species’ SLA over the canopy.

In general, and unlike *L. salicaria*, *I. pseudacoris* showed no clear signs of shade acclimation in lower leaf positions. The light response curves showed that *I. pseudacorus* did not reach complete light saturation in any leaf position even at 2000 μmol PAR m⁻² s⁻¹, revealing that this species is well-adapted to high light environments throughout its canopy. Such a phenomenon is usually observed in C4-photosynthetic species, but C3 species, like *I. pseudacorus*, can well have high assimilation rates and productivity, due to high inherent photosynthetic traits like carboxylation rate and electron transport rate (Webster et al., 2016). The overall higher chlorophyll, carotenoid and N concentrations of *I. pseudacorus* compared to *L. salicaria* suggested relatively larger amounts of photosynthetic enzymes and chloroplasts (Evans, 1989; Sun et al. 2016). However, net assimilation rates of light response curves showed stronger differences at different leaf positions in *I. pseudacorus* than *L. salicaria*, as a stronger decrease in C assimilation was a response by the less shade-acclimated, lower leaf positions in *I. pseudacorus*.

Long leaves, such as those of *I. pseudacorus* are partitioned during leaf development, with the growing base and the aging tip functioning as sinks to photosynthetic products and the middle of the leaf as transition zone from sink to source (Li et al. 2010). While we tried to ensure to cover a light gradient on leaf parts of similar age by measuring on similar green areas, we cannot completely rule out a certain age-effect over the three leaf
positions on *I. pseudacorus*. However, our light response results strongly suggest that light differences were the main regulating factor, not age. Otherwise, the middle position would have yielded the highest photosynthetic activity, which was not the case. Also, we would have detected much higher respiration rates in the lower leaf position, due to high metabolic activity in developing leaf parts (Li et al. 2010).

The cardinal points derived from the light response curves reveal further differences in photosynthetic light acclimation strategies between the two species. Thus, the light compensation point (*I*<sub>c</sub>) increased with lower leaf position in *I. pseudacorus* while it decreased in *L. salicaria*. Light compensation point defines the light intensity at which photosynthesis and dark respiration (*R*<sub>d</sub>) are balanced, and net assimilation rates are zero. *Iris pseudacorus* had similar *R*<sub>d</sub> at all leaf positions while photosynthesis decreased towards the bottom of the canopy. Hence, net C gain was greater in the upper canopy compared to the lower canopy where respiration was higher relative to photosynthesis rate. In contrast to this, *L. salicaria* had decreasing Φ, *R*<sub>d</sub> and *A*<sub>max</sub> towards the bottom of the canopy, resulting in decreasing *I*<sub>c</sub>, a typical acclimation to shading (Herrmann et al. 2019). Reduced *R*<sub>d</sub> in shade leaves is symptomatic for a lower metabolic activity and decreased need for protection from high irradiance (Plaxton, 1996; Herrmann et al. 2019). Morphologically, *I. pseudacorus* is potentially exposed more often to higher radiation in the lower canopy, due to its lanceolate leaves, in contrast to *L. salicaria*, in which leaves at the bottom of the canopy are mostly self-shaded. Hence, higher *R*<sub>d</sub> in *I. pseudacorus* may facilitate protection from solar radiation and support the lower SLA and higher structural support needed. Some caution of interpreting C gain based on *A*<sub>max</sub> and *R*<sub>d</sub> is, however, advised. Day respiration in light is not necessarily equal to *R*<sub>d</sub>, since part of the CO<sub>2</sub> released during daytime, by day respiration or photorespiration, can be re-captured within the photosynthetic cell (Tcherkez et al., 2017). Also, day respiration is inhibited in the light (Heskel and Tang, 2018; Keenan et al., 2019).
There is no convenient, easily implemented, and accurate enough method of determining day respiration (Tcherkez et al., 2017). However, the potential bias resulting especially from light inhibition of day respiration, would only emphasize our findings: in high light at the top of the canopy, respiration rates may in fact have been lower and C gain higher during the day, while that would not have been the case in a self-shaded canopy.

The light saturation point \((I_k)\) is defined as the light intensity where assimilation rates become less limited by electron transport rates and more by Calvin Cycle reactions (Herrmann et al. 2019). As \(I_k\) was highest in middle leaf positions in \(I. \) pseudacorus, this species had the highest light demand until approaching light-saturation in the middle of its canopy, providing evidence that it is indeed adapted to high light environments with minimal shading. Contrastingly, decreasing \(I_k\) over the canopy of \(L. \) salicaria, and the distinct light-saturated phase of the species’ light response curves, demonstrated that especially the lower leaves of \(L. \) salicaria were adapted to low light intensities (Dias-Filho, 1997, 2002). The shading that \(L. \) salicaria is exposed to may be primarily provided by its own canopy since this species is often found in light-open habitats, for example on river banks (Mitich, 1999).

No statistical evidence was found that \(L. \) salicaria was better adapted to low than high temperatures with regard to gas exchange traits. Different metabolic acclimation strategies, such as regulation of the electron transport capacity or the capacity for storage of carbon compounds, may lead to similar photosynthetic responses even under different growth temperatures, as long as those conditions are not extreme (Herrmann et al. 2019). Nonetheless, we detected a tendency for negative warming impacts on the lower leaves grown at 25 ºC, which was evident in considerably lower light-response curves, \(\Phi\), \(R_d\), \(A_{\text{max}}\) and PNUE. This finding was in accordance with our expectation that light attenuation would affect gas exchange of \(L. \) salicaria more than \(I. \) pseudacorus. Generally, photosynthetic responses to different light availability seem to be similar in species with similar morphology,
even in closely related species from contrasting habitats (da Costa et al., 2019). Moreover, warming resulted in higher SLA, especially in *L. salicaria*. Leaves in the high temperature treatment were therefore thinner than in the low temperature treatment, which could possibly be explained by faster growth rates in leaves with fewer structural components, allowing a larger investment of resources in other structures, such as flowers (Perez-Hardguindreuguy, 2013). We indeed observed more flowering individuals of *L. salicaria* at 25 ºC compared to 15 ºC.

Opposite to our expectation, no evidence was found that *I. pseudacorus* was more susceptible to photoinhibition at low temperature than at high temperature, as there was no significant difference in *A*$_{\text{max}}$ and Φ between temperatures. A likely explanation could be effective quenching of excess light energy, such as chlorophyll fluorescence, ridding the photosynthetic apparatus of excess excitation energy and, preventing photoinhibition in the species (Jespersen et al., 2017; Powles, 1984; Sello 2019). The shape of the light response curves for *I. pseudacorus* were very similar across temperature treatments even though curves were slightly flatter at high light intensities in the low temperature, at least for the upper leaf position. Nonetheless, *I. pseudacorus* had higher biomass production and greater final shoot height in the high temperature, compared to the low temperature. In addition, its chlorophyll concentration and PNUE were higher at high temperature, indicating a photosynthetic apparatus adapted to warm, high-light environments (Webster et al., 2016). *Lythrum salicaria* was less responsive to temperature than *I. pseudacorus* in its vegetative growth, but its flower development was considerably enhanced unlike growth at 15 ºC. Hence, although the lower leaves of the species at 25 ºC indeed seemed to have lower assimilation rates, confirming our expectation, the overall performance of *L. salicaria* was positively affected by the higher temperature.
Both species are therefore capable of developmental acclimation and likely to follow the general pattern of shifting habitats to higher latitudes and elevations in a warming climate (Athanasiou et al. 2010). *Lythrum salicaria* in Canada is known to be limited in its northern distribution by cold temperatures (Thompson et al., 1987), and with temperatures rising most dramatically in the Arctic and subarctic regions, its range is likely to extend rapidly to the north. Moreover, faster flowering development may increase the invasive hazard of the species as the climate warms. A range extension to the north is also likely for *I. pseudacorus* with rising temperatures, both in Canada and Scandinavia, as the species showed increased growth at high temperature. Management of both invasive species should take this forecast into account.

In conclusion, our study indicated that increased temperature within the range used in this experiment will benefit these two species, despite their different morphology, and even though their photosynthetic traits were less affected. Light response curves revealed that *L. salicaria* allocated more photosynthetic pigments and N to lower leaf positions than *I. pseudacorus*, and was therefore better acclimated to low light intensities than *I. pseudacorus*. Both species are usually found in the same light-open type of habitat. We therefore suggest that *L. salicaria* showed an adaptive response to self-shading in its canopy. Both species should continue to be recognised as highly invasive under future climate scenarios in management plans, and the impending advancement of their northern ranges seen as a threat to the health of native ecosystems.

**DATA**

All data necessary for the reproduction of this study is provided as supplementary material.

**FUNDING**

F. Eller was funded by the Carlsberg Foundation (grant number CF15-0330).
ACKNOWLEDGEMENTS

We thank the staff at our department for technical support as well as help with data collection. We also thank Påskehøjgård research farm for propagating the seedlings used in this experiment.
LITERATURE CITED

Aro E, Virgin I, Andersson B. (1993). Photoinhibition Of Photosystem-2 - Inactivation, Protein Damage And Turnover. *Biochimica Et Biophysica Acta, 1143*(2), 113–134.

Athanasiou K, Dyson BC, Webster RE, Johnson GN. (2010). Dynamic acclimation of photosynthesis increases plant fitness in changing environments. *Plant Physiology, 152*(1), 366-373. https://doi.org/10.1104/pp.109.149351

Barkley TM, Holm L, Pancho JV, Herberger JP, Plucknett DL. (1980). A Geographical Atlas of World Weeds. *Brittonia.* https://doi.org/10.2307/2806777

Berry J, Bjorkman O. (1980). Photosynthetic Response and Adaptation to Temperature in Higher Plants. *Annual Review of Plant Physiology, 31*(1), 491–543. https://doi.org/10.1146/annurev.pp.31.060180.002423

Berry CZ, Goldsmith GR. (2019). Diffuse light and wetting differentially affect tropical tree leaf photosynthesis. *New Phytologist, 225*(1), 143-153.

Blondeel H, Perring MP, De Lombaerde E et al. (2020). Individualistic responses of forest herb traits to environmental change. *Plant biology.* https://doi.org/10.1111/plb.13103

Bjorkman O. (1981). Responses to different quantum flux densities. In ‘Physiological plant ecology I: responses to the physical environment, 12, 57-107. Springer, Berlin, Heidelberg.

Blossey B, Skinner LC, Taylor J. (2001). Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. *Biodiversity and Conservation, 10*(10), 1787–1807. https://doi.org/10.1023/A:1012065703604

Brodersen CR, Vogelmann TC, Williams WE, Gorton HL. (2008). A new paradigm in leaf-level photosynthesis: direct and diffuse lights are not equal. *Plant, Cell and Environment 31* (1), 159-164.

Bunce JA. (1989). Growth rate, photosynthesis and respiration in relation to leaf area index.
Annals of Botany, 63(4), 459-463.

da Costa GS, Dalmolin AC, Schilling AC et al. (2019). Physiological and growth strategies of two Cariniana species in response to contrasting light availability. Flora, 258, 151427. Doi: 10.1016/j.flora.2019.151427.

Daehler CC. (2003). Performance comparisons of co-occurring native and alien invasive plants: Implications for Conservation and Restoration. Annual Review of Ecology, Evolution, and Systematics, 34(1), 183-211.

https://doi.org/10.1146/annurev.ecolsys.34.011802.132403

Department of Environmental Affairs (South Africa). (2016). Alien and invasive species list. National environmental management: Biodiversity act, 2004 (Act no. 10 of 2004), 864(40166), 25657. Retrieved from https://www.environment.co.za/wp-content/uploads/2017/03/nemba10of2004_alienandinvasive_specieslists2016.pdf (December 2019)

Dias-Filho MB. (1997). Physiological response of Solanum crinitum Lam to contrasting light environments. Pesquisa Agropecuária Brasileira, 32(8), 789.

Dias-Filho MB. (2002). Photosynthetic light response of the C4 grasses Brachiaria brizantha and B. humidicola under shade. Scientia Agricola, 59(1), 65–68.

https://doi.org/10.1590/S0103-90162002000100009

Earles JM, Theroux-Rancourt G, Gilbert ME, McElrone AJ, Brodersen CR. (2017). Excess diffuse light absorption in upper mesophyll limits CO₂ drawdown and depresses photosynthesis. Plant Physiology, 174, 1082-1096.

Evans, JR. (1989). Photosynthesis and Nitrogen Relationships in Leaves of C₃ Plants. International Association for Ecology, 78(1), 9–19.

Fernández de Castro AG, Navajas A, Fagúndez J. (2018). Changes in the potential distribution of invasive plant species in continental Spain in response to climate change.
Flexas J, Barbour MM, Brendel O et al. (2012). Mesophyll diffusion conductance to CO2: An unappreciated central player in photosynthesis. *Plant Science*, 193–194, 70–84. https://doi.org/10.1016/j.plantsci.2012.05.009

Forest Operations Lands & Natural Resource (B. C. Canada). (2019). Weed Control Act. Retrieved from http://www.bclaws.ca/Recon/document/ID/freeside/10_66_85 (December 2019)

Gaskin JF, Pokorny ML, Mangold JM. (2016). An unusual case of seed dispersal in an invasive aquatic; yellow flag iris (*Iris pseudacorus*). *Biological Invasions*, 18(7), 2067–2075. https://doi.org/10.1007/s10530-016-1151-0

Gritti ES, Smith B, Sykes MT. (2006). Vulnerability of Mediterranean Basin ecosystems to climate change and invasion by exotic plant species. *Journal of Biogeography*, 33(1), 145–157. https://doi.org/10.1111/j.1365-2699.2005.01377.x

Herrmann HA, Schwartz JM, Johnson GN. (2019). From empirical to theoretical models of light response curves - linking photosynthetic and metabolic acclimation. *Photosynthesis Research*, 1–10. https://doi.org/10.1007/s11120-019-00681-2

Heskel MA, Tang J. (2018). Environmental controls on light inhibition of respiration and leaf and canopy daytime carbon exchange in a temperate deciduous forest. *Tree Physiology*, 38, 1886-1902.

Hirose AT, Werger MJA. (1987). Maximizing Daily Canopy Photosynthesis with Respect to the Leaf Nitrogen Allocation Pattern in the Canopy. *Oecologia*, 72(4), 520–526. https://doi.org/10.1007/BF00378977

Hoober JK, Eggink LL, Chen M. (2007). Chlorophylls, ligands and assembly of light-harvesting complexes in chloroplasts. *Photosynthesis research*, 94(2-3), 387-400.
Hovick SM, Bunker DE, Peterson CJ, Carson WP. (2011). Purple loosestrife suppresses plant species colonization far more than broad-leaved cattail: Experimental evidence with plant community implications. *Journal of Ecology*, 99(1), 225–234. https://doi.org/10.1111/j.1365-2745.2010.01754.x

IPCC. (2014). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. https://doi.org/10.1017/CBO9781107415324

Jacobs J, Pokorny M, Mangold J, Graves-Medley M. (2011). Biology, ecology and management of yellow flag iris (*Iris pseudacorus L*). *Extension publication EB203, Montana State University Extension, Bozeman, MT, USA*.

Jespersen E, Brix H, Sorrel BK. (2017). Acclimation to light and avoidance of photoinhibition in *Typha latifolia* is associated with high photosynthetic capacity and xanthophyll content. *Functional Plant Biology*, 44, 774-784.

Keenan TF, Migliavacca M, Papale D et al. (2019). Widespread inhibition of daytime ecosystem respiration. *Nature Ecology & Evolution*, 3, 407-415.

Li P, Ponnala L, Gandotra N et al. (2010). The developmental dynamics of the maize leaf transcriptome. *Nature Genetics*, 42(12), 1060-1067.

Lichtenthaler HK. (1987). Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes, *148*, 350–382.

Lichtenthaler HK, Buschmann C, Döll M et al. (1981). Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis Research*, 2(2), 115–141.

Lobo F, de Barros MP, Dalmagro HJ et al. (2013). Fitting net photosynthetic light-response curves with *Microsoft Excel* — a critical look at the models. *Photosynthetica*, 51, 445–
Long SP, Humphries S. (1994). Photoinhibition of photosynthesis in Nature. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 45, 633–662. https://doi.org/10.1146/annurev.pp.45.060194.003221

Mack RN, Simberloff D, Mark Lonsdale W, Evans H, Clout M, Bazzaz FA. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological applications, 10*(3), 689-710. https://doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

Mal TK, Lovett-doust J, Lovett-doust L et al. (1992). The Biology of Canadian weeds. 100. *Lythrum salicaria*, 1330, 1305–1330.

Melis A, Harvey GW. (1981). Regulation og photosystem stoichiomyelry, chlorophyll a and chlorophyll b content and relation to chloroplast ultrastructure. *Biochimica et Biophysica Acta*, 637, 138–145. https://doi.org/10.1016/0005-2728(81)90219-X

Ministry for Primary Industries. (2017). Unwanted Organisms Register. Retrieved from https://www1.maf.govt.nz/uor/ (December 2019)

Ministry of the Environment (Japan). (2005). Information and caution about alerted alien species. Retrieved from http://www.env.go.jp/nature/intro/1outline/files/siteiisyu_list_e.pdf (in Japanese) (December 2019)

Mitich LW. (1999). Purple loosestrife, *Lythrum salicaria* L. *Weed technology*, 13(4), 843-846. https://doi.org/10.1017/S0890037X00042330

Moran EV, Alexander JM. (2014). Evolutionary responses to global change: Lessons from invasive species. *Ecology Letters, 17*(5), 637–649. https://doi.org/10.1111/ele.12262

Mullin BH. (1998). The Biology and Management of Purple Loosestrife (*Lythrum salicaria*) *Weed Technology*, 12(2), 397-401. https://doi.org/10.1017/S0890037X00043992

Niu S, Li Z, Xia J, Han Y, Wu M, Wan S. (2008). Climatic warming changes plant
photosynthesis and its temperature dependence in a temperate steppe of northern China.  

*Environmental and Experimental Botany, 63*(1–3), 91–101.  

https://doi.org/10.1016/j.envexpbot.2007.10.016  

Ocheltree TW, Nippert JB, Prasad PVV. (2012). Changes in stomatal conductance along grass blades reflect changes in leaf structure. *Plant, Cell and Environment, 35*(6), 1040–1049.  

https://doi.org/10.1111/j.1365-3040.2011.02470.x  

Onoda Y, Hikosaka K, Hirose T. (2004). Allocation of nitrogen to cell walls decreases photosynthetic nitrogen-use efficiency. *Functional Ecology, 18*(3), 419-425.  

https://doi.org/10.1111/j.0269-8463.2004.00847.x  

Perez-Hardguindeguy, N. (2013). New Handbook for Standardised Measurement of Plant Functional Traits Worldwide. *Australian Journal of Botany, 1–9.*  

https://doi.org/10.1071/BT12225  

Plaxton WC. (1996). The Organization and Regulation of Plant Glycolysis. *Annual Review of Plant Physiology and Plant Molecular Biology, 47*(1), 185–214.  

https://doi.org/10.1146/annurev.arplant.47.1.185  

Poorter H, Evans JR. (1998). Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. *Oecologia, 116*(1-2), 26-37.  

https://doi.org/10.1007/s004420050560  

Powles SB. (1984). Photoinhibition of Photosynthesis Induced by Visible Light. *Annual Review of Plant Physiology, 35*(1), 15–44.  

https://doi.org/10.1146/annurev.pp.35.060184.000311  

Prioul JL, Chartier P. (1977). Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO2 fixation: a critical analysis of the methods used. *Annals of Botany, 41*(4), 789-800.  

https://doi.org/10.1093/oxfordjournals.aob.a085354
Sello S, Meneghesso A, Alboresi A, Baldan B, Morosinotto T. (2019). Plant biodiversity and regulation of photosynthesis in the natural environment. *Planta*, 249(4), 1217-1228. https://doi.org/10.1007/s00425-018-03077-z

Sun J, Ye M, Peng S, Li Y. (2016). Nitrogen can improve the rapid response of photosynthesis to changing irradiance in rice (Oryza sativa L.) plants. *Scientific Reports*, 6(1), 1–10. https://doi.org/10.1038/srep31305

Takahashi S, Murata N. (2008). How do environmental stresses accelerate photoinhibition? *Trends in Plant Science*, 13(4), 178–182. https://doi.org/10.1016/j.tplants.2008.01.005

Tcherkez G, Gauthier P, Buckley TN et al. (2017). Leaf day respiration: low CO₂ flux but high significance for metabolism and carbon balance. *New Phytologist*, 216, 986-1001.

Thompson DQ, Stuckey RL, Thompson EB. (1987). Spread, Impact, and Control of Purple Loosestrife (*Lythrum salicaria*) in North American Wetlands. *U.S. Fish and Wildlife Service*.

Turnbull MH, Murthy R, Griffin KL. (2002). The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. *Plant, Cell and Environment*, 25(12), 1729–1737. https://doi.org/10.1046/j.1365-3040.2002.00947.x

Urban O, Klem K, Holisova P et al. (2014). Impact of elevated CO₂ concentration on dynamics of leaf photosynthesis in *Fagus sylvatica* is modulated by sky conditions. *Environmental Pollution* 185, 271-280.

USDA (United States Department of Agriculture). (2013). Weed Risk Assessment for Iris pseudacorus, *I*. Retrieved from

https://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/wra/Iris_pseudacorus_WRA.pdf (December 2019)

Walther GR, Roques A, Hulme PE et al. (2009). Alien species in a warmer world: risks and opportunities. *Trends in Ecology and Evolution*, 24(12), 686–693.
Webster RJ, Driever SM, Kromdijk J et al. (2016). High C3 photosynthetic capacity and high intrinsic water use efficiency underlies the high productivity of the bioenergy grass *Arundo donax*. *Scientific Reports*, 6, 20694. Doi: 10.1038/srep20694

Weraduwage SM, Chen J, Anozie FC et al. (2015). The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. *Frontiers in Plant Science* 6: 167. Doi: 10.3389/fpls.2015.00167

Yamori W, Noguchi K, Terashima I. (2005). Temperature acclimation of photosynthesis in spinach leaves: Analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. *Plant, Cell and Environment*, 28(4), 536–547. https://doi.org/10.1111/j.1365-3040.2004.01299.x
FIGURE LEGENDS

**Fig. 1:** Growth parameters of *Iris pseudacorus* and *Lythrum salicaria*. Mean ± standard deviation of above ground biomass (A) and final shoot height (B) of both species grown at 15 °C and 25 °C, respectively. Different letters indicate statistically significant differences between groups provided by Tukey’s honestly significant differences test at a 0.05 significance level. DW: dry weight.

**Fig. 2:** Photosynthetic parameters from light response curves of *Iris pseudacorus* and *Lythrum salicaria*. Mean ± standard deviation of light compensation point $I_c$ (A), light saturation point $I_k$ (B), specific leaf area SLA (C), light-saturated photosynthetic rate $A_{max}$ (D), quantum yield $\Phi$ (E) and dark respiration rate $R_d$ (F) of both species grown at 15 °C and 25 °C, and measured at three different leaf positions, respectively. Different letters indicate statistically significant differences between groups provided by Tukey’s honestly significant differences test at a 0.05 significance level. PAR: photosynthetic active radiation.

**Fig. 3:** Light response curves of *Iris pseudacorus* and *Lythrum salicaria*. Mean ± standard deviation photosynthetic rates, $A$, measured at different light intensities, $I$, of photosynthetic active radiation (PAR) for both species grown at 15 °C and 25 °C, measured at three different leaf positions, respectively. Data were fitted using a nonlinear hyperbolic regression.

**Fig. 4:** Photosynthetic pigments of *Iris pseudacorus* and *Lythrum salicaria*. Mean ± standard deviation of total chlorophyll concentration $\text{Chl}_{a+b}$ (A), total carotenoid concentration $\text{C}_{x+c}$ (B), chlorophyll/ carotenoid ratio $\text{Chl}_{a+b}/\text{C}_{x+c}$ (C), chlorophyll a/b ratio $\text{Chl}_a/\text{Chl}_b$ (D) of both species grown at 15 and 25 °C, and measured at three different leaf
positions; upper, middle and lower, respectively. Different letters indicate statistically significant differences between groups provided by Tukey’s honestly significant differences test at a 0.05 significance level. DW: dry weight.

**Fig. 5:** Carbon and nitrogen concentration of *Iris pseudacorus* and *Lythrum salicaria*.

Mean ± standard deviation of leaf nitrogen concentration N (A), leaf carbon concentration C (B), leaf carbon/nitrogen ratio (C), photosynthetic nitrogen use efficiency PNUE (D) of both species grown at 15 °C and 25 °C, and measured at three different leaf positions (upper, middle and lower), respectively. Different letters indicate statistically significant differences between groups provided by Tukey’s honest significant differences test at a 0.05 significance level. DW: dry weight.
Figure 1

A

Biomass (g DW)

I. pseudacorus  L. salicaria

B

 Shoot height (cm)

I. pseudacorus  L. salicaria

Temperature 15 °C  25 °C
Figure 2

A

B

C

D

E

F

I. pseudacorus  
L. salicaria

I. pseudacorus  
L. salicaria

I. pseudacorus  
L. salicaria

I. pseudacorus  
L. salicaria

I. pseudacorus  
L. salicaria

I. pseudacorus  
L. salicaria

_l_c (μmol PAR m^-2 s^-1)

_l_k (μmol PAR m^-2 s^-1)

SLA (m^2 g^-1 DW)

A_max (μmol CO_2 m^-2 s^-1)

ϕ (μmol CO_2 μmol^-1 PAR)

Temperature (°C)

Leaf position  
Upper  
Middle  
Lower
Figure 3

- **L. salicaria at 15 °C**
  - Graph A

- **L. salicaria at 25 °C**
  - Graph B

- **I. pseudacorus at 15 °C**
  - Graph C

- **I. pseudacorus at 25 °C**
  - Graph D

Leaf position — Upper — Middle — Lower
Figure 5

A

I. pseudacorus

L. salicaria

N (% DW)

15 25

15 25

ab abc bcd cde

a ab

B

I. pseudacorus

L. salicaria

C (% DW)

15 25

15 25

a ab bcd ab ab c
d

bc bc bcd abc abc cd

C

I. pseudacorus

L. salicaria

C/N

15 25

15 25

cd cd cd d cd

a a a a

D

I. pseudacorus

L. salicaria

PNUE (umol CO₂ mol⁻¹ N s⁻¹)

15 25

15 25

ab abc bcd cd d

ab a cd cd

Leaf position

Upper  Middle  Lower
Table 1. Nutrient concentrations used during the experiment. *Iris pseudacorus* and *Lythrum salicaria* grown at 15 °C and 25 °C received 100 mL of the solution twice per week.

| Macro nutrient | Concentration (mg L\(^{-1}\)) | Micro nutrient | Concentration (%) |
|----------------|---------------------------------|----------------|-------------------|
| NO\(_3\)-N      | 59.5                            | B              | 0.32              |
| NH\(_4\)-N      | 37.0                            | Cu             | 0.13              |
| Total-N         | 96.5                            | Fe             | 1.62              |
| P              | 11.5                            | Mn             | 0.63              |
| K              | 77.0                            | Mo             | 0.06              |
| Mg             | 15.0                            | Zn             | 0.32              |
| S              | 19.5                            | -              | -                 |
Table 2. Light intensities at three leaf positions in *Lythrum salicaria* and *Iris pseudacorus* grown at two temperatures. “Upper”, “Middle” and “Lower”: light intensities at upper, middle and lower leaf position, respectively. $\Delta_{\text{Upper - middle}}$: average difference in light intensity between upper and middle leaf positions, $\Delta_{\text{Upper - lower}}$: average difference in light intensity between upper and lower leaf positions. Light intensities are given as mean ± standard deviation. PAR: photosynthetically active radiation.

| Leaf position | I. pseudacorus | L. salicaria |
|--------------|----------------|-------------|
|              | 15 °C          | 25 °C       | 15 °C       | 25 °C       |
| Upper        | 348.8 ± 42.6   | 283.4 ± 76.6 | 393.1 ± 37.0 | 290.0 ± 55.2 |
| Middle       | 335.1 ± 38.0   | 256.0 ± 72.3 | 348.1 ± 54.9 | 204.8 ± 75.3 |
| Lower        | 214.1 ± 69.3   | 103.0 ± 65.9 | 197.5 ± 42.0 | 175.2 ± 52.5 |
| $\Delta_{\text{Upper - middle}}$ | 13.7           | 27.4         | 45.0         | 85.2         |
| $\Delta_{\text{Upper - lower}}$ | 134.7          | 180.4        | 195.6        | 114.8        |
Table 3: F-values of analysis of variance (ANOVA) for growth and photosynthetic parameters. Degrees of freedom indicated as “df”. Species: *Iris pseudacorus* or *Lythrum salicaria*. Temperature: 15°C or 15°C, Leaf position: upper, middle or lower. BM: above ground biomass, SH: shoot height, \(A_{\text{max}}\): maximum photosynthesis rate, \(R_d\): dark respiration rate, \(I_c\): light compensation point, \(\Phi\): quantum yield, \(I_k\): light saturation point, SLA: specific leaf area, Chl\(_{a+b}\): total chlorophyll concentration, C\(_{x+c}\): total carotenoid concentration, Chl\(_{a+b}\)/C\(_{x+c}\): chlorophyll/carotenoid ratio, Chl\(_d\)/Chl\(_b\): Chlorophyll a/b ratio, N: leaf nitrogen concentration, C: leaf carbon concentration, C/N: leaf carbon/nitrogen ratio, PNUE: photosynthetic nitrogen use efficiency. Significant explaining factors are marked with boldface. Level of significance is marked with asterisks (*: \(P < 0.05\); **: \(P < 0.01\); ***: \(P < 0.001\)).

| Parameter | Source of variation | Interactions |
|-----------|---------------------|--------------|
|           | Species (df = 1)    | Temperature (df = 1) | Leaf position (df = 2) | Species x temperature (df = 1) | Species x leaf position (df = 2) | Temperature x leaf position (df = 2) | Species x temperature x leaf position (df = 2) |
| BM        | 137.919***          | 6.778*       | -                  | 0.384                        | -                              | -                          | -                          |
| SH        | 12.966**            | 30.784***    | -                  | 0.815                        | -                              | -                          | -                          |
| \(A_{\text{max}}\) | 36.082***         | 3.525        | 5.741**            | 0.00840                      | 0.887                         | 1.977                      | 0.379                      |
| \(R_d\)   | 1.389               | 0.124        | 0.441              | 0.0829                       | 2.884                         | 0.0327                     | 1.883                      |
| \(I_c\)   | 2.432               | 0.188        | 0.864              | 0.173                        | **6.961***                     | 0.296                      | 0.816                      |
| \(\Phi\)  | 21.251***           | 0.00120      | 5.722**            | 1.053                        | 2.744                         | 0.337                      | 1.549                      |
| \(I_k\)   | 19.159***           | 0.898        | 6.667**            | 0.0280                       | **4.246***                     | 0.715                      | 0.0536                     |
| SLA       | 5.768*              | 7.691**      | 3.805*             | 2.324                        | **20.149***                    | 0.281                      | 2.857                      |
|                              |        |        |        |        |        |        |
|------------------------------|--------|--------|--------|--------|--------|--------|
| **Chl_{a+b}**                | 110.333*** | 36.756*** | 5.740**  | 2.248  | 8.186*** | 6.931**  | 1.691  |
| **C_{x+c}**                  | 100.039*** | 10.258**  | 4.204*   | 0.109  | 3.954*   | 5.200**  | 1.391  |
| **Chl_{a+b}/C_{x+c}**        | 15.239*** | 6.612*   | 3.074   | 1.116  | 0.620    | 0.448    | 0.356  |
| **Chl_{a}/Chl_{b}**         | 0.894   | 20.174*** | 0.876   | 0.0262 | 2.837    | 0.683    | 1.138  |
| **N**                        | 71.085*** | 2.273   | 4.602*   | 0.223  | 3.871*   | 1.297    | 0.133  |
| **C**                        | 16.209*** | 2.460   | 9.677*** | 3.190  | 3.788*   | 0.652    | 0.0485 |
| **C/N**                      | 76.090*** | 1.870   | 2.138   | 1.168  | 3.312*   | 0.757    | 0.275  |
| **PNUE**                     | 4.580*   | 9.493**  | 4.426*   | 1.082  | 3.248*   | 1.487    | 1.465  |