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Research Article

No time to dye: dye-induced light differences mediate growth rates among invasive macrophytes

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Abstract

Invasive, submerged macrophytes negatively alter aquatic ecosystems and biodiversity through disruption of ecological structure and functioning. These plants are especially challenging and costly to control, with relatively few successful eradications. We examine the efficacy of dye treatments to control three invasive, submerged macrophyte species: *Elodea canadensis* Michx., *Elodea nuttallii* (Planchon) H. St. John and *Lagarosiphon major* (Ridley). Using an experimental mesocosm approach, growth rates of each species were monitored in relation to five light treatment groups: light, 1×, 2×, 3× dye dosage, and complete darkness (range: 270 to 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Dye presence did not negate growth in any of the tested species, but the effects of treatments on invasive macrophyte growth rates differed across species. In dyed conditions, *E. canadensis* exhibited significantly greater increases in length compared to *E. nuttallii* and *L. major*, whilst *E. nuttallii* and *L. major* were lower and statistically similar. However, *L. major* significantly increased length relative to *Elodea* spp. in dark conditions. Similarly, for biomass changes, *Elodea* spp. gained significantly more biomass than *L. major* under light and dyed conditions, but not in the dark. Our findings suggest that the tested dye concentrations are not sufficient to halt the growth of these plants. However, under certain conditions, they could potentially help to reduce densities of invasive macrophytes by slowing growth rates and reducing biomass in select species. Differential responses to light could also help explain species replacement dynamics under varying environmental contexts. Overall, while further empirical research is required, management actions that reduce light could help control aquatic macrophytes in combination with other actions, but could also simultaneously mediate shifts in community assembly.

Key words: *Elodea canadensis*, *Elodea nuttallii*, freshwaters, *Lagarosiphon major*, plant control, relative growth rates

Introduction

Invasive aquatic species can adversely impact the physical, chemical and biological processes of freshwater ecosystems (Dudgeon et al. 2006;

Simberloff et al. 2013; Piria et al. 2017). In particular, invasive macrophytes can negatively alter community dynamics and ecosystem function, in turn threatening native biodiversity (Schultz and Dibble 2012; Kuehne et al. 2016; Lu et al. 2018). Furthermore, invasive macrophytes often represent a substantial economic burden, with large monospecific swards inhibiting recreation and commercial activities and increasing the risk of floods (Lafontaine et al. 2013; Cuthbert et al. 2021). Effective management techniques are urgently required to minimise these impacts whilst taking into account international regulations such as the European Union (EU) regulation 1143/2014, designed for the prevention and management of the introduction and spread of invasive alien species (Coughlan et al. 2020).

A number of management options exist for the control of aquatic macrophytes (e.g., physical, chemical, biological), however, it is widely accepted that full-scale eradication is rarely feasible or cost-effective other than for small waterbodies, and therefore reducing the size of populations is a more realistic objective (Vernon and Hamilton 2011; Hussner et al. 2017). Physical control methods, including manual cutting or pulling, ploughing, harrowing, trailing blades and other mechanical techniques, are widely used in macrophyte control (Murphy 1988; Newman 2009; Newman and Duenas 2010; Zehnsdorf et al. 2015). The efficacy of these methods has been questioned, however, as mechanical methods usually produce large numbers of plant fragments that can spread within or between sites, fostering new populations (Hussner et al. 2017; Coughlan et al. 2018). Herbicides have been used for the removal of invasive aquatic plants for decades (e.g., diquat, endothall and fluridone), and are comparatively successful (Skogerboe et al. 2004; Clayton and Matherson 2010). However, the use of herbicides for submerged vegetation in waterbodies has been banned by the EU since 2010 owing to legislative restrictions concerning their impacts on the environment (Hussner et al. 2017). Glyphosate is authorised for use on emergent species in the EU, but is not suitable for submerged macrophytes because it is water soluble (Hussner et al. 2017). Therefore, herbicide control is no longer a viable aquatic plant control technique in many countries. A myriad of natural enemies have been identified as part of biocontrol attempts to manage invasive macrophytes (e.g., Pieczyńska 2003; Baars et al. 2010; Gettys et al. 2020). However, in many geographic regions candidate biocontrol agents are largely untested, are prohibited, or discouraged if alien. Indeed, some natural enemies which cause fragmentation might even accelerate macrophyte propagation rates (Crane et al. 2020b).

Reductions in light intensity cause shading, which can help control aquatic invasive macrophytes (Dawson and Hallows 1983; Newman and Duenas 2010; Caffrey et al. 2010) and algae (Gettys et al. 2020). Light reduction can be achieved by a number of methods including the use of a benthic barrier such as biodegradable jute matting (Caffrey et al. 2010).

Another light-reducing technique that has been suggested for small, static waterbodies (e.g. ponds) is the use of dye, which acts by absorbing the part of light spectrum that plants use to photosynthesise, thereby reducing the depth of the photic zone (Newman 2011; Vernon and Hamilton 2011; Gettys et al. 2020). Blue, neutral and black dyes have been demonstrated to effectively suppress non-invasive submerged weeds in the UK and The Netherlands, and dye has been a longstanding approach in the USA for several decades; however, efficacies against invasive aquatic plants have seldom been demonstrated (Hussner et al. 2017). By reducing light, dyes have the potential to reduce efficiency of chlorophyll, thereby impeding photosynthesis, and underwater light levels have been reduced to below 5% of surface levels (Bornette and Puijalon 2011). Limitations, however, include the need for repeat applications to account for precipitation and UV degradation. The approach is best suited towards small waterbodies and non-toxic dyes have reportedly few long-lasting negative ecosystem effects (Hussner et al. 2017). Very few studies have compared the responses of dye among invasive submerged plants, and its implications for potential competitive interactions among these species (Denys et al. 2014).

The present study thus examines the effects of pond dye on the growth rates (both length and biomass) of three morphologically-similar submerged invasive macrophytes: *Elodea canadensis* Michx., *E. nuttallii* (Planchon) H. St. John and *Lagarosiphon major* (Ridley). *Elodea canadensis* is a submerged macrophyte, widely established in Europe, that spread rapidly in the UK and Ireland following its introduction in the early 1800s and, although it continues to cause problems in some locations, it is now regarded as naturalised and therefore a low-impact invader in part of its non-native range (Newman and Duenas 2010). *Elodea nuttallii* and *L. major* are fast-growing invasive alien species that can outcompete other vegetation, modify macrophyte community composition by forming dense mats, and cause considerable problems in freshwater systems (e.g. Stiers et al. 2011; Hussner 2014). Owing to their impacts, both species were listed in the updated and consolidated list of Invasive Alien Species of Union Concern in 2017 (EU Regulation 1143/2014). This legislation addresses the problem of invasive alien species, their prevention and management (Coughlan et al. 2020). To date, the effect of dye treatments on growth of these three invasive alien species has not been assessed. For these three species, we hypothesised that colouring water using biodegradable blue dye, at 1, 2 and 3 times the recommended dose, would increasingly dampen their growth by reducing light levels. Furthermore, we concurrently tested growth responses to both light (i.e., control) and fully dark conditions. Owing to reported competitive dynamics among these species (Barrat-Segretain and Elger 2004; Kelly et al. 2015), we expected invasive *E. nuttallii* and *L. major* to outperform the benign *E. canadensis* in clear to moderate light conditions. However, as water brownification and eutrophication have

been suggested to decrease the competitive performance of select invasive macrophytes (Xu et al. 2018), we expected dye presence to mediate differential responses among species. In essence, although there are some parallels in other work for these species considering light (but not dye), our study is novel in several aspects: it considers these three species concurrently, with dye, and the focus is control-orientated.

Materials and methods

Sample collection and cultivation

Elodea canadensis was collected in Tully Mill Lough, Northern Ireland (54°15′32.34″N; 7°42′50.88″W) and *E. nuttallii* was collected in Lough Erne, Northern Ireland (54°17′07.89″N; 7°32′52.61″W). *Lagarosiophion major* was collected from Lough Corrib, County Galway, Ireland (53°26′36.9″N; 9°19′17.5″W). Following collection of unrooted fronds, all species were transported in source water to Queen's Marine Laboratory (QML), Portaferry, Northern Ireland. The plant stock was maintained from March onwards in large, aerated, outdoor aquaria, filled with lake water (Lough Cowey: 54°24′41.79″N; 5°32′25.96″W) and displayed excellent survival and sustained growth during a six month cultivation period prior to commencement of the experiment. Apical plant fragments were harvested 48-hrs prior to experimentation and transferred indoors to aerated aquaria with lake water to allow them to acclimate to laboratory conditions. Fragments were cultured at a temperature of 12 °C ± 1 °C and photon flux density of 270 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (measured at the bottom of the aquaria, *c.* 15 cm) using a cosine-corrected quantum sensor (Skye SKP 215; Llandrindod Wells, Powys, UK). We acknowledge that light readings may differ according to depth, but were measured for all species and dye levels at the same depth. This temperature corresponds to spring-like conditions in the study area, while greater growth rates could be exhibited at higher temperatures (e.g. James et al. 2006). Irradiance was supplied from above by four 52 W Arcadia 1200 mm Marine Stretch LED lamps (16:8 light to dark regime). All waste invasive plant material was destroyed by autoclaving.

Experimental design

The efficacy of dye to inhibit the growth of *E. canadensis*, *E. nuttallii* and *L. major* was examined using Dyofix Pond Blue (Triaryl Methane Dye Preparation): a food-grade dye that, according to the suppliers, is non-toxic to fish, fowl, other wildlife, domestic animals or to the environment in general. However, a degree of caution must ensue when applying dye to aquatic ecosystems. The supplier (Town End, Leeds) state that they have carried out an extensive series of tests (*unpublished*) to establish the dosage of Dyofix. Taking into account the practical needs of water users to maintain native plant life in the shallows and on the waterbody margins,

Table 1. Light levels associated with each experimental treatment (μmol·m^{-2}·s^{-1}). Each dye dose corresponds to the manufacturer's recommendation (0.71 mL per 500 mL of pond/lake water).

Treatment	Light level (μmol·m^{-2}·s^{-1})	Relative proportional light availability (%)	Light intensity
No dye	270	100	High
1 dye dose	220	82	Moderate–High
2 dye doses	190	70	Moderate
3 dye doses	140	52	Low
Complete darkness	0	0	None

the company recommends a dilution ratio of 10 mL dye to seven litres water to provide a barrier to solar radiation (i.e. 0.71 mL per 500 mL).

In all cases, 8 cm apical fragments were harvested from healthy, mature plants. Where possible, fragments were cut from unbranched sections of stem; however, if present, axillary side shoots were carefully removed. Plant fragments were randomly selected from holding aquaria and excess media was gently removed by manually spinning individual fragments, ten times in both directions, within a handheld centrifugal spinner. Fragment biomass (fresh wet mass) was then recorded: *E. canadensis* 0.22 ± 0.07 g; *E. nuttallii* 0.12 ± 0.02 g; *L. major* 0.73 ± 0.3 g. We acknowledge differences in starting biomass among plants, but opted to standardise based on length primarily (see above) such that plants were positioned similarly in the water column at the start of the experiment. The base of each individual fragment was protected using a small piece of cotton wool, before being wrapped with a small aquarium weight to keep the base of the fragment at the bottom of the mesocosm and the apical section positioned vertically. While fragments were not rooted in the experiment, the weighting helped to mimic their positioning in waterbodies when rooted. Each weighted fragment was placed in the centre of a 550 mL glass chamber with 500 mL of unfiltered pond water and air lines delivered oxygen and water motion for the duration of the experiment (one plant fragment per chamber, replicated three times per dye treatment and species). Water and dye were exchanged every seven days and the experiment was initiated in October 2018.

Each plant species was subject to five treatments: light (no dye) and three dye (i.e., intermediate light regimes) treatments, as well as complete darkness (achieved using an aluminium foil covering). The completely dark regime was selected to capture a full spectrum of light regimes. The three dye treatments included 1, 2 and 3 times the recommended dose (1 being the recommended dose of 0.71 mL per 500 mL). The light regimes associated with each of the experimental treatments are shown in Table 1, measured at the bottom of the glass chamber (*c.* 15 cm) with a cosine-corrected quantum sensor. Although plants were weighted to the bottom of each chamber where the light was measured, growth towards the surface over time could have permitted additional light to reach fronds during the course of the experiment. There were three replicates per treatment in a randomised array. Biomass and length assessments of individual fragments were made following the removal of excess water using a handheld centrifugal

spinner at the beginning and end of the experiment. The experiment was conducted over six weeks, with measurements at the beginning and end.

Statistics

Relative growth rates (RGRs) were estimated between the start and end of the experiment for both length (cm) and biomass (g): RGR = (ln(f) – ln(i))/t, where f = final values, i = initial values and t = time interval (42 days) (Hunt 1982).

Length- and biomass-based RGRs were analysed using separate linear models, with plant species (3 levels) and dye treatment (5 levels) included as independent and interacting factors. Likewise, biomass change per unit length change was examined according to the same variables, by dividing the absolute change in biomass by the corresponding absolute change in length per replicate. Dye treatment and plant species were included as fixed and interacting effects. Normality and homogeneity assumptions were checked using residual diagnostic plots. Tukey tests were implemented *post hoc* for pairwise comparisons (lsmeans; Lenth 2016). Non-significant terms were removed such that the final models included only significant terms and interactions. Statistical analyses were performed using R v3.4.4. (R Core Development Team 2018).

Results

Considering plant length, dye treatment did not significantly affect growth rates overall ($F_{4, 30}$ = 1.91, p = 0.13), but growth rates differed significantly among plant species ($F_{2, 30}$ = 21.95, p < 0.001). A significant interaction effect indicated that the three plant species' lengths responded differentially to the dye treatments ($F_{8, 30}$ = 7.93, p < 0.001) (Figure 1). *Elodea canadensis* lengths increased significantly more than *L. major* in all light and dye treatments (p < 0.05), with on average 4- to 13-times higher RGRs (Figure 1). Under those same treatments (270–140 μmol·m^{-2}·s^{-1}), *L. major* and *E. nuttallii* were always statistically similar (all p > 0.05). *Elodea canadensis* significantly outgrew *E. nuttallii* under the three dye treatment levels (220–140 μmol·m^{-2}·s^{-1}) (p < 0.05). Conversely, under complete darkness, *L. major* grew significantly more in length than both *Elodea* species (both p < 0.001), with 4-times greater growth than *E. canadensis*, whilst *E. nuttallii* RGR became negative on average (Figure 1). Within species, growth rates in *E. canadensis* were always significantly reduced in the dark (all p < 0.01), whereas *E. nuttallii* was significantly reduced in the dark compared to only 220 μmol·m^{-2}·s^{-1} (p < 0.05). Growth in *L. major* was significantly enhanced in the dark compared to all light treatments (except 140 μmol·m^{-2}·s^{-1}) (all p < 0.01).

For plant fresh biomass changes, growth rates differed significantly according to dye treatment ($F_{4, 30}$ = 64.67, p < 0.001) and species ($F_{2, 30}$ = 57.53, p < 0.001). Again, a significant interaction term indicated differential responses

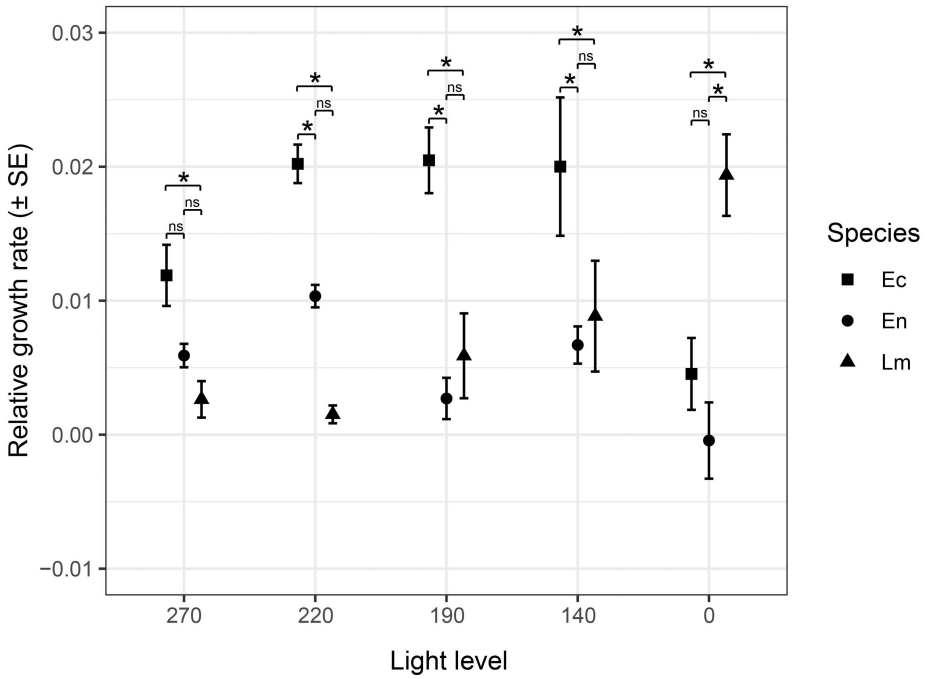

Figure 1. Mean (± standard error) daily relative growth rates of plant length (recorded in cm) for *Elodea canadensis* (Ec), *Elodea nuttallii* (En) and *Lagarosipon major* (Lm) according to light regime (μmol·m^{-2}·s^{-1}). Asterisks denote significant pairwise differences among species within light level groups (*, $p < 0.05$; ns, non-significant).

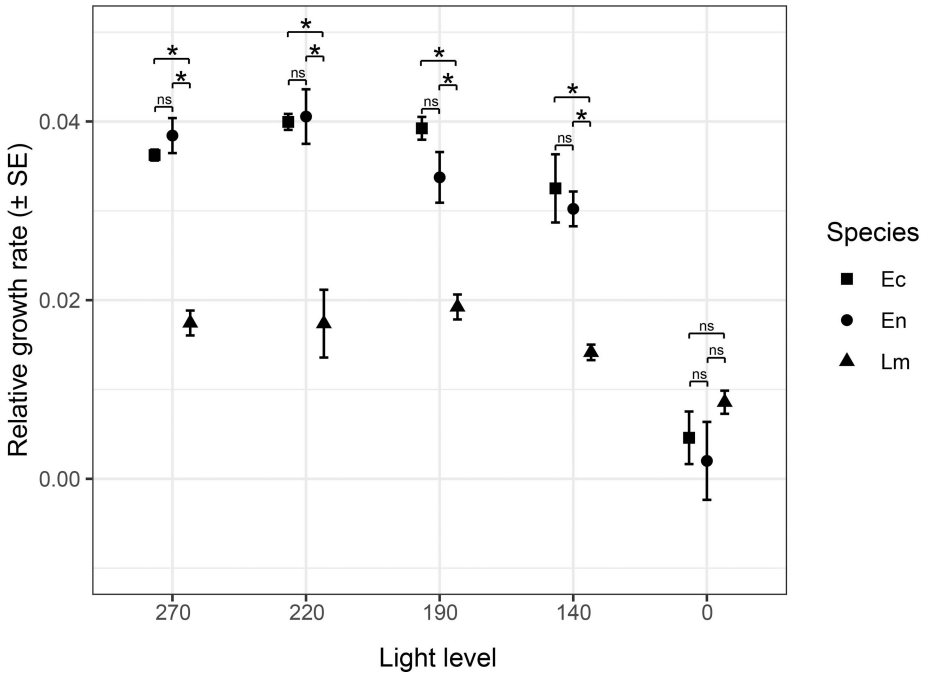

Figure 2. Mean (± standard error) daily relative growth rates for biomass production (recorded in g) by *Elodea canadensis* (Ec), *Elodea nuttallii* (En) and *Lagarosipon major* (Lm) biomasses according to light regime (μmol·m^{-2}·s^{-1}). Asterisks denote significant pairwise differences among species within light level groups (*, $p < 0.05$; ns, non-significant).

among macrophyte species to dye and light treatments ($F_{8,\ 30} = 7.33$, $p < 0.001$) (Figure 2) with respect to relative growth rates in plant biomass. Here, both *Elodea* species increased biomass significantly more than *L. major* under all light and dye treatments (270–140 μmol·m^{-2}·s^{-1}) (all $p < 0.001$),

with approximately double the biomass gain on average (Figure 2). However, under completely dark conditions, biomass growth of all species was statistically similar (all $p > 0.05$), and thus *L. major* was relatively less affected by darkness in terms of biomass RGR than *Elodea* spp. Biomass-based RGRs between *E. canadensis* and *E. nuttallii* were never statistically different (all $p > 0.05$) (Figure 2). Within species, *E. canadensis* and *E. nuttallii* growth rates were significantly reduced in the dark compared to all other groups (all $p < 0.001$), whereas *L. major* growth was only significantly reduced in the dark compared to 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($p < 0.05$).

Biomass changes per unit length growth were not significantly affected by dye treatment ($F_{4, 38} = 2.16$, $p = 0.09$). Biomass-length relationships differed significantly among species ($F_{2, 42} = 11.13$, $p < 0.001$), however, with *L. major* gaining significantly more biomass per unit length growth than both *Elodea* spp. (both $p < 0.01$; nine-fold higher than *E. canadensis* and four-fold than *E. nuttallii*), which were in turn more similar ($p > 0.05$). There was no significant interaction between dye treatment and species considering biomass-length relationships ($F_{8, 30} = 1.91$, $p = 0.10$). Underlying experimental data are provided in Supplementary material Table S1.

Discussion

Water clarity as well as the amount and wavelength of light that reaches macrophytes governs their abundance, composition, distribution and invasion success (Barko et al. 1986; Chambers 1987; Sand-Jensen and Madsen 1991; Dar et al. 2014; Lougheed et al. 2001; Szabó et al. 2018). In conditions with high light availability, submerged macrophytes, such as those examined here, often extend their range into deeper water since they are capable of apical elongation through phenotypic responses (Barko and Smart 1981; Dar et al. 2014; Maberly and Madsen 2002; Szabó et al. 2018). In this study, both length and biomass growth rates were affected by dye treatments and thus light levels, with *Elodea* spp. tending to perform best at all except complete dark conditions, where *L. major* was least affected.

Emergent effects were prevalent here, which indicated differential responses to treatment groups among tested species, and which likely reflect different light compensation points. While *E. nuttallii* has been suggested to require higher light levels, with its growth being concentrated in apical elongation, our results conversely suggest the congeneric *E. canadensis* has a greater capacity to elongate under the majority of light regimes, and therefore factors outside of light may mediate their competitive interactions (e.g. Crane et al. 2020a). In turn, under dark conditions, the performance of both these species deteriorated, whereas *L. major* displayed a growth advantage. Although not assessed directly here, differences in growth forms between length and biomass may mediate competitive interactions among these co-occurring invasive macrophytes.

Species which elongate rapidly may not display a commensurate growth in biomass owing to etiolation. Inversely, biomass might disproportionately exceed length increases owing to lateral spread through branching under certain conditions. In general, in response to reduced light levels, macrophytes devote more energy to elongating main stems, while reducing branching and altering biomass allocated to roots (Barko and Smart 1981; Riis et al. 2012; Zefferman 2014), and dye may thus be more effective towards algae (Gettys et al. 2020), for example. Interestingly, for invasive rather than native species, previous studies have shown reductions in lateral spread and branching in macrophytes, leading to an increase in main stem length in response to shading (Kankanamge et al. 2019). Accordingly, an ability to switch between growth forms might confer invasion success to macrophytes and mediate their competitive dynamics (Kankanamge et al. 2020). Given the present study did not consider effects of interspecific competition on growth directly by exposing plant species to dye in different combinations, our work could be further improved by examining effects of both dye and different plant ratios or compositions on growth rates and related traits.

Interspecific differences in growth rates were substantially altered in darkness, with *L. major* exhibiting greatest length increases, and relatively unaffected biomass changes, in darkened conditions. Moreover, *L. major* gained significantly more mass per unit growth in length than the other species, indicating that biomass and length do not respond linearly owing to plant traits and physiological responses among species. This suggests that in light conditions, *L. major* tends to produce side shoots more than apical extension growth, or could be investing in thicker, heavier stems. Although not formally recorded, in light conditions, we generally observed that *L. major* tended to produce side shoots more than apical extension growth (K. Crane *pers. obs.*). Under decreasing intensities of light, the stem elongation of *L. major* then becomes greater, with fewer side shoots produced. Accordingly, the significant increase in length, but not biomass, compared to the other species in *L. major* under dark conditions suggests that this plant has a high capacity to etiolate. This heightened ability to lengthen in dark conditions may enhance the capture of available light and the competitive ability of *L. major* over *Elodea* species. Hussner et al. (2010) also found that, although *E. nuttallii* is capable of photosynthesis under lower light intensities, it is less efficient when acclimatising to high light levels following shading. Length extension is thus an essential trait for reaching the water surface quickly, as species that are capable of rapid canopy formation may out-compete other macrophytes by shading effects (Barrat-Segretain and Elger 2004). Previous studies have also found invasive *E. nuttallii* to exhibit decreased competitive ability compared to native species in brownified conditions (Xu et al. 2018). This corroborates our

results, where the benign *E. canadensis* significantly outgrew *E. nuttallii* under all dye treatments in terms of length.

Lagarosiphon major has been reported to grow most vigorously in the darker, colder months between October and May in some regions, and therefore its tolerance to lower light intensities is not unexpected (Caffrey et al. 2011). James et al. (1999) suggested that *L. major* can photosynthesise at a higher rate than both *Elodea* spp. and in some habitats is competitively superior to *Elodea*. Based on the rapid stem extension observed under very low light conditions in this experiment, and its known traits for photosynthesis, it would seem reasonable to suggest that *L. major* could out-compete *E. nuttallii* and *E. canadensis* should they co-occur under completely dark conditions, or seasonally as light regimes shift, and may help explain natural species displacement dynamics. Contrastingly, the superior growth rates of *E. canadensis* over *E. nuttallii* in terms of length appear to contradict patterns of replacement in the field and previous laboratory experiments, suggesting the presence of additional context-dependencies that mediate interspecific interactions, such as biotic interactions, water quality and nutrient availability (Crane et al. 2020a, b; Szabó et al. 2020). However, other studies have found negative photosynthesis or growth rates in darkness, such as for *Egeria densa* (Rodrigues and Thomaz 2010), *E. canadensis* (Madsen and Sand-Jensen 1994; Szabó et al. 2020), *E. nuttallii* (Szabó et al. 2020), *Hydrilla veriticillata* (Van et al. 1976; Bowes et al. 1977), *L. major* (Bickel 2011), *Ceratophyllum demersum*, and *Myriophyllum spicatum* (Van et al. 1976). Our measuring of fresh biomass as opposed to dry biomass of plants may have affected these trends; however, the nature of such biomass measures was consistent among species and treatments in the present study, ensuring comparability.

According to our results (albeit with plants treated in single-species assays), dye treatments, and thus light regimes, might alter competitive dynamics among these macrophyte species, as has been shown in previous works (Kankanamge et al. 2020). Nevertheless, assessment of *in situ* biotic and abiotic effects requires consideration. For example, increased water temperatures in many regions will exceed the 12 °C used in the present study, and this will likely cause greater plant growth (e.g. Barko et al. 1982). Water clarification effects may also arise from the breakdown of the dye as well as other anthropogenic management practices, such as for improving swimming or drinking water. Nevertheless, the specific effects of dye treatments did not fully negate growth of macrophytes over the experimental period, under our applied conditions with relatively low water volume, and thus the use of dye alone is unlikely to effectively control macrophyte populations *in situ* via light reductions. However, we also note that we used unrooted (but weighted) plants in shallow water, and dye effects could have been stronger on plants at greater depths.

Nevertheless, using higher concentrations of dye could aid management as these levels might have a stronger effect, as evidenced by the capacity of the completely dark treatment to significantly reduce growth rates, particularly for *Elodea* spp. in the present study. Concentrated dye effects on native communities, including native submerged plants and cascading effects, should, however, also be considered. Furthermore, synergistic control strategies could be used to control invasive macrophytes, such as light reductions in combination with nutrient alterations (Hussner et al. 2017). However, whilst nutrient concentrations are important for productivity of submerged aquatic macrophytes more generally, eutrophication alone has previously been shown to not explain empirical replacement dynamics among *E. canadensis*, *E. nuttallii* and *L. major* (James et al. 2006). Therefore, future studies should examine light regime and nutrient levels in concert to further understand this interaction, as elevated nutrient levels might compensate for reduced light, and vice versa. In this context, a recent study found the light compensation point and biomass loss in darkness to be higher, and photochemical efficiency and chlorophyll concentration lower, for *E. canadensis* compared to *E. nuttallii* across ranging nitrogen levels, with the latter also having a higher nitrogen removal efficiency (Szabó et al. 2020). This suggests that *E. nuttallii* exhibits better performance than *E. canadensis* in hypertrophic conditions that are shaded. Formally considering other traits such as lateral branching, dry weight and dry matter content would also provide useful insights, given their importance for invasion success, as well as additional ecophysiological measures. Moreover, testing plant responses in deeper mesocosms with multiple water levels (and levels of dye) would help determine the depth and concentrations at which the dye is most effective, as well as under different nutrient regimes. This could then be tested in small lakes over a longer period of time, and through all seasons where plants may display differing levels of dormancy, while also accounting for natural shading by trees, for example. Furthermore, even though dyes are reportedly non-toxic food-grade substances that may have no long-lasting effects on aquatic ecosystems (Hussner et al. 2017), research on any collateral damage to other plants and organisms should be explored. For example, the presence of dye could impede efficiency of visual predators (Cuthbert et al. 2018), and potentially alter populations of epipelic diatoms and epiphytic algae which could in turn mediate macrophyte growth. Equally, the direct effects of dye on native macrophytes should be explored to understand potential non-target impacts on other submerged plants, for which recovery may be of interest post-treatment. In this vein, future experiments at the community-scale could allow for potential cascades to be elucidated, for example, where reduced macrophyte abundances under low light conditions could lead to low light-tolerant phytoplankton dominance.

Conclusion

Dye-mediated light reductions did not negate growth for all three assessed macrophytes. We found striking evidence for sustained growth over an extended period of time, even under very low light levels in these species, suggesting that management strategies that manipulate light differ in efficacy among taxa. Nonetheless, they could be employed for a longer period of time (several months at least) and at greater concentrations of dye than employed here for potentially better efficacy in controlling invasive macrophytes. Moreover, the differential responses to light intensity evidenced among *L. major*, *E. canadensis* and *E. nuttallii*, suggest that dye-induced regime shifts could foster changes in community composition and dominance, with *L. major* in particular performing well under completely dark conditions. Therefore, the use of dye treatments may have implications for communities where two or more of these species coexist. We recommend that further studies be undertaken to examine effects of higher dye concentrations and different dye colours on relative growth rates and species succession in aquatic macrophytes, implications for wider ecological communities, and the influence of water depth on efficacy of applied dye.

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Author contribution

KC and LK: research conceptualisation; sample design and methodology. KC: investigation and data collection. RNC: data analysis and interpretation. AR, HJM and JTAD: funding provision. KC, RNC and NEC: writing – original draft. All authors: writing – review and editing.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Original data with plant species, dye treatments and growth measurements (length (cm) and biomass (g)) at the beginning and end of experimentation.