



Response rate of submerged macrophyte chlorophyll content under changing light conditions

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Abstract

Lake browning, i.e., a gradual and long-term increase in the water colour, is a recognized and topical water quality problem that has received much less attention in water protection than eutrophication. Thus, new bioindicators that respond to environmental stress are needed to monitor lake browning. Recent study suggests that the chlorophyll content of macrophytes is a promising indicator for assessing lake browning, yet little is known about how quickly the changes in the total chlorophyll content of aquatic plants occur. For instance, it is not known whether the plants adjust their chlorophyll content to the daily changes in light availability or to the longer-term light environment in the lake. Therefore, the response time of macrophytes to changes in light availability needs to be clarified to assess their suitability for detecting changes in water quality. The objectives of this experimental study were to investigate the chlorophyll content of *Egeria densa* and their response time to changes in light availability. No significant change in total chlorophyll content was observed in *E. densa*, but our study showed a change in macrophyte chlorophyll *a:b* ratio within 5–7 days of light intensity alternation, suggesting the chlorophyll ratio can be a more promising indicator for the light conditions varying on the daily scale than the snapshot weather monitoring. Thus, our study corroborates the idea of using macrophytes as bioindicators of browning.

Keywords Water colour · Lake browning · Aquatic plants · Water framework directive

Introduction

In the northern hemisphere, coloured, dissolved organic matter and iron (Fe) inputs to surface waters have increased over recent decades (Kritzberg and Ekström 2012; Monteith et al. 2007), causing severe and strong effects on aquatic ecosystems (Arzel et al. 2020; Houser 2006; Weyhenmeyer et al. 2016). The substantial increase in terrestrially derived, coloured, dissolved organic carbon (DOC) and Fe in surface waters is referred to as “browning”. Climate warming, increased precipitation, land use and decreased atmospheric sulphate deposition have been related to this phenomenon, but the drivers vary geographically (Estlander et al. 2021;

Evans et al. 2005; Weyhenmeyer et al. 2016). Lake browning has far-reaching consequences for ecosystem structure and functioning, such as primary production and nutrient cycles (Horppila et al. 2023; Seekell et al. 2015) and biodiversity (Arzel et al. 2020; Urrutia-Cordero et al. 2017). In addition, the browning of lakes interferes with different kinds of human water use e.g., recreation, fisheries and drinking water supply (Kritzberg et al. 2020).

Although lake browning is a recognized and topical water quality problem, lake browning is not consistently monitored, for example, in European Water Framework Directive (WFD) 2000/60/EC monitoring programmes (European Commission 2000; Horppila et al. 2024; Sepp et al. 2018). The aim of the WFD is to evaluate the ecological state of surface waters (rivers, lakes, transitional waters, and coastal waters) within the European Union. The ecological status of lakes has been monitored through phytoplankton, phyto-benthos, macrophyte, zoobenthos and fish communities (European Commission 2000). However, most of the biological quality elements in the WFD are focused on assessing eutrophication, whereas other stressors, such as lake browning, have received considerably less attention. In addition,

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only a few studies have assessed the suitability of potential lake browning bioindicators (Rajala et al. 2024; Strandberg et al. 2023).

The use of bioindicators is considered to have advantages over traditional water sampling undertaken at certain intervals because local prevailing biological species reflect environmental conditions over a longer period of time and can be used to assess biotic responses to environmental stress. As primary producers, aquatic macrophytes are sensitive to a variety of abiotic and biotic factors, such as light (Choudhury et al. 2019), nutrient concentration (Schneider and Melzer 2004) and pH (Trempe and Kohler 1995). Especially for submerged macrophytes, light availability is one of the most important environmental factors that affect growth and morphology (Barko et al. 1982). The underwater light condition also affects physiological functions such as photosynthetic rate and the concentrations of photosynthetic pigments of primary producers (Barko and Filbin 1983; Goldsborough and Kemp 1988). Since water colour controls the downward light penetration in terms of its intensity and quality in the water column (e.g., Effler et al. 2010; Horppila et al. 2023), the light sensitivity of macrophytes makes them promising ecological indicators to monitor lake browning. For example, Rajala et al. (2024) suggested that the photosynthetic pigments of macrophytes respond differently to eutrophication and browning and that the chlorophyll content of macrophytes may be a valid bioindicator of lake browning.

It is known that macrophytes typically growing in low light intensity have higher chlorophyll content compared with macrophytes growing in high light intensity (Macauley et al. 1988) and that the chlorophyll *a:b* (Chl *a:b*) ratio lowers with decreasing light intensity (Barko and Filbin 1983; Kordyum and Klimenko 2013). The use of the Chl *a:b* ratio as an indicator is based on the principle that the amount of chlorophyll *b* (Chl *b*) varies as a function of light availability and quality (Yamazaki et al. 2005), and under low light availability macrophytes tend to increase their Chl *b* concentration relative to Chl *a* concentration (Rajala et al. 2024). Thus, in clear water with high light availability, the Chl *a:b* ratio is expected to be greater than in high-coloured water with low light availability.

Horppila et al. (2022) showed with floating-leaved species *Nuphar lutea* (L.) that the Chl *a:b* ratio decreased in petioles of *N. lutea* with increasing water colour, and Rajala et al. (2024) also showed that the Chl *a:b* ratio fluctuated reciprocally with water colour during the growing season. However, the effects of browning on macrophytes have been relatively little studied overall (Blanchet et al. 2022). For instance, very little is known about how rapidly the changes in the total chlorophyll content of aquatic plants occur. Periods from one day to weeks have been suggested in terrestrial plants (Špundová et al. 2003) and periods from 3 to 6 days in certain macrophyte species in marine environment

(Goldsborough and Kemp 1988). The response time must however be measured when using the chlorophyll content of macrophytes to monitor temporal changes in water quality.

Here, we studied the chlorophyll content response time of the submerged macrophyte-dense waterweed *Elodea densa* (Planch.) Casp., 1857 in aquariums under high and low light intensity. Since total chlorophyll in submersed macrophytes should be inversely related to light intensity (Barko and Filbin 1983), we expected the chlorophyll *a + b* (Chl *a + b*) concentration to increase and the Chl *a:b* ratio to decrease in decreased light conditions (e.g., Horppila et al. 2022; Marcus 1980; Rajala et al. 2024). In addition, we expected *E. densa* to display a response time somewhat similar to 3 days for *Potamogeton perfoliatus* L. reported by Goldsborough and Kemp (1988).

Materials and methods

Experimental set-up

The submerged macrophyte *E. densa* is a freshwater perennial herb native to South America and has been introduced in many countries around the world (Cabrera Walsh et al. 2013) and commonly used in experimental studies. It can inhabit waters with a wide range of temperatures, has a low light requirement and can thrive in turbid environments and is therefore sustainable under varying environmental conditions (e.g., Yarrow et al. 2009). Specimens of *E. densa* used in this study consisted of cultivated individuals from a plant nursery. Upon arrival, the shoots of *E. densa* (length c. 20 cm) were placed in aquariums in bundles of 20 shoots and attached to submerged stainless steel grates to keep them in a natural position near the water surface. The plants were left to acclimatize in a 16:8 h light:darkness cycle ($50 \mu\text{E m}^{-2} \text{s}^{-1}$) at 18 °C for 48 h before the experiments.

This experimental study was conducted in three separate aquarium tanks (500 L) divided in half by an opaque wall so that water was allowed to flow freely between the compartments. Two light levels (high and low) in a 16:8 h light:darkness cycle were provided through the use of a black PVC cover in the other half of each of the three aquariums. The light levels were adjusted with photosynthetically active light (PAR) LED lamps, using a light quantum sensor (LI-1400 DataLogger with an LI-192SA Quantum Sensor, LICOR Biosciences, NE, USA), resulting in a $50 \mu\text{E m}^{-2} \text{s}^{-1}$ light intensity at 50 cm depth in the ‘high-light’ compartment with no cover and a $3 \mu\text{E m}^{-2} \text{s}^{-1}$ intensity at 50 cm depth in the ‘low-light’ compartment with cover. These light intensities were chosen to reflect the light levels measured at 50 cm depth in typical clear and humic water Finnish lakes, respectively (Horppila et al. 2022). Three replicate treatments were carried out for both light levels.

The aquariums were filled to a depth of 80 cm with chlorine-free tap water and incubated for 1 week before the experiments. Aeration and water circulation were provided by an external filter system with a ceramic gravel material filter and an aquarium bubble air stone. The mixture of nutrients (Seachem Flourish Phosphorus + Nitrogen, Seachem Laboratories, GA, USA, Aquatic Nature Aqua-Plant + Activator, Aquatic Nature, Belgium and Tetra PlantaMin mineral solution for trace minerals Fe, manganese and potassium, Tetra GmbH, Germany) was added to avoid nutrient limitation during the experiment. The nutrient concentration was monitored daily by water test kits, JBL PO₄ Test (JBL GmbH & Co. KG, Germany), Tetra Test NO₃ and Tetra Test Fe (Tetra GmbH, Germany), to maintain the concentrations of Fe at 0.5 mg L⁻¹, total phosphorus at 18–20 µg L⁻¹ and total nitrogen at 700–800 µg L⁻¹, which simulate the nutrient levels commonly found in Finnish lakes (Estlander et al. 2021). At the end of the last treatment, the total nitrogen (TN) and total phosphorus (TP) concentrations were determined from the samples with a Lachat Autoanalyzer (QuikChem Series 8000, Lachat Instruments, Hach Co., USA), resulting in 700–760 µg L⁻¹ and 16–17 µg L⁻¹ for TN and TP, respectively. The water temperature was kept constant at 18 °C throughout the experiments. The aquariums were kept clean by scraping the walls to remove epiphytes every other day during the experiment.

First, shoots of *E. densa* were moved from the acclimatization unit to the aquariums and attached to stainless steel grates such that the tops of the plants were at a depth of 50 cm. Samples for Chl *a* and *b* were taken at the onset of the experiment (day 0) to determine the initial chlorophyll content of the macrophytes. After starting the treatment, chlorophyll samples were taken 24 h later and thereafter at 2-day intervals: days 1, 3, 5 and 7. After day 7, light conditions were alternated between two compartments in each tank: a shading PVC cover was placed over each high-light compartment and the covers were removed from the low-light compartments. Light levels thus changed from 50 to 3, and 3 to 50 µE m⁻² s⁻¹, to measure the response time of macrophytes under the alternation of light conditions. After the alteration of light conditions, chlorophyll samples were taken in the same as the former experiment. At each sampling time, three replicate samples for chlorophyll were taken by cutting 5 cm from the top of each randomly selected shoot. The same shoots were not used repeatedly for the chlorophyll sampling during the experiment, that is, samples were always taken from uncut shoots. The samples were rinsed with tap water and gently wiped visually clean with a soft brush to remove periphyton from the surface of the plant shoots and immediately wrapped in aluminium foil and stored in a freezer (-18 °C) before analysis.

Chlorophyll analysis

The extraction of chlorophyll from macrophyte samples was conducted according to Horppila et al. (2022). Samples were first weighed (wet weight), then macerated, and subsequently extracted in 95% ethanol at 75 °C for 5 min. To ensure extraction, the samples were also treated with ultrasound. The extract was filtered to remove particulate matter, and the concentrations of Chl *a* and Chl *b* were determined with an F-4000 fluorescence spectrophotometer (Hitachi Ltd, Japan) and calculated using the equations by Lichtenthaler and Wellburn (1983) as follows:

$$C_{Chla} = 13.95 \times A_{665nm} - 6.88 \times A_{649nm} \quad (1)$$

$$C_{Chlb} = 24.96 \times A_{649nm} - 7.32 \times A_{665nm} \quad (2)$$

where *C* is the chlorophyll µg ml⁻¹ plant extract, and *A* is the measured absorbance at different wavelengths.

Statistical analysis

Between-treatment differences in the chlorophyll content of *E. densa* shoots (Chl *a* + *b*, Chl *a*:*b*) were tested with a two-way repeated-measures analysis of variance (ANOVA), along with a Tukey HSD post hoc test. Sampling day was specified as the repeated factor (within subjects) and light treatment as a fixed effect (between subjects). For Chl *a* + *b* and Chl *a*:*b* the analyses included pairwise treatment comparisons separately for each time point.

Results

In contrast to expectations, but in line with existing knowledge (Horppila et al. 2022; Rajala et al. 2024), Chl *a* + *b* showed no significant variation between light treatments or over the duration of the experiment (Fig. 1, Table 1). The Chl *a* + *b* was on average 0.96 ± 0.05 µg mg⁻¹ in the high-light treatments and 1.02 ± 0.09 µg mg⁻¹ in the low-light treatments. When the light treatments were reversed from high to low light, the Chl *a* + *b* was on average 1.06 ± 0.26 µg mg⁻¹, and when they were reversed from low to high light, the Chl *a* + *b* was on average 1.01 ± 0.18 µg mg⁻¹ (Fig. 1). In contrast, as we hypothesized, the Chl *a*:*b* ratio was significantly higher in the high-light treatments compared with the low-light treatments, only after 7 days of light treatment (Fig. 1, Tables 1, 2), concluding that the time for Chl *a*:*b* ratio of *E. densa* to respond to decreasing light was 7 days. Also, post hoc comparisons using the Tukey HSD test indicated that the mean Chl *a*:*b* ratio of the low-light treatment (mean difference = 0.30, SD = 0.04) was significantly lower than the

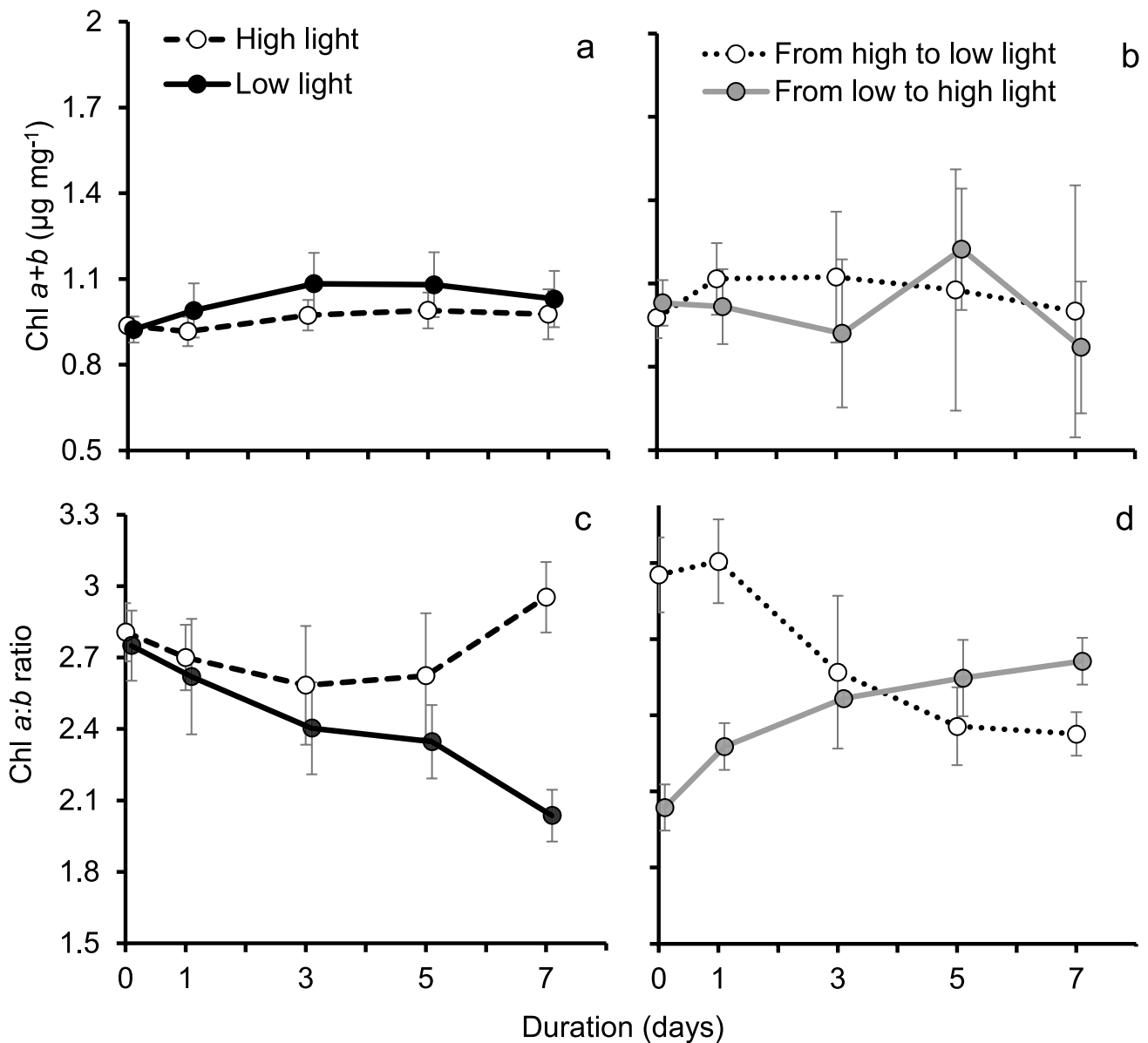


Fig. 1 Variation of Chl ($a+b$) concentration (**a**, **b**) and Chl $a:b$ ratio (**c**, **d**) with $\pm 95\%$ confidence intervals in *Egeria densa* shoots during the 7-day experiment under different light treatments

Table 1 Results on two-way repeated-measures ANOVA for Chl $a+b$ or Chl $a:b$ of *Egeria densa* shoots in an upper model (Day) to test temporal variation within each treatment and in a lower model (Light treatment $\times$ Day) to test between-treatment differences on each sampling day

	Chl $a+b$				Chl $a:b$		
	DF	F	<i>p</i>		DF	F	<i>p</i>
Day	4	1.29	0.296		4	3.78	0.013
Light treatment $\times$ day	12	0.61	0.61		12	9.19	<0.01

Statistically significant *p* values are shown in bold

high-light treatment ($p < 0.01$). After the treatments were reversed, the differences in the Chl $a:b$ ratio between high- and low-light treatments were almost eliminated on day 3

(Fig. 1, Table 2), and on day 7 the *E. densa* Chl $a:b$ ratio was on average higher in the low-to-high-light treatments compared with the high-to-low-light treatments. However,

Table 2 Pairwise comparison based on estimated marginal means of Chl *a:b* ratio of *E. densa* shoots between two-time points of time in each light treatment. Given *p*-values are based on the repeated-measures ANOVA

		High light	Low light	High-to-low light	Low-to-high light
Day 0	Day 1	1.000	1.000	1.000	1.000
	Day 3	0.975	0.195	0.122	0.069
	Day 5	1.000	0.151	0.019	0.046
	Day 7	1.000	< 0.001	0.001	0.001
Day 1	Day 3	1.000	1.000	0.097	1.000
	Day 5	1.000	0.956	0.020	0.990
	Day 7	0.107	0.001	< 0.001	0.023
Day 3	Day 5	1.000	1.000	1.000	1.000
	Day 7	0.088	0.092	0.535	1.000
Day 5	Day 7	0.305	0.390	1.000	1.000

where significant differences are shown in bold

a post hoc Tukey HSD test indicated that there were no significant differences in the Chl *a:b* ratios between the high-to-low-light and high-light treatments (mean difference = -0.09, SD = 0.04, $p = 0.213$) nor between the low-to-high-light and low-light treatments (mean difference = -0.04, SD = 0.04, $p = 0.735$), suggesting that reversing the light treatment had no significant effect on Chl *a:b* ratios.

When the response time was estimated within treatments, a significant change from the initial value on day 0 occurred on day 5 in low-to-high-light treatment (Fig. 1, Table 2). However, *E. densa* shoots that had been under the low-light treatment and then reversed to high light did not reach as high a Chl *a:b* level on 5–7 days as initially measured in the high-light treatments, resulting in the ratio lower by 0.34 than the initial level. Correspondingly, changing the treatment from a high to low light level did not decrease the Chl *a:b* ratio to as low a level as in the low-light treatment on 7 days (Fig. 1).

Discussion

Plants can respond with a variety of biochemical and physiological reactions to changing light intensity on timescales from less than a second to a season (Assmann and Wang 2001). Lake browning bioindicators should however reflect changes in water quality or light availability in water and not to other environmental conditions such as sudden changes in weather. We demonstrated that the change in macrophyte chlorophyll content in response to light intensity variation occurred within 5–7 days, suggesting that short-term environmental changes, such as daily changes in the weather, should not be indicated by the change in the chlorophyll content of macrophytes. Thus, our study corroborates the idea of using macrophytes as bioindicators of browning (Rajala et al. 2024).

Light availability in water is highly variable because of variations in season, weather conditions, water

transparency, depth and waves. Therefore, as sessile organisms, phenotypically adapting to changes in the environment is crucial for macrophytes (Kirk 1994). Different types of adaptive response to light variation have evolved in macrophytes: e.g., morphology adjustment, biomass reallocation and changes in the content of photosynthetic pigments (Goldsborough and Kemp 1988; Strand and Weisner 2001). The total Chl *a + b* content of plants generally increases with decreasing light intensity due to compensation for photosynthesis (Barko and Filbin 1983). An opposite or non-significant relationship between light intensity and the chlorophyll concentration of aquatic macrophytes has also been reported (Marcus 1980). In our study, the 7-day low-light treatment had no significant effect on the total Chl *a + b* content of *E. densa*, which varied within the range of values that reported in previous studies on this species (Ellawala et al. 2011). Our results are also in line with Horppila et al. (2022), Marcus (1980) and Rajala et al. (2024), who found no clear correlation between light intensity and the content of either Chl *a* or Chl *b* but that the Chl *a:b* ratio decreased with decreasing light intensity. The change in the Chl *a:b* ratio was also dependent on the period of exposure to low light intensity, as in the case of *E. densa* in which a significant change in the Chl *a:b* ratio occurred within a week.

It appears that *E. densa* acclimatize to low light rather by increasing Chl *b* relative to Chl *a* than by increasing the total chlorophyll content, corroborating previous studies with macrophytes (Horppila et al. 2022). It should be noted, however, that submerged macrophyte *E. densa* is adapted to a lower light level overall in its natural growth environment than, for example, floating-leaved macrophytes. For example, the total chlorophyll content was almost 10 times higher in the *E. densa* studied here than in that reported in the petioles of *N. lutea* (Horppila et al. 2022; Rajala et al. 2024). This is in accordance with Dar et al. (2013), who suggested that floating-leaved macrophyte species have a lower total chlorophyll content than submerged macrophytes, as

the floating-leaved species are adapted to an environment with higher light intensities.

Interestingly, our results indicated that the Chl *a:b* ratio of *E. densa* did not respond in the same manner under reversed light treatments as observed in the initial light treatments, contrary to the patterns reported in earlier studies with other macrophyte species (Goldsborough and Kemp 1988). Our experiment revealed that the Chl *a:b* ratio of *E. densa* did not return to the level at the beginning of light treatment, whether it is high-to-low or low-to-high, within at least 7 days needed for the Chl *a:b* response to the low light condition. Possible reasons for this are depletion of micronutrients and the incubation condition should equally stresses to all individuals. A critical difference between the latter from the former experiments is switching between extreme light conditions, which can be a physiological stress to plants. Although the nutrient concentrations (N and P) remained constant throughout the experiment, we did not monitor micronutrients such as magnesium or copper. In addition, it is likely that phytoplankton were present in the experimental units. Phytoplankton, for instance, have been shown to compete with macrophytes for essential resources such as light and nutrients (Barrow et al. 2019). The chlorophyll concentration of the water was not analysed here, but any potential turbidity caused by phytoplankton was not reflected in the light levels measured in the experiment, which remained constant throughout the duration of the study. In addition, the level of the high-light treatment ($50 \mu\text{E m}^{-2} \text{s}^{-1}$) and the temperature (18°C) in our experiments were relatively low compared with the optimum levels (25°C , $80\text{--}100 \mu\text{E m}^{-2} \text{s}^{-1}$) reported for *E. densa* (Rodrigues and Thomaz 2010). Since plants commonly respond to environmental stress by altering chlorophyll content (Agathokleous et al. 2020), we suspect that some stressors experienced by *E. densa* under the experimental conditions, potentially exacerbated by micronutrient deficiencies, may have influenced the response of macrophyte Chl *a:b*, particularly with the elapsed time of our experiment.

The light climate of lakes has been studied extensively, but few articles have focused on daily change (Alvarez-Cobelas et al. 2002), which is significant when considering the chlorophyll content of macrophytes as an indicator of water quality. Generally, short-term (daily) variation in the DOC concentration of lakes is relatively small (Eloranta 1999), while Alvarez-Cobelas et al. (2002) showed that the within-lake fluctuations in water colour usually take several days. Rajala et al. (2024) observed that the Chl *a:b* ratio of *N. lutea* responded to water quality variations within the lakes during the growing season when the sampling interval was 2 weeks. Both the findings by Alvarez-Cobelas et al. (2002) and Rajala et al. (2024) are in line with our observation that the Chl *a:b* ratio of macrophytes can serve as a successful bioindicator of lake browning in terms of response time.

However, it should be noted that in addition to browning, other factors also affect underwater light, such as turbidity caused by phytoplankton or clay particles. According to Rajala et al. (2024), turbidity can disturb the use of chlorophyll content of macrophytes as an indicator of lake browning. They also note that macrophyte Chl *a:b* responses to turbid water and water colour were differentiated depending on differences in light spectral distribution between coloured and turbid lakes. Other environmental stressors, such as salinity and pollutants, have been shown to affect the Chl *a:b* ratio in terrestrial plants (Li et al. 2015; Ranjbarfordoei et al. 2006). However, the effects of these factors on aquatic plants are largely unexplored and warrant future investigation.

Given the response time for the Chl *a:b* ratio of macrophytes studied here, we conclude that it is a potential bioindicator to detect relatively short-term changes in water quality, i.e., changes that take place in a week. This type of response could be considered an ‘early-warning’ indicator of environmental changes and be suitable for use in reactive management strategies to alleviate the effects of any further changes in water quality (Cooper et al. 2009). In particular, the Chl *a:b* ratio of macrophytes is interesting, because different types of photosynthetic pigments respond differently to the spectral composition of light (Gao et al. 2023; Yamazaki et al. 2005). Anthropogenic disturbances, such as eutrophication and browning, have differential effects on the spectral composition of the underwater light climate (Eloranta 1999). In addition to the quantity, the quality of light affects the Chl *a:b* ratio in macrophytes (Horppila et al. 2022; Klimenko 2012; Rajala et al. 2024). Thus, the long-term effects of browning, light quality and possible genetic adaptations of macrophytes to low light intensities should be investigated in the future.

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Author contributions S.E., E.P. and J.H. conceptualized the study and S.E. and E.P. conducted the experimental work. S.E. analysed the data and wrote the first draft of the manuscript. S.R., J.H. and L.N. reviewed and provided substantive revisions to the manuscript.

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Data availability Data are available from the authors upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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