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SECRETS OF SHALLOW LAKES

Spatial and temporal variation of *Nuphar lutea* pigment content in small boreal lakes: effect of water colour and phosphorus concentration

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Abstract The spatial and temporal variation of *Nuphar lutea* chlorophyll content was studied in 12 small lakes in Finland to clarify the bioindicator potential of *N. lutea* chlorophyll content for monitoring lake brownification. Significant in-lake variation in water colour and total phosphorus concentration was observed in a few study lakes. At highest, the in-lake variation for water colour was 13% and for total phosphorus concentration 27%. However, the in-lake variation in water quality did not coincide with significant in-lake variation of *N. lutea* chlorophyll content. The total chlorophyll concentration (chlorophyll $a+b$) varied in a few lakes, but the chlorophyll *a* and *b* ratio (chlorophyll *a:b*) did not change within the lakes. The chlorophyll *a:b*, however, varied between lakes and decreased significantly with increasing water colour and total phosphorus concentration in *Nuphar lutea* individuals collected near the lake outflow. The chlorophyll content of *N. lutea* did not show significant temporal variation during the one-week sampling period despite variable weather conditions. Chlorophyll *a:b* of *N. lutea* seems to be a possible bioindicator for monitoring brownification, as it can differentiate lakes from each other based on a small difference in water colour but does not react to short-term in-lake variations in the water quality.

Keywords Macrophytes · Brownification · Spatial variation · Temporal variation · Water colour · Chlorophyll

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Introduction

Freshwaters are confronting numerous human-induced changes. One of these changes is lake brownification, i.e. a substantial increase in the water colour, which has been observed in temperate and northern regions of the world during recent decades (Monteith et al., 2007; Weyhenmeyer et al., 2016). Brownification is mainly caused by increased loading of dissolved organic carbon (DOC) from catchments, but also iron (Fe) has a significant role (Kritzberg & Ekström, 2012; Creed et al., 2018). Increased precipitation and elevated temperature caused by climate change (Weyhenmeyer & Karlsson 2009; de Wit et al., 2016), land-use change, especially peat production and forest ditching (Estlander et al., 2021), and a decrease in atmospheric sulphate deposition (Evans et al., 2012; Finstad et al., 2016) have been suggested to be the main drivers behind brownification.

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Brownification has diverse impacts on lake ecosystems. Since DOC effectively absorbs short-wavelength light, which is especially important for photosynthesis, the amount of photosynthetically active radiation is decreased in the water column, which in turn limits primary production (Seekell et al., 2015; Bergström & Karlsson, 2019). Brownification also causes a decrease in phytoplankton and zooplankton biodiversity (Urrutia-Cordero et al., 2017; Estlander & Horppila, 2023), turns lakes more heterotrophic (Creed et al., 2018), decreases the abundance of aquatic invertebrates (Arzel et al., 2020) and the predation efficiency of visually oriented fish (Estlander et al., 2012), as well as increases fish mortality during the ice-cover season (Hedström et al., 2017).

To prevent the deterioration of the status of water bodies, the European Water Framework Directive (WFD) was established in the year 2000, and since then it has been the main policy tool for water protection in Europe (Reyjol et al., 2014). The WFD requires European Union Member States to use their river basin management plans and programmes to protect and restore water bodies in order to reach good ecological and chemical status by 2027 (Reyjol et al., 2014). In the WFD, there are several biological parameters that are related to pristine conditions to define an ecological quality ratio, which is a comparable measure of ecosystem quality (Reyjol et al., 2014). However, current biological parameters, for instance phytoplankton biomass, are mainly targeted to describe eutrophication and, as such, are not suitable to monitor the effects of brownification (Reyjol et al., 2014; Sepp et al., 2018; Albrecht et al., 2023; Horppila et al., 2024). The ecological quality class of a lake may thus remain unchanged even if the lake ecosystem changes due to brownification, and therefore there is an urgent need to develop new biological indicators specifically for brownification (Albrecht et al., 2023).

Macrophytes are widely used bioindicators for monitoring eutrophication (Haury et al., 2006; Penning et al., 2008). In the WFD, the use of macrophytes is based on displayed biotic responses measured as changes in the macrophyte community structure (Penning et al., 2008; Vuori et al., 2009). However, it is most likely challenging to distinguish eutrophicated and brownificated lakes from each other based on the responses in macrophyte community structure. Since both eutrophication and brownification reduce water transparency, the colonization area especially for submerged species is diminished and less light-limited life-forms, floating-leaved and emergent macrophytes, often dominate in both eutrophicated and in brownificated lakes (Egertson et al., 2004; Estlander et al., 2009). Therefore, the effects of brownification and eutrophication on macrophyte community structure are likely to be partly similar, and thus we have suggested that the macrophyte-based indicator of brownification could rather be developed based on the chlorophyll (CHL) content of macrophytes (Horppila et al., 2022; Rajala et al., 2024).

Macrophytes adapt to variations in light conditions by changing their CHL content (Kume et al., 2018). The adaptation is based on the fact that different CHL pigments have different absorption maxima for wavelengths of light (Kume et al., 2018). For example, CHL *b* absorbs short-wavelength light more effectively than CHL *a* (Yamazaki et al., 2005). In brownificated lakes the short-wavelength light is effectively absorbed by DOC, and thus the amount of this photosynthetically important light is scarce (Eloranta, 1999). Therefore, as an adaptive response to decreased short-wavelength light, macrophytes increase their CHL *b* concentration relative to their CHL *a* concentration, causing a decrease in the ratio of CHL *a* to CHL *b* (CHL *a:b*) (Barko & Filbin, 1983; Horppila et al., 2022; Rajala et al., 2024). Because the spectral composition of light differs in brownificated and eutrophicated lakes, CHL *a:b* of the yellow water-lily (*Nuphar lutea* (L., 1753), Sm., 1809) seems to differentiate the effects of brownification from those of eutrophication (Horppila et al., 2022; Rajala et al., 2024).

However, there are still some aspects that require clarification when considering the bioindicator potential of *N. lutea* CHL *a:b*. Firstly, the in-lake variation of the *N. lutea* CHL content remains unknown. In the littoral zone, the chemical oxygen demand is often noted to be higher than in the open water area (Yin et al., 2011), indicating higher organic matter concentration and water colour near the shoreline. In addition, due to the land–water interface in the littoral zone, the effect of DOC and Fe loading from the catchment is stronger than in the pelagic zone (Håkanson, 1991; Wetzel, 2001). In-lake water colour differences may also originate from the land-use history of the catchment area (Brandão et al., 2022). Additionally, due to resuspension-induced internal

loading, phosphorus (P) concentrations in the shallow littoral zone may exceed the concentrations in the pelagic zone, thus causing in-lake variation in nutrient concentrations (Kristensen et al., 1992; Evans, 1994). This phenomenon is especially important in areas devoid of macrophytes or with sparse macrophyte coverage (Wetzel, 2001; Horppila & Nurminen, 2005). Macrophyte stands trap fine sediment particles, thus limiting sediment resuspension (Madsen et al., 2001; Reitsema et al., 2020; Thomaz, 2023), but compared with other life-forms, the sediment trapping capacity is weaker among the floating-leaved stands, typically dominating in high-coloured lakes (Horppila & Nurminen, 2005). Therefore, sediment resuspension in the littoral zone of high-coloured lakes can be more prominent, potentially causing higher in-lake variation in water quality.

Secondly, temporal variation in the CHL content of macrophytes due to weather variation is poorly understood**.** For example, the rate of photosynthesis of macrophytes can show daily and even hourly variation, depending on the prevailing solar radiation (Figueroa et al., 1997). Macrophytes have different mechanisms for adapting to changing light conditions. One common light acclimation strategy is to transfer excess energy from CHL to carotenoid (CAR), thus causing a decrease in CHL:CAR (Franklin & Forster, 1997; Schneider et al., 2006; Ruban et al., 2007). However, CHL:CAR varies faster than the macrophyte CHL content (Goldsborough & Kemp, 1988; Howarth & Durako, 2013), and therefore we postulate that the CHL content could reflect changes in water quality, rather than rapid changes in weather conditions, and thus work as a more reliable indicator of water quality than CHL:CAR.

In this study, we investigated if the total concentration of CHL *a* and CHL *b* (CHL *a*+*b*) and the CHL *a:b* of the floating-leaved *N. lutea* petiole display in-lake and between-lake variation in lakes with different water colour. In addition to water colour, macrophyte CHL *a:b* has been noted to be higher in lakes with high nutrient concentration (Hu et al., 2008). Therefore, we also studied whether the CHL content of *N. lutea* is related to the total P concentration in the littoral zone. In addition, we studied the short-term changes in *N. lutea* CHL content (CHL *a*+*b,* CHL *a:b*) with changing weather conditions. The aim was to clarify, from which part of the lake the *N. lutea* samples must be collected so that they would reflect the average water quality of the lake, and to investigate, whether short-term changes in weather conditions affect the CHL content of *N. lutea* and complicate its use as a bioindicator for long-term environmental changes. The specific study questions were: (1) Do the water quality (water colour, total P concentration) and CHL content (CHL *a*+*b*, CHL *a:b*) of *N. lutea* display between- and in-lake variation? (2) Does the in-lake growing site of *N. lutea* affect its ability to reflect the average water quality (water colour, total P concentration) of the lake? (3) Does the CHL content (CHL *a*+*b*, CHL *a:b*) of *N. lutea* show short-term variation according to changes in weather conditions, mainly solar irradiation? We hypothesize that possible in-lake differences in water quality (water colour, total P concentration) can be detected as differences in the *N. lutea* CHL content (CHL *a*+*b*, CHL *a:b*). In addition, we hypothesize that short-term variation in the solar irradiance does not affect the CHL content of *N. lutea*.

Materials and methods

Study lakes

The study was conducted in June–July 2022 in 12 lakes situated in southern Finland (Table 1), and the samplings were done once for each lake. The lakes are small, ranging in area from 1.0 to 48.8 ha. The size of the catchment area varies between 0.2 and 66.5 km^2 (Table 1). The study lakes were chosen to represent a range of water quality, which has been investigated thoroughly in previous studies (Estlander et al., 2021; Horppila et al., 2023), with water colour ranging from 14 to 314 mg Pt l^{-1} and total P concentration from 9 to 47 µg l^{-1} (Horppila et al., 2023). The water quality of the study lakes varies due to differences in catchment characteristics (Table 1). Peatland coverage of the lake catchment varies from 0 to 74% and the density of ditches in the catchment from 0.1 to 6.8 km^2 (Table 1). Both peatland coverage and ditch density in the lake catchment have been shown to have a significant effect on the water quality (Estlander et al., 2021).

Table 1 Background data from the study lakes and their catchments (Estlander et al. 2021)

Lake	Location	Area (ha)	Catchment area (km^2)	Peatland coverage (%)	Ditch density ($km\ km^{-2}$)
Alinen Rautjärvi	61° 11′ N, 25° 05′ E	48.8	66.5	9.0	4.5
Haukijärvi	61° 13′ N, 25° 08′ E	2.2	5.6	21.8	6.3
Hokajärvi	61° 14′ N, 25° 06′ E	8.4	6.3	9.3	4.1
Iso Valkjärvi	61° 11′ N, 25° 06′ E	3.8	0.2	0	2.6
Kalliojärvi	61° 13′ N, 25° 01′ E	4.9	1.3	9.1	3.4
Käkilammi	60° 57′ N, 24° 43′ E	9.9	0.6	34.4	4.0
Kärppäjärvi	62° 12′ N, 22° 15′ E	19.6	10.9	71.0	6.8
Neva-Lyly	62° 11′ N, 22° 50′ E	10.7	3.1	74.3	5.3
Rahtijärvi	61° 13′ N, 25° 08′ E	12.0	30.5	13.2	5.1
Valkea Mustajärvi	61° 13′ N, 25° 07′ E	13.1	0.5	0	0.1
Vähä Koukkujärvi	61° 11′ N, 25° 04′ E	1.0	0.3	0	3.2
Vähäjärvi	61° 10′ N, 25° 11′ E	7.9	0.7	0.1	4.4

Water sampling

Three sampling stations were selected from each lake, located near each lake's inflow and outflow, and in the intermediate area between these. From each sampling station, three replicate water samples were taken with a Limnos tube sampler (volume 2.8 l, Limnos). From water samples ($n = 108$), water colour and total P concentration were analysed. Water colour was measured spectrophotometrically as the absorbance of light at 410 nm wavelength and converted to mg Pt l^{-1}, according to standard SFS-EN ISO 7887 (Finnish Standards Association, 2011), and total P concentration was analysed with a flow injection analyser (Gallery Plus, Lachat QuikChem).

Macrophyte sampling and chlorophyll analyses

To investigate the in-lake variation of *N. lutea* CHL content, three replicates of *N. lutea* were collected from each lake from the same sampling stations where the water samples were collected ($n = 108$). For the temporal study, three *N. lutea* replicates were collected daily from Haukijärvi for one week (17–23 July 2022) ($n = 21$). Sampling was thus conducted during the highest CHL content of *N. lutea* (Dar et al., 2013). Macrophytes for the temporal study were always collected from the same sampling station situated approximately 5 m from the shore, near the outflow of Haukijärvi.

Macrophyte sampling was done by cutting the petiole of each plant from the sediment–water interface and placing the plant in a dark plastic bag after sampling (Horppila et al., 2022). In the laboratory, the plants were gently wiped to remove the periphyton (Horppila et al., 2022). For the CHL analysis, a 1 cm piece of the petiole from 10 cm below the floating leaf was cut from each plant, wrapped in aluminium foil and frozen. The petiole of *N. lutea* was studied instead of floating or submerged leaves because the petiole has been shown to be the most reliable indicator of water colour (Rajala et al., 2024).

The CHL extraction was prepared by macerating samples and extracting weighted samples in 95% ethanol at 75 °C for 5 min. To ensure the CHL extraction, samples were also treated with ultrasound (Horppila et al., 2022), and to remove particulate matter, the extract was filtered. The concentrations of CHL *a* and CHL *b* were calculated using the method by Lichtenthaler and Wellburn (1983).

Statistical analyses

To investigate whether the water colour, total P concentration and *N. lutea* CHL content (CHL $a+b$, CHL *a:b*) show between- and/or in-lake variation, a nested analysis of variance and pairwise Bonferroni *t*-tests were computed with ln-transformed data. The analysis included pairwise comparisons between all three sampling stations, separately for each lake. In addition, linear regression analysis was used to

determine whether the in-lake growing site of *N. lutea* affects its ability to reflect average lake water quality (water colour, total P content). In the regression analysis the *N. lutea* CHL content (CHL $a+b$, CHL *a:b*) of each sampling station (inflow, outflow, intermediate area) was separately compared to the average water quality of the lake (water colour, total P concentration). For the linear regression analysis, CHL data were ln-transformed to improve normality.

Finally, the temporal variation of the CHL content of *N. lutea* in the 7-day sampling period was analysed with a one-way repeated measures analysis of variance. To evaluate the effects of weather conditions, solar irradiation data from the closest meteorological station (60.32937°N, 24.97274°E) were obtained (Finnish Meteorological Institute open data), and the relationship of CHL $a+b$ and CHL *a:b* to solar irradiance was examined with a Pearson correlation analysis.

Results

Between- and in-lake variation of water colour and total phosphorus concentration

Water colour differed significantly between study lakes ($P<0.001$) (Table 2). The lowest water colour was observed in Valkea Mustajärvi (27 mg Pt l^{-1}) and highest in Käkilammi (319 mg Pt l^{-1}) (Fig. 1). Water colour showed significant in-lake variation within 4 (Iso Valkjärvi, Hokajärvi, Kärppäjärvi and Haukijärvi) out of 12 study lakes ($P<0.001$) (Table 2). In Iso Valkjärvi, the range in water colour was 10 mg Pt l^{-1} ($P<0.001$), in Hokajärvi 4 mg Pt l^{-1} ($P<0.001$), in Kärppäjärvi 26 mg Pt l^{-1} ($P<0.001$), and in Haukijärvi 12 mg Pt l^{-1} ($P<0.001$) (Fig. 1).

Total P concentration of study lakes also varied significantly between lakes ($P<0.001$) (Table 2). The total P concentration was lowest in Vähäjärvi (8 µg l^{-1}) and highest in Käkilammi (46 µg l^{-1}) (Fig. 1). Total P concentration also showed significant in-lake variation ($P<0.001$) (Table 2). The in-lake variation was significant in 7 (Iso Valkjärvi, Neva-Lyly, Alinen Rautjärvi, Rahtijärvi, Kärppäjärvi, Vähä Koukkujärvi and Käkilammi) out of 12 study lakes. The range in total P concentration in Iso Valkjärvi was 2 µg l^{-1} ($P=0.01$), in Neva-Lyly 3 µg l^{-1} ($P=0.01$), in Alinen Rautjärvi 3 µg l^{-1} ($P<0.001$), in Rahtijärvi 6 µg l^{-1} ($P<0.001$), in Kärppäjärvi 8 µg l^{-1} ($P<0.001$) in Vähä Koukkujärvi 2 µg l^{-1} ($P=0.04$) and in Käkilammi 14 µg l^{-1} ($P<0.001$) (Fig. 1).

Between- and in-lake variation of *N. lutea* chlorophyll content (CHL $a+b$, CHL *a:b*)

CHL $a+b$ concentration varied significantly between lakes ($P<0.001$), being highest in Neva-Lyly (0.29 µg mg^{-1}) and lowest in Rahtijärvi (0.14 µg mg^{-1}) (Table 2; Fig. 1). CHL $a+b$ concentration also showed significant in-lake variation, but only in 2 (Neva-Lyly and Vähäjärvi) out of 12 study lakes ($P<0.001$) (Table 2; Fig. 1). The range

Table 2 Results from the nested analysis of variance: between- and in-lake variation of water quality parameters (water colour, total phosphorus concentration) and *Nuphar lutea* chlorophyll content (CHL $a+b$, CHL *a:b*)

Variation	Sum of squares	Degrees of freedom	Mean square	*F*	*P*
Water colour					
Between-lake	67.90	11	6.17	42 194.87	<0.001
In-lake	0.12	24	0.01	34.51	<0.001
Total phosphorus					
Between-lake	33.96	11	3.09	594.73	<0.001
In-lake	0.78	24	0.03	6.24	<0.001
N. lutea CHL $a+b$					
Between-lake	2.16	11	0.20	3.98	<0.001
In-lake	4.30	24	0.18	3.65	<0.001
N. lutea CHL *a:b*					
Between-lake	0.19	11	0.02	16.51	<0.001
In-lake	0.03	24	0.01	1.14	0.330

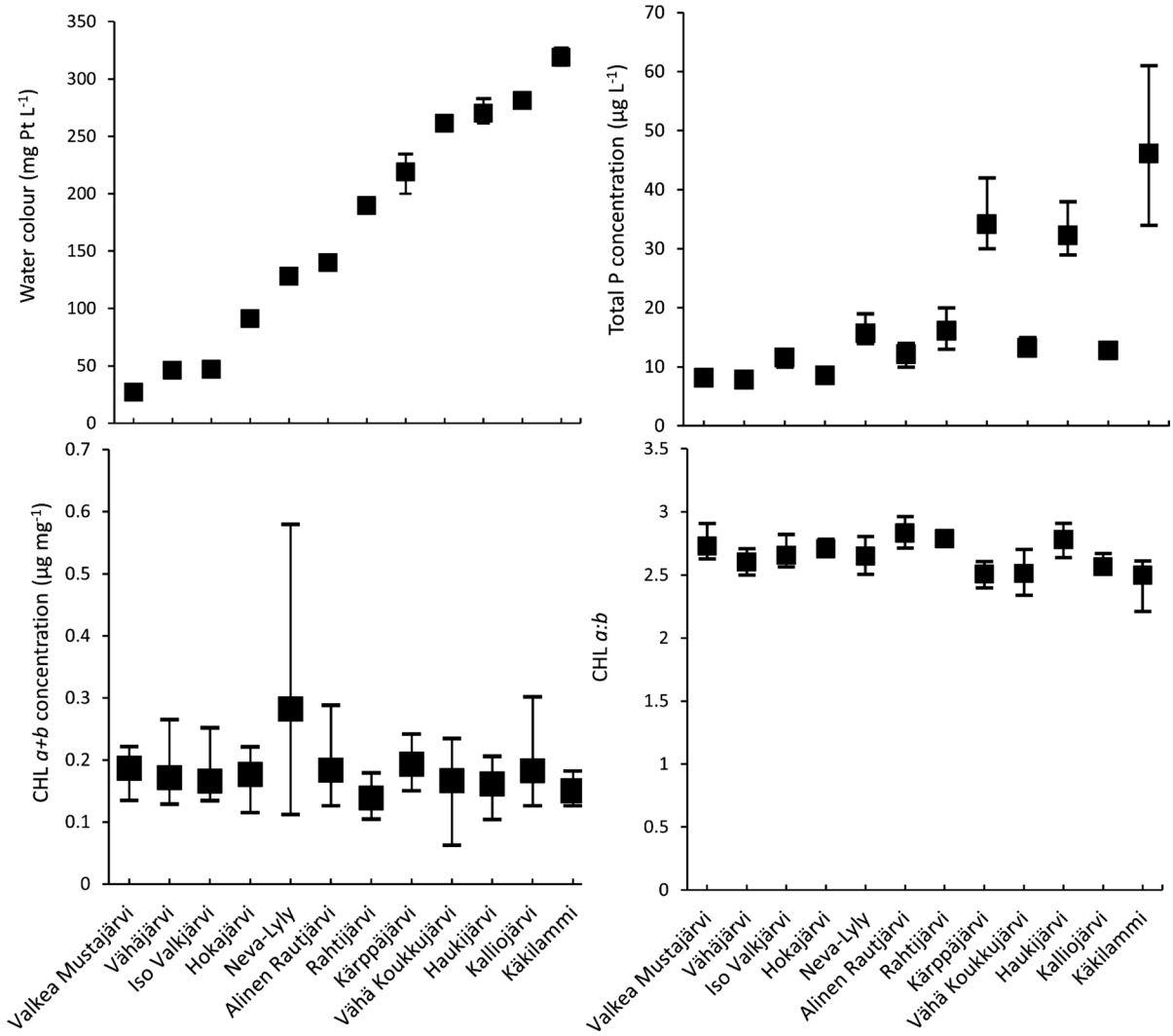


Fig. 1 Average water colour (top left), total phosphorus concentration (top right), average total chlorophyll concentration (CHL $a+b$) (bottom left) and average CHL *a:b* (bottom right) of *Nuphar lutea* together with their minimum and maximum values

in CHL $a+b$ concentration was 0.33 µg mg^{-1} in Neva-Lyly ($P<0.001$), and 0.09 µg mg^{-1} in Vähäjärvi ($P=0.016$).

For CHL *a:b*, the highest ratio was observed in Alinen Rautjärvi, where CHL *a:b* was on average 2.83, and the lowest CHL *a:b* in Käkilammi (2.50) (Fig. 1). The variation was again significant between lakes ($P<0.001$) (Table 2), but no in-lake variation was found ($P=0.330$) (Table 2).

The effect of water quality (water colour, total P concentration) on *N. lutea* chlorophyll content (CHL $a+b$, CHL *a:b*) in different growing sites

The average water colour or total P concentration of the lake did not affect *N. lutea* CHL $a+b$ concentration in any of the growing sites (Table 3). Instead, the CHL *a:b* of *N. lutea* collected near the lake outflow decreased significantly with increasing average water

Table 3 Results from the linear regression analysis on the effect of the average water colour and total phosphorus (P) concentration on *Nuphar lutea* CHL *a*+*b* and CHL *a:b* collected from the lake inflow, outflow and from the intermediate area

Dependent variable	Independent variable	$F_{1,34}$	R^2	*P*
CHL *a*+*b* inflow	Average water colour	0.166	0.005	0.687
CHL *a*+*b* outflow	Average water colour	2.601	0.071	0.116
CHL *a*+*b* intermediate area	Average water colour	0.000	0.000	0.996
CHL *a*+*b* inflow	Average total P concentration	0.408	0.012	0.527
CHL *a*+*b* outflow	Average total P concentration	0.160	0.005	0.691
CHL *a*+*b* intermediate area	Average total P concentration	0.364	0.011	0.550
CHL *a:b* inflow	Average water colour	3.404	0.091	0.074
CHL *a:b* outflow	Average water colour	5.648	0.142	**0.023**
CHL *a:b* intermediate area	Average water colour	3.921	0.103	0.056
CHL *a:b* inflow	Average total P concentration	1.565	0.044	0.219
CHL *a:b* outflow	Average total P concentration	7.335	0.177	**0.011**
CHL *a:b* intermediate area	Average total P concentration	3.321	0.089	0.077

Significant *P*-values are bolded

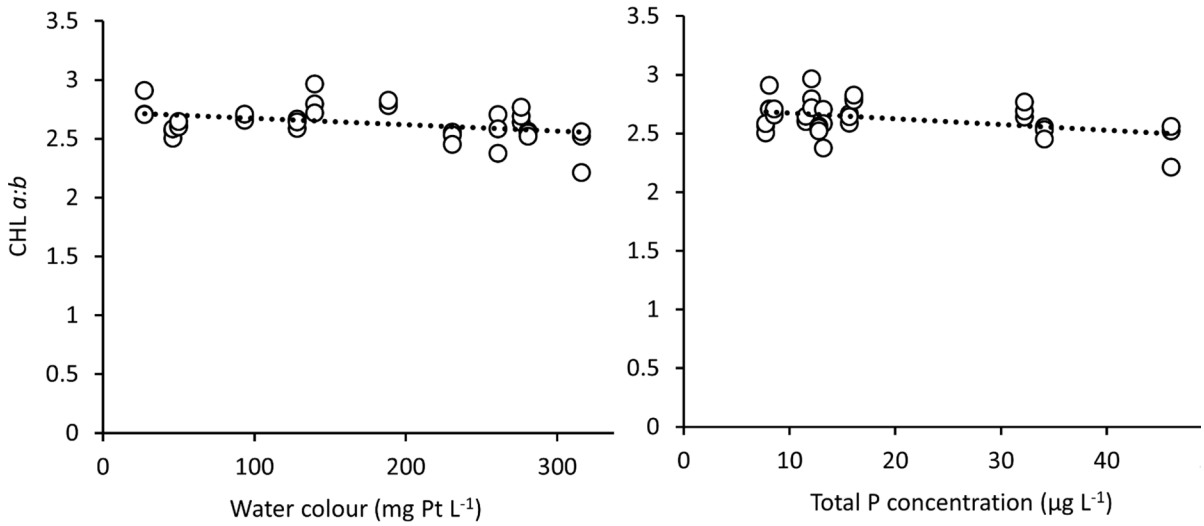


Fig. 2 The relationship between the average water colour (left figure) and total P concentration (right figure) and CHL *a:b* of *N. lutea* collected near the lake outflow

colour ($P=0.023$) and with increasing average total P concentration ($P=0.011$) (Table 3; Fig. 2). The average water colour or total P concentration of the lake did not have a significant effect on the CHL *a:b* of *N. lutea* individuals collected near the inflow or from the intermediate area (Table 3).

Temporal variation of *N. lutea* chlorophyll content (CHL *a*+*b*, CHL *a:b*)

The daily average solar irradiance varied from 428 to 912 W m^{-2} during the sampling period (Fig. 3). Other weather conditions varied too: the first two days and the last day of the sampling period were rainy and cool (15 °C), but in the middle of the sampling period the weather was sunny, and the air temperature varied from 21 to 29 °C.

During the one-week sampling period in Haukijärvi, the *N. lutea* petiole average CHL *a*+*b* concentration was 0.16 µg mg^{-1} and the CHL *a:b* 2.71 (Fig. 3). Despite the variable weather, no significant temporal variation was observed in CHL *a*+*b* ($F_{6,14}=0.797$, $P=0.588$) or CHL *a:b* ($F_{6,14}=1.061$, $P=0.430$) of *N. lutea* (Fig. 3). Neither CHL *a*+*b* ($r=-0.60$, $P=0.153$) nor CHL *a:b* ($r=0.45$,

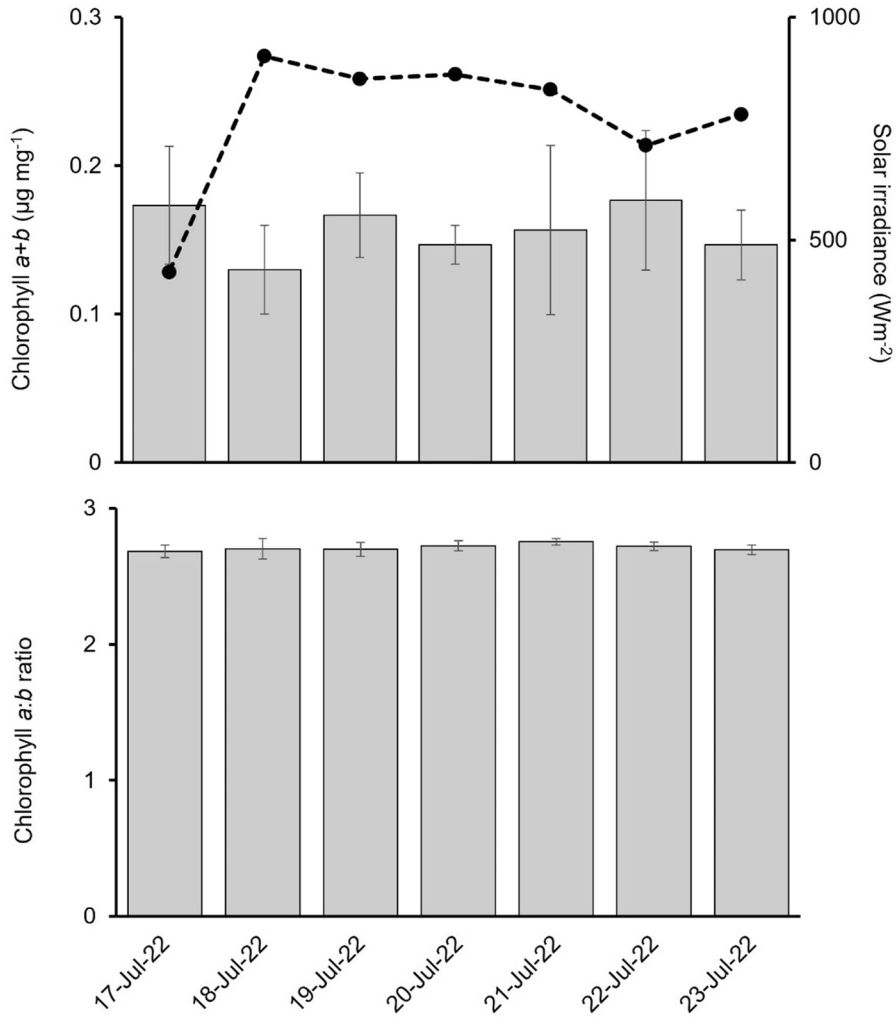


Fig. 3 Total chlorophyll concentration (CHL $a+b$) (top) and CHL *a:b* (bottom) in *Nuphar lutea* and daily average solar irradiance (dashed line, top) over a 7-day sampling period in Haukijärvi. CHL values are means with ± 95% confidence limits ($n=3$)

$P=0.314$) showed a significant correlation with solar irradiation.

Discussion

In this study, we aimed to clarify the bioindicator potential of *N. lutea* CHL content in monitoring lake brownification by solving, from which part of the lake the *N. lutea* samples must be collected so that they would reflect the average water quality of the lake. In addition, we wanted to find out, whether short-term changes in the weather conditions affect *N. lutea* CHL content. Contradicting to our hypothesis, the in-lake variation in the water quality did not coincide with the in-lake variation of *N. lutea* CHL content, but we found out that the CHL *a:b* of *N. lutea* individuals collected near the lake outflow best reflects the average water colour of the lake. In addition, according to our hypothesis, short-term variation in the solar irradiance did not coincide with significant changes in the *N. lutea* CHL content, thus confirming the use of *N. lutea* CHL *a:b* as an indicator of brownification.

Between- and in-lake variation of water quality and *N. lutea* chlorophyll content (CHL $a+b$, CHL *a:b*)

Both water colour and total P concentration displayed significant in-lake variation. On average, the water colour in the study lakes was highest near the inflow and lowest near the outflow. This was especially observed in Kärppäjärvi, where the inflow

ditch is descending from the peat production area. The total P concentration, instead, was on average highest near the outflow and lowest near the inflow. In addition, the in-lake variation in total P concentration was more prominent than for water colour. The higher in-lake variation of total P concentration could be due to, for example, horizontal variation of sediment P concentration (Nõges & Kisand, 1999) and variation in the sediment resuspension (Kristensen et al., 1992; Evans, 1994). Also, both macrophyte community composition and plant density in the littoral zone affect sediment resuspension and the release of P (Horppila & Nurminen, 2005). Thus, it can be suggested that the in-lake variation in total P concentration does not only originate from the differences in the catchment, but also from the in-lake processes (Kristensen et al., 1992; Evans, 1994; Horppila & Nurminen, 2005), which explains why total P concentration was not highest near the inflow area, like water colour.

The in-lake variation of water colour or total P concentration, however, did not coincide with the significant variation of the *N. lutea* CHL $a+b$ concentration. Although the CHL $a+b$ concentration displayed in-lake variation in two of the study lakes (Neva-Lyly and Vähäjärvi), it was not in concordance with the variation of water quality. Among these two lakes, only Neva-Lyly showed in-lake variation also in water quality (total P concentration). However, whereas the CHL $a+b$ concentration varied between all three sampling stations in Neva-Lyly, the total P concentration only varied between two stations. In previous studies, different responses of nutrient concentration on CHL $a+b$ concentration have been found (Hu et al., 2008; Horppila et al., 2022), and therefore the effect of nutrient concentration on CHL $a+b$ remains somewhat obscure. In addition, variations in water quality and light availability affect the depth distribution of CHL $a+b$ in *N. lutea* petioles (Horppila et al., 2022), and thus the effect of water quality may not be detected when the CHL $a+b$ samples are taken from the fixed depth (Horppila et al., 2022; Rajala et al., 2024). Also, for example, plant age and plant diseases can affect the CHL $a+b$ concentration (Wood & Bachelard, 1969; Kok et al., 1990; Yahya et al., 2020).

Compared to CHL $a+b$ concentration, the CHL *a:b* of *N. lutea* varied more consistently according to water quality. Interestingly, our results seem to indicate that CHL *a:b* of *N. lutea* can differentiate lakes from each other based on a rather small difference in water colour, whereas within a lake, CHL *a:b* does not significantly react to the same difference in water colour, as no significant in-lake variation in CHL *a:b* was found. For example, in Kärppäjärvi, the water colour between sampling points differed by up to 26 mg Pt l^{-1} (13%) but no significant difference in CHL *a:b* of *N. lutea* between sampling points could be observed. However, CHL *a:b* of *N. lutea* was significantly higher for example in Rahtijärvi than in Kärppäjärvi, although the difference in average water colour between the lakes was 29 mg Pt l^{-1} (15%). In addition, Horppila et al. (2022) observed a significant decrease in *N. lutea* CHL *a:b* with increasing water colour with the wide water colour range (4.4–505 mg Pt l^{-1}) of the study lakes, and Rajala et al. (2024) observed a significant difference in CHL *a:b* between lakes with an approximately 25% difference in water colour.

The reason why *N. lutea* CHL *a:b* responds to relatively small differences in water colour between lakes but not to the same magnitude of water colour differences within the same lake is probably due to the short-term nature of water colour variation within the study lakes. Even though the reproduction of *N. lutea* is mostly vegetative (Hart & Cox, 1995; Schoelynck et al., 2014), and thus plant individuals living in different parts of the lake are most likely derived from the same gene pool (Schoelynck et al., 2014), it is unlikely that the same genetic background would prevent the photoadaptation of *N. lutea* and be the cause of the lack of in-lake variation. Firstly, CHL *a:b* of *N. lutea* has been shown to change significantly during the growing season in macrophytes collected from the same sampling site of a lake, indicating that CHL *a:b* is regulated according to changes in environment (Rajala et al., 2024). Secondly, as macrophytes are sessile organisms, it is essential for their survival to express phenotypic plasticity (Barrett et al., 1993; Pintado et al., 1997). CHL content must be precisely regulated through various biochemical pathways, enabling the photoadaptation of plants (Tanaka et al., 1998; Zhao et al., 2020). Short-term changes in the water quality, instead, are common, and they can be caused by weather events, such as high precipitation, causing increased loading of allochthonous matter from the catchment (Pace & Cole 2002;

Brandão et al., 2022). In Kärppäjärvi, for example, the highest water colour was determined from the sampling station located near the inflow ditch descending from the nearby peat production area, corroborating Brandão et al. (2022), who found a link between catchment land use and spatial water quality variability. The effect of ditching on water quality is also strong in lakes with high peatland coverage in their catchments, as in Kärppäjärvi (Estlander et al., 2021). However, in the case where the in-lake variations in the water colour and total P concentration are more permanent, for example due to catchment land-use (Brandão et al., 2022), the gradients can express more ecological significance for macrophytes. Because macrophytes must adapt to changes in the light conditions in order to sustain effective photosynthesis (Tanaka et al., 1998; Zhao et al., 2020), large enough gradients in water quality can cause adaptive responses in macrophytes, consequently resulting to in-lake variations in their chlorophyll content.

The effect of average lake water quality on N. lutea CHL content (CHL a + b, CHL a:b) in different growing sites

Corroborating previous studies (Horppila et al., 2022; Rajala et al., 2024), CHL *a:b* of *N. lutea* decreased significantly with increasing water colour. The effect of average water colour of the three sampling stations of each lake on *N. lutea* CHL *a:b* was only significant in *N. lutea* individuals collected near the lake outflow. Due to variations in the discharge from the catchment area, short-term water quality fluctuations are largest near the inflow (Yin et al., 2011). Therefore, macrophytes growing near the inflow area do not reflect the average water quality of the lake as asccurately as macrophytes collected near the outflow. Based on these results, it is thus recommendable to collect the samples near the lake outflow, if *N. lutea* CHL *a:b* is used as an indicator of brownification.

CHL *a:b* of *N. lutea* also decreased significantly with increasing total P concentration, but it was most likely due to high correlation between water colour and total P concentration, because in previous studies light has been the main factor causing changes in the CHL *a:b* ratio of macrophytes (Barko & Filbin, 1983; Horppila et al., 2022; Rajala et al., 2024).

The effect of short-term changes in weather conditions on *N. lutea* CHL content (CHL $a+b$, CHL *a:b*)

Despite varying weather conditions, no significant changes in the CHL $a+b$ concentration or CHL *a:b* could be observed during the one-week sampling period in Haukijärvi, thus confirming our hypothesis that the CHL content of macrophytes reflects changes in water quality rather than changes in weather conditions. The CHL $a+b$ concentration displayed more variation, than CHL *a:b*, but it did not correlate with solar irradiance. Again, the variation of CHL $a+b$ concentration can therefore be assumed to be background variation due to, for example, differences in plant age (Wood & Bachelard, 1969; Kok et al., 1990). Changing the CHL *a:b* requires alterations in the plants' gene expression, which can take several days (Ruban, 2009), and therefore a longer response time is needed to see changes in the CHL *a:b* with changing light conditions. Therefore, CHL *a:b* rather reflects acclimatization to a longer-term environmental change than a short-term adaptation (Ruban, 2009). For short-term adaptation, macrophytes have other mechanisms, such as shoot elongation (Barko & Smart, 1981) and chloroplast movement (Lechowski & Białczyk, 1992; Kagawa & Wada, 2002), ensuring their photosynthetic efficiency in various light environments.

Conclusions

CHL *a:b* of *N. lutea* collected near the lake outflow reflects the average water quality of the lake better than CHL $a+b$ concentration. *N. lutea* CHL *a:b* can distinguish lakes from each other based on a rather small difference in the water colour, but it does not change according to short-term changes in the water quality or in weather conditions. CHL $a+b$ concentration, instead, displays more background variation. Therefore, CHL *a:b* of *N. lutea* is a promising indicator of lake brownification that can be used together with direct water quality measurements to indicate long-term changes in lake ecosystems. In addition, this study points out the importance of littoral area when assessing the lakes water quality, as the in-lake variation in the water quality between littoral areas can be prominent.

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Author contributions S.R., J.H., S.E., and L.N. planned the study together. S.R., S.E., and L.N. performed most of the field and laboratory work. S.R. conducted the statistical tests of the data. S.R. wrote the paper with input from all authors.

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Data availability Data are available from the corresponding author upon reasonable request.

Declarations

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

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