Taking over the dominance of the macrophyte community by *Elodea nuttallii* (Planch.) H. St. John is poorly reflected in ecological status assessment results

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**Abstract**

Nuttall’s waterweed (*Elodea nuttallii*) is an aquatic plant native to North America. In Poland, the species was first identified in the early 1990s and since then it has started to spread in surface waters. We investigated one of the six lakes in Poland (Probarskie Lake), which were reported to be invaded by *E. nuttallii* among all the lakes monitored in the period 2005–2016. Based on our field survey data (2019 and 2020) and historical monitoring data (2011 and 2017), we explored the rate of invasion, the effects on taxonomic composition, spatial structure, and abundance of biological assemblages, and the ecological status assessment of this new incomer. We surveyed the lake for macrophytes, phytoplankton and macrozoobenthos for two subsequent years using the field protocols applied in lake monitoring in Poland. Water physicochemistry, planktonic algae and benthic invertebrates were sampled at the sites that were invaded and uninvaded by *E. nuttallii*, and phytoplankton and physicochemistry also in pelagic sites. Nuttall’s waterweed was identified in Probarskie Lake in 2017 for the first time, occupying about 30% of the phytolittoral area, while in 2019 and 2020, it dominated the macrophyte community, occupying over 80% of the total hydrophyte area. The appearance of the alien invader and its relatively fast taking over of the dominance in the macrophyte community were not reflected in the bioassessment results. None of the analysed biological assemblages truly mirrored the severe changes in macrophyte taxonomic composition among the sampling sites as well as over the subsequent years. As the spread of *E. nuttallii* is anticipated to increase in Polish waters, there is an urgent need for verification of ecological status assessment methods to improve their diagnostic capacity to capture the problem of ecological invasions.

**Key words:** Nuttall’s waterweed, invasive species, alien hydrophyte, assessment methods, lakes, Poland

**Introduction**

Nuttall’s waterweed (*Elodea nuttallii* (Planch.) H. St. John) is an aquatic species (hydrophyte), occurring in natural and artificial reservoirs, e.g., lakes, ponds, dam reservoirs, pits, slowly flowing rivers and channels. It also occurs in coastal lakes and bays. The species is native to North America and it was likely introduced to Europe only in the 20th century. The first reports on its establishment in Europe originate from Great Britain in 1914 (originally identified as *Hydrilla verticillata*).
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In 1974; Josefsson 2011). Currently, *E. nuttallii* is reported as invasive in 23 European countries (GBIF 2021).

In Poland, Nuttall’s waterweed was identified for the first time in 1990–1993 in the oxbow lake of Biebrza near Goniądz (Barendregt and Wassen 1994). In 2007, it was found in the waters of Vistula (Kamiński 2010), and since then it has rapidly spread along large rivers. This species reproduces vegetatively through fragmentation. Its propagules spread passively along with the water current. Therefore, flowing waters are usually more prone to rapid expansion than lakes, especially those more isolated, located on the edge of the catchment area. Aquatic animals, mainly birds, are also active vectors for the spread of the species (Figuerola and Green 2002; Coughlan et al. 2017). Field observations of the species distribution in Poland indicate that *E. nuttallii* spreads mostly in the valleys of large rivers: the Vistula (mainly in its middle course), the estuary of the Oder, and sporadically the Warta (Middle Warta Valley region) (Solarz et al. 2020). Most of the localities were found after the year 2000. This is likely that it was frequently confused with the similar, more common species, the Canadian waterweed *E. canadensis* Michx. Therefore, the reports on the species occurrence in Poland may be under reported, and in fact, it may occur more abundantly and in a larger number of sites than commonly believed.

Alien species, if established in a territory and entering the aggressive expansion phase, may pose serious ecological and economic threats (Larson 2003). The majority of environmental problems posed by Nuttall’s waterweed result from its ability for massive growth. The species can develop dense monospecific stands, which may overgrow the entire water column or form dense floating mats with a strong shading effect (Simpson 1984; Barrat-Segretain 2005). The mass development impedes recreational and aquacultural use of reservoirs, causes pipe blockage, impairs the operation of boat engines, and hinders sailing, swimming, angling and fishing (Sand-Jensen 2000; Josefsson 2011).

Dense populations of plants reduce the water movement, reduce light penetration, produce anoxic conditions and trap sediments (Jones et al. 1996; Simpson and Duenas 2011; Carey et al. 2016). Decomposition of the large amounts of biomass at the end of the growing season significantly contributes to the deterioration of water quality and intensification of secondary eutrophication altering the nutrient balance of the entire ecosystem (Sand-Jensen 2000; Barrat-Segretain 2005; Thiébaut and Di Nino 2009). Further, the species affects local populations of aquatic plants and animals. Dense monospecific communities of *E. nuttallii* can displace native species and contribute to the reduction of taxonomic diversity of aquatic vegetation. For macrophytes, this effect is usually related to competition for space, light and nutrients, which leads to the displacement of the native species by the invading species and contributes to the reduction of taxonomic diversity of aquatic vegetation (Simpson 1984; Barrat-Segretain 2005). The
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effect on phytoplankton is mainly due to the allelopathic properties of *E. nuttallii* by limiting the growth of eukaryotic algae and cyanobacteria (Erhard and Gross 2006; Hilt and Gross 2008; Vanderstukken et. al. 2014). A negative impact of Nuttall’s waterweed on populations of aquatic animals, i.e., fish, zooplankton or benthic macroinvertebrates was also demonstrated (Erhard et al. 2007; Simpson and Duenas 2011; Schulz and Dibble 2012). Erhard et al. (2007) demonstrated the inhibiting effect of the unknown chemical compounds of *E. nuttallii* on the growth and survival of Lepidoptera larvae, leading these herbivores to avoid consuming this plant species. Likewise, results by Thiébaut and Gierlinski (2007) and Thiébaut and Di Nino (2009) indicated reduced herbivory on *E. nuttallii* compared to the one on species native to Europe, which may suggest limited food resources for macrozoobenthos in the invaded ecosystems. As the appearance of a new alien invader usually modifies the structure and functioning of aquatic biological communities (Strayer 2010), the change in the ecological status of a waterbody is expected. On the other hand, studies on the appearance of Nuttall’s waterweed in the lakes of Northern Ireland showed a small ecological effect of the invasion on aquatic biota, i.e., macrophytes, algae and macrozoobenthos assemblages (Kelly et al. 2015).

Reports on the ecology and behaviour of Nuttall’s waterweed in Europe come from research conducted mainly outside of Poland. To date, scientific reports on the performance of the species in Poland are limited (Kolada et al. 2018; Solarz et al. 2020; Dembowska et al. 2021). There are rationales for the highly invasive nature of this species, its expansive growth and displacement of native species of aquatic flora. However, the species most likely is still in its expansion phase in Poland, spreading to new areas, and the research from the country is still pioneering.

We screened the national monitoring database from the years 2005–2016 (740 lake-years with the available data on macrophytes) and found six lakes that were inhabited by Nuttall’s waterweed. To date, these lakes have been the only lakes in Poland surveyed under the state environmental monitoring for ecological status assessment as required by the Water Framework Directive (WFD; European Community 2000), where *E. nuttallii* has been identified. Of these lakes, Probarskie Lake was selected as an object of the study. During the monitoring survey conducted in 2011, the lake was free of *E. nuttallii* and its littoral zone was dominated by stands of *Myriophyllum spicatum* and *Ceratophyllum demersum*. The presence of *E. nuttallii* was identified first in 2017 and the species covered 33% of the phytolittoral area.

We investigated Probarskie Lake during two subsequent vegetation seasons and analysed the former monitoring data to explore the rate of invasion, potential threat to the native aquatic flora and the impact on the lakes’ ecological status of this new alien hydrophyte in Poland. We attempted to explore the potential effects of *E. nuttallii* on taxonomic and spatial structure, and the abundance of lake biological assemblages, i.e., macrophytes,
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Figure 1. Location of the Lake Probarskie in Poland (inset) and the distribution of sampling sites in 2019 and 2020: 1–16 – transects for the macrophyte survey; 1–3En – littoral sites dominated by *E. nuttallii*; 1–3noEn – littoral sites without *E. nuttallii*; 1–3Pel – pelagic sites; (B)En – littoral site for macrozoobenthos survey dominated by *E. nuttallii*; (B)noEn – littoral site for macrozoobenthos survey without domination of *E. nuttallii* (macrozoobenthos studied only in 2019).

We assumed that the appearance and spread in an ecosystem of the alien hydrophyte invader would be reflected by the results of the national methods for the ecological status assessment.

**Materials and methods**

*Sampling and biocenotic analyses*

Probarskie Lake is located in north-eastern Poland (Figure 1). It has an area of 2.0 km², with a maximum depth of 31.0 m, and an average depth of 9.2 m. The lake is a hydrologically isolated, drainage ecosystem with a small phytoplankton and macroinvertebrates, as well as the ecological status of the invaded lake.
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Catchment (6.88 km²) dominated by agricultural lands (58%). The monitoring results derived from the state environmental monitoring surveys conducted in 2011 and 2017 indicated the meso-eutrophic waters and good overall ecological status.

The field surveys on Probarskie Lake were conducted in 2019 and 2020 using field protocols and procedures that are routinely applied in the state monitoring. They included research on aquatic and rush vegetation, sampling of the phytoplankton and water quality parameters in summer, at the peak of the growing season (August 2019 and August 2020) and sampling of littoral macrozoobenthos in early autumn (September 2019).

**Macrophytes**

The macrophyte surveys were carried out using the national monitoring method, the Ecological Status Macrophyte Index (ESMI; Ciecierska and Kolada 2014). Macrophytes are surveyed from a boat with a grapnel and/or a bathyscope within transects, which are set perpendicularly to the lake shoreline. Each transect is approximately 30 m wide and with the length corresponding to the total extent of the phytolittoral zone, from the shore to the maximum colonisation depth. The number of transects depends on the lake size and the shoreline length (Jensén 1977; Keskitalo and Salonen 1994). For Probarskie Lake, the research was carried out on 16 transects, set in the same locations as determined in the previous surveys conducted within the state monitoring (Figure 1). The surveys covered all groups of aquatic and rush vegetation, i.e., sedge rush and rush (helophytes), floating leaf vegetation (nymphaeids/lemnids), submerged vascular vegetation (elodeids/potamids), charophytes and mosses, with the special focus on *E. nuttallii*, which belongs to the vascular rooted vegetation (elodeids).

Since macrophyte monitoring in Poland employs the synecological approach (Braun-Blanquet 1964), plant communities, not species, are considered. The term “community” (syntaxa) is used for homogenous and uniform vegetation stands (phytocoenoses *sensu* Westhoff and van der Maarel 1973, after Jensén 1977), named after a dominating species. For the vascular plant communities, the syntaxonomic systems established by Brzeg and Wojterska (2001) and Matuszkiewicz (2002) were adopted. On each transect, the depth range of macrophytes, the total vegetation coverage, the plant taxonomic composition and the relative cover of all plant communities (syntaxa) in relation to the total vegetation coverage were recorded.

Based on the data collected from 16 transects, the macrophyte indicators for the lake were calculated, i.e., the average maximum colonisation depth of vegetation ($C_{\text{max}}$ [m]), mean vegetation coverage (%COV), the absolute area covered by vegetation (N [ha]), number of all plant communities ($S_{\text{TOT}}$), number of hydrophyte communities ($S_{\text{HYDR}}$) and the relative cover of each plant community (%N) in relation to N = 100%. The multimetric ESMI was calculated using the formula [Eq. 1]:
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\[ ESMI = 1 - \exp \left[ -J \times \frac{N}{\text{isob}2.5} \times \exp \left( \frac{N}{P} \right) \right] \]  

[Eq. 1]

where \( J \) (Pielou’s evenness index; Pielou 1975) is a ratio of phytocenotic diversity index \( H' \) (Shannon and Weaver 1949) and the maximum biocenotic diversity index \( H_{\text{max}} (= \ln S) \), \( N \) is the total vegetated area, isob2.5 is the lake area bounded by the 2.5 m isobath (potential phytolittoral area with a depth of less than 2.5 m) and a factor \( (N/P) \) is a ratio of the total vegetated area and the total lake area (for more details see Ciecierska and Kolada 2014). The index ranges from 0 to 1, where 0 is a degraded state and 1 denotes the reference conditions, and, therefore, is considered the Ecological Quality Ratio (EQR) *sensu* WFD. The lake classification system based on ESMI is provided in Supplementary material Table S1.

**Phytoplankton**

Samples for the taxonomic composition and biomass of phytoplankton estimation were collected from nine sites that differed in the macrophyte coverage and dominance of *E. nuttallii*. Three sampling points were located in the littoral, with submerged vegetation dominated by *E. nuttallii* (1En, 2En, 3En), three in the littoral, with submerged vegetation dominated by other species of vascular plants and/or charophytes (1noEn, 2noEn, 3noEn), and three in the pelagial zone (1Pel, 2Pel, 3Pel) (Figure 1). Water samples for quantitative laboratory analyses were collected using a Ruttner sampler (Hydrobios) of 2 dm³ capacity. To provide comparability among all sampling sites, all samples were collected from the layer 0–1 m considered representative of epilimnion. Moreover, to avoid a strong local modifying effect of macrophyte stands on phytoplankton and water characteristics, the littoral samples were taken from sites with at least 3–4 m depth, high above the macrophyte layer. Phytoplankton samples were fixed with Lugol’s solution and preserved with 4% formalin.

Microscopic analysis of phytoplankton abundance and biomass was done following the Utermöhl method (Utermöhl 1958). For counting, the samples were transferred to a settling chamber and at least 100 individuals (i.e., the so-called units, which were single cells, cenobia, colonies and filaments) of the most numerous algae were counted per sample as described by Lund et al. (1958). The units were calculated for different magnification levels. Large taxa were calculated using 100-fold magnification, inspecting the whole bottom of the chamber, and so were the medium-sized ones under 200-fold magnification in 2–4 slant belts and nanoplankton under 400-fold magnification in 100 fields. Biovolumes (mm³ L⁻¹) were determined according to Hillebrand et al. (1999) and converted to biomass (mg fresh weight L⁻¹). For taxonomic analysis, additional samples were obtained with the 10-µm plankton net and for *in vivo* analysis net samples were not preserved.
To perform an ecological status assessment based on phytoplankton, the national monitoring method, the Phytoplankton Metric for Polish Lakes (Hutorowicz and Pasztaleniec 2014) requires four samplings during the season as a minimum. Therefore, in this study the classification of lake ecological status based on phytoplankton was performed using one of the components of the PMPL, i.e., cyanobacterial metric \( Y_{CY} \), which enables the approximate assessment of the lake status based on a single, summer sampling. The metric \( Y_{CY} \) was calculated using the formula [Eq. 2]:

\[
Y_{CY} = 1,4113 \times \ln \left( \frac{B_{CY} + B_{CY} \times \left( \frac{B_{CY}}{B_{FL}} \right)^2}{2} \right) + 1,8112 \quad [\text{Eq. 2}]
\]

where \( B_{CY} \) is the total biovolume of the cyanobacteria and \( B_{FL} \) is the total biovolume of the phytoplankton community in the summer period based on the single sampling (mid-June to mid-September). To provide comparability with other biological metrics analysed in this study, the value of \( Y_{CY} \) was recalculated to EQR using the formula: EQR \( Y_{CY} = -0.2 \times Y_{CY} + 1 \) (Hutorowicz and Pasztaleniec 2014). The metric EQR values range from 0 to 1, where 1 denotes the reference conditions and 0 is a highly degraded state. The metric was calculated for both study seasons, 2019 and 2020, as well as for the years 2011 and 2017 using the data derived from the monitoring database. The lake classification system for the cyanobacterial metric is provided in Table S1.

Macrophytes

The macrophytes were surveyed at two sites in the littoral area, first where \( E. \) nuttallii dominated in macrophyte patches (B)\( \text{En} \) and second where the species was scarce (B)\( \text{noEn} \) (Figure 1). Samples were taken in accordance with the field procedure relevant for the monitoring method Lake Macroinvertebrate Index (LMI; Bielczyńska et al. 2020). Each sampling site was 15 m wide along the shore and expanded 1–2 m lakeward. For safety reasons, the sampling depth did not exceed 1 m. An integrated sample from all habitats present at the site was collected using the “kick sampling” method (García-Criado and Trigal 2005; Böhmer et al. 2014; Porst et al. 2016), using a hydrobiological net with a mesh size of 500 µm. The sampling time was one minute, and this time was distributed among all the habitats present on the site, proportionally to their share. The material collected in the field was preserved with 96% ethanol. In the laboratory, animals were identified at the family level, in general, as required by the LMI method.

For each sampling site, the Lake Macrozoobentic Index (LMI; Bielczyńska et al. 2020) for ecological status assessment was calculated, which is based on four compositional metrics, i.e., the Average Score Per Taxon adapted to Polish conditions (ASPT-PL; Kownacki et al. 2004), the Shannon-Wiener Diversity Index (\( H' \); Shannon and Weaver 1949), the percentage
share of individuals of Diptera in the total number of fauna individuals (%Diptera) and the absolute number of families of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata (EPTCBO).

To provide comparability among compositional metrics of different ranges and response directions, each metric was transformed to EQR ranging from 0 (most disturbed) to 1 (least disturbed conditions) using the approach by Hering et al. (2010) and formulas: \( \text{EQR } \text{ASPT-PL} = 0.7927 \times \text{ASPT-PL} \) – 3.5406; \( \text{EQR } H' = 0.6531 \times H - 0.7197 \); \( \text{EQR } \%\text{Diptera} = -0.0146 \times \%\text{Diptera} + 1.0491 \); \( \text{EQR } \text{EPTCBO} = 0.0909 \times \text{EPTCBO} - 0.3636 \) (Bielczyńska et al. 2020). The multimetric LMI is a weighted average of four EQRs of composition metrics calculated using the formula [Eq. 3]:

\[
LMI_{\text{site}} = \frac{2 \times \text{EQR ASPT-PL} + \text{EQR } H' + 2 \times \text{EQR } \%\text{Diptera} + \text{EQR } \text{EPTCBO}}{6}
\]  

[Eq. 3]

The LMI values range from 0 (most disturbed) to 1 (least disturbed conditions). For a lake, LMI is an arithmetic average of multimetrics for all sampling sites within a lake. The classification system based on LMI is provided in Table S1.

Physicochemical water properties

The in-situ measurements of water properties were made along with and at the same nine sampling sites as for phytoplankton sampling (August, 2019 and August, 2020). They included water temperature (Temp), pH, conductivity (Cond), chlorophyll \( a \) (Chla), oxygen concentration (DO) and oxygen saturation (%O\(_2\)) determined using a multi-parameter water quality probe YSI 6600 V2 (Yellow Springs, OH, USA). Measurements were conducted in the surface layer (0–1 m). The visibility of the Secchi disc (SD) was measured only in pelagic sites.

Water for laboratory chemical analyses was filtered through a Whatman GF/F glass fibre filter. In filtrate, the concentration of ammonium ions was analysed with the phenol hypochlorite method after Solórzano (1969). Nitrates were determined by phenyldisulphonic acid (Standard Methods 1960), while total Kjeldahl nitrogen and total phosphorus (TP) were determined after mineralisation of unfiltered water with concentrated sulphuric acid (Golterman and Clymo 1969) followed by spectrophotometric analyses of resulting ammonium ions and phosphates, respectively. Total nitrogen (TN) is the sum of total Kjeldahl nitrogen and nitrates concentration. Data for Cond only for 2019, while for TP only for 2020 were available.

Data analysis

The variability of physicochemical and biological parameters among study sites and habitat types (with \( E. \) nuttallii, without \( E. \) nuttallii and pelagic sites) was analysed for each year separately, using the coefficient of variation (CV) and the statistical significance of differences was examined using the
non-parametric Kruskall-Wallis test (KW). The differences between mean values of physicochemical parameters of water quality in two research years were tested using the non-parametric Mann-Whitney U test (M-W). All statistical calculations were performed in STATISTICA v. 12.5 (StatSoft Inc. 2013).

The spatial effect of the presence of Nuttall’s waterweed on the phytoplankton and macrozoobenthos communities was analysed using the Principal Component Analysis (PCA) in the Canoco for Windows 4.5 software (ter Braak and Šmilauer 2002).

To analyse the temporal effects of the appearance of plant invader on aquatic biota, the taxonomic composition and indicator values for all analysed biological elements were compared between two research years and the previous studies conducted within state monitoring in 2011 and 2017. For physicochemical parameters and phytoplankton, only data collected in August 2011 and 2017 were analysed to ensure comparability with the data collected in 2019 and 2020. For macrophytes and phytoplankton monitoring data from the years 2011 and 2017, while for macrozoobenthos only from 2017 were available (method LMI has been included in the state monitoring from 2013). While the composition of multimetric LMI changed between the sampling years 2017 (Soszka et al. 2012) and 2019 (Bielczyńska et al. 2020), to enable comparison between the two sampling periods, the updated formula of LMI [Eq. 3] was used for both years, 2017 and 2019.

**Results**

**Water quality parameters**

The physicochemical parameters of Probarskie Lake in summer 2019 and 2020 indicated the waters as moderately fertile, and slightly eutrophic (Table 1). In the pelagic zone, the pH was relatively high (9.0–9.4), while Cond was relatively low (170.2–171.8 µS/cm, data available only for 2019). The total phosphorus concentrations ranged between 40 and 56 µg L\(^{-1}\) in 2020. The total nitrogen concentration was generally low and ranged between 0.39 and 0.83 mg L\(^{-1}\). The visibility of the Secchi disc varied from 2.3 m to 3.3 m. The oxygenation of the lake waters was generally high with open water %O\(_2\) of about 120% (11.0 mg O\(_2\) L\(^{-1}\)) and 100% (8.9 mg O\(_2\) L\(^{-1}\)) in two subsequent years, respectively. The variation in physicochemical parameters among three pelagic sites was low (CV ca. 0.5–3.0%), except for more variable total nitrogen concentration (CV from 7% to 24%).

The statistically significant differences in water quality parameters in the pelagic zone between 2019 and 2020 were demonstrated for the water temperature, pH and oxygenation, which were lower in 2020 than in 2019 (Table 1). For total nitrogen, although the concentrations in 2020 were noticeably lower than in 2019, the difference was statistically insignificant (p = 0.081). Likewise, no difference was found between the studied years regarding Secchi disk visibility (p = 0.369).
Table 1. Comparison of the mean values of water quality parameters among different habitat types in Probarskie Lake surveyed in August 2019 and 2020; habitat types: En – littoral sites dominated by *E. nuttallii*, noEn – littoral sites without *E. nuttallii*, Pel – pelagic sites; p(KW) – results of the Kruskal-Wallis test among habitat types (n = 3 per habitat type); p(M-W) – results of the Mann-Whitney test between 2019 and 2020 (n = 9 per year).

| Parameter [unit] | 2019 | 2020 | p(M-W) |
|------------------|------|------|--------|
|                  | Pel  | noEn | En    | Pel  | noEn | En    |        |
| SD [m]           | 2.6  | n.a. | n.a.  | 3.0  | n.a. | n.a.  | 0.3686 |
| Temp [°C]        | 21.8 | 22.1 | 22.2  | 0.110| 20.8 | 20.8  | 0.770  | 0.0003 |
| pH               | 9.2  | 9.2  | 9.1   | 0.067| 9.3  | 9.3   | 0.780  | 0.0005 |
| Cond [µS cm⁻¹]   | 170.9| 162.7| 170.6 | 0.110| n.a. | n.a.  | –      | –      |
| %O₂ [%]          | 124.4| 122.2| 121.0 | 0.105| 100.2| 95.9  | 96.7   | 0.590  | 0.0004 |
| DO [mg L⁻¹]      | 11.0 | 10.8 | 10.7  | 0.512| 8.9  | 8.6   | 8.6    | 0.079  | 0.0004 |
| TP [mgP L⁻¹]     | n.a. | n.a. | n.a.  | 0.048| 0.048| 0.051 | 0.628  | –      | –      |
| TN [mgN L⁻¹]     | 0.50 | 6.17 | 2.85  | 0.027| 0.77 | 0.77  | 0.80   | 0.413  | 0.0808 |

The comparison of physicochemical parameters values among different types of habitats (*En, noEn, Pel*), performed separately for each year, showed statistically significant differences only for the total nitrogen in the first study year (2019: KW-H (2; 9) = 7.2, p = 0.027). In this year, the total nitrogen concentrations varied highly among the habitat types and their maximum values (mean 6.17 mg L⁻¹, σ = 0.073) at *noEn* sites, while minimum values (mean 0.50 mg L⁻¹, σ = 0.098) at *Pel* sites were noted (Table 1). In 2020, no significant differences among studied habitats for the total nitrogen or all the other parameters were found (Table 1). Slight variation among sites of the littoral habitats compared to pelagic ones was also observed for temperature (up to 4%) and oxygen concentrations (up to 6%). The other physicochemical features in the littoral sites were generally similar to those of the pelagic waters.

The comparison of the physicochemical parameters in the study years with those from the previous years (August) demonstrated a significant increase in TP, a slight increase in pH and chlorophyll *a*, fluctuations in generally low TN and no significant changes in the Cond and SD over the last decade (Table 2). A significant increase in TP may indicate progressive eutrophication of lake waters, which, however, was not confirmed by the other water quality parameters.

**Macrophyte characteristics**

In the growing season of 2019, 16 plant communities, while in 2020, 19 plant communities were identified (Table S2). In 2020, five communities not identified in 2019 were found and they were *Nitelletum flexilis, Potametum perfoliati, Lemno-Hydrocharitetum morsus-ranae, Nupharo-Nymphaetum albae* form with *Nymphaea alba* and *Stratiotetum aloidis*. In 2019, two communities were identified that were not found in 2020, i.e., *Potametum compressi* and *Scirpetum lacustris*. The contribution of all these communities to the total vegetated area in the years of their occurrences was negligible (< 1%).
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Compared with the data collected in the years 2011 and 2017, the number of all macrophyte communities $S_{TOT}$ and the number of hydrophyte communities $S_{HYDR}$ fluctuated, being the highest in 2017 and the lowest in 2019 (Table 2). The contribution of syntaxonomic groups to the total phytolittoral area was similar in all the analysed years (Figure 2, Table S2). A slight increase in the share of charids (a group of structural algae sensitive to eutrophication) and a decrease in the share of floating leaf vegetation with an almost unchanged share of submerged vascular and rush vegetation between 2011 and 2020 were observed.

The most apparent change in the macrophyte syntaxonomic structure in Probarskie Lake between 2011 and 2020 concerned the change in the dominant species. In 2011, in the submerged vegetation the community of Ceratophylletum demersum dominated inhabiting 35% of the total phytolittoral area and 47% of the area covered by the submerged vegetation. The only hydrophyte species alien to Poland was Elodea canadensis, whose phytocoenoses covered less than 5% of the total area vegetated (Table S2). In 2017, the community of E. nuttallii dominated in the hydrophyte zone inhabiting 33% of the total phytolittoral area and 55% of the area covered by the submerged vegetation. Between 2017 and 2020, a strong increasing

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**Table 2.** Comparison of the physicochemical and biological indicators used for the ecological status assessment of Probarskie Lake in subsequent years of the research; ESMI – Ecological State Macrophyte Index; Y$_{CY}$ – metric “Biomass of cyanobacteria”; EQR – Ecological Quality Ratio (metrics normalised to the range [0–1]); LMI – Lake Macroinvertebrate Index.

| Quality element | Parameter | 2011* | 2017* | 2019** | 2020** |
|-----------------|-----------|-------|-------|--------|--------|
| Physicochemistry (pelagial zone in August) | pH | 8.8   | 8.4   | 9.2    | 9.3    |
|                 | Conductivity [$\mu$S cm$^{-1}$] | 178   | 191   | 171    | n.a.   |
|                 | Secchi disk visibility [m] | 2.5   | 2.6   | 2.6    | 3.0    |
|                 | Total nitrogen [mg L$^{-1}$] | 0.33  | 0.81  | 0.59   | 0.78   |
|                 | Total phosphorus [mg L$^{-1}$] | 0.021 | 0.018 | n.a.   | 0.049  |
| Macrophytes     | Total number of syntaxa $S_{TOT}$ | 22    | 28    | 16     | 19     |
|                 | Number of hydrophyte syntaxa $S_{HYDR}$ | 15    | 19    | 11     | 15     |
|                 | Diversity index for all taxa $H'$$_{TOT}$ | 1.81  | 2.00  | 1.23   | 1.36   |
|                 | Diversity index for hydrophyte taxa $H'$$_{HYDR}$ | 1.45  | 1.71  | 0.83   | 0.87   |
|                 | Max colonisation depth $C_{max}$ [m] | 4.8   | 5.0   | 5.0    | 5.0    |
|                 | Vegetation coverage $\%COV$ [%] | 94.7  | 87.2  | 85.0   | 85.0   |
|                 | Vegetated area $N$ [ha] | 59.7  | 55.8  | 54.4   | 54.4   |
|                 | ESMI | 0.639 | 0.618 | 0.471  | 0.509  |
|                 | Ecological status based on ESMI | good | good | good   | good   |
| Phytoplankton   | Chlorophyll $a$ [µg L$^{-1}$] | 8.00  | 8.90  | 6.40   | 10.10  |
|                 | Total biomass [mg L$^{-1}$] | 1.00  | 1.32  | 7.52   | 1.87   |
|                 | Biomass of cyanobacteria [mg L$^{-1}$] | 0.60  | 0.17  | 6.21   | 0.90   |
|                 | Total number of taxa | 22    | 21    | 43     | 34     |
|                 | Diversity index $H'$ | 0.834 | 0.727 | 0.728  | 0.777  |
|                 | EQR $Y_{CY}$ | 0.72  | 1.00  | 0.02   | 0.61   |
|                 | Ecological status based on $Y_{CY}$ | good | high | bad    | good   |
| Macrozooobenthos | EQR ASPT-PL | n.a.  | 0.414 | 0.624  | n.a.   |
|                 | EQR $H'$ | n.a.  | 0.832 | 0.762  | n.a.   |
|                 | EQR $\%$Diptera | n.a.  | 0.848 | 0.418  | n.a.   |
|                 | EQR EPTCBO | n.a.  | 0.424 | 0.410  | n.a.   |
|                 | LMI | n.a.  | 0.630 | 0.544  | n.a.   |
|                 | Ecological status based on LMI | –     | good  | moderate | –     |

* monitoring data derived from the State Monitoring Program  
** own data
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Figure 2. Relative abundance (%COV) of the macrophyte groups in the total phytolittoral area of Probarskie Lake in August in the subsequent surveys; En – *E. nuttallii*, noEn – without *E. nuttallii*.

A trend in proportion of *E. nuttallii* was observed, up to 63% of the total vegetated area and over 80% of the area covered by hydrophytes (Figure 2). The contribution of the other communities to the total vegetated area, including the one formed by *E. canadensis*, was marginal, ranging from less than 1% to about 6% of the total phytolittoral area (Figure 2, Table S2). The expansion of Nuttall's waterweed phytocoenoses caused a decrease or extinction of native species, e.g., the disappearance of communities of pondweeds such as *Potamogeton compressus, P. friesii, P. perfoliatus, P. crispus, P. rutillus* and *Stuckenia pectinata* (Table S2).

Changes in the syntaxonomic structure were reflected in the diversity index calculated for both all syntaxa ($H'_{TOT}$) and hydrophyte syntaxa ($H'_{HYDR}$) being considerably lower in 2019 and 2020 than before the invasion of *E. nuttallii*, and particularly, for $H'_{HYDR}$ it was almost two times lower. Despite the significant changes in compositional indices, the abundance indices, i.e., $C_{max}$ and $N$, remained at a similar level in all years of the study (Table 2). The macrophyte colonisation depth reached 5.0 m on average (mean of 16 transects) and was similar in all study years. The vegetation coverage of the littoral was generally high, however, it decreased from almost 95% in 2011 to 85% in 2019 and 2020. Inevitably, the estimated total area vegetated decreased in this period from about 60 ha to 55 ha.

The macrophyte ecological status index ESMI decreased from 0.639 in 2011 to 0.471 in 2019, and then it slightly increased to 0.509 in 2020. These fluctuations did not affect the classification of the macrophyte-based lake ecological status, which was good in all the analysed years.

**Phytoplankton characteristics**

The total biomass of phytoplankton at study sites ranged from 1.4 mg L$^{-1}$ (site 3Pel in 2020) to 9.3 mg L$^{-1}$ (site 3En in 2019). The variation of total
biodiversity (CV) among sites of the same habitat ranged from 10% to 15% in 2019, and from 26% to 33% in 2020. No significant differences were noted among habitats in both years studied (the KW-H (2;9) = 5.6, p = 0.060 and KW-H (2;9) = 0.62, p = 0.730, respectively). At all sites, the biomass values were higher in 2019 than in 2020, and for overall biomass this difference was statistically highly significant based on the Mann-Whitney U test (p < 0.0003; Figure 3, inset). The similarity of the taxonomic structure was relatively high, at the level of approx. 70%, both among the sites and the analysed habitat types.

Three groups dominated the taxonomic structure: cyanobacteria, cryptophytes and green algae, while taxa belonging to dinophytes, chrysophytes, euglenoids or diatoms were less numerous (category “Other” in Figure 3). Among cyanobacteria, the species from the order Oscillatoriales, *Lyngbya* cf. *hieronymusii* Lemmermann 1905 (synonym of *Limnoraphis hieronymusii* (Lemmermann) J.Komárek, E.Zapomelová, J.Smarda, J.Kopecký, E.Rejmánková, J.Woodhouse, B.A.Neilan & J.Komárková, 2013) constantly dominated constituting from 40% to 92% of the total biomass. This taxon was abundant in the phytoplankton community in all types of analysed habitats (*En, noEn, Pel*). In the first study year, its biomass was lower at *noEn* sites (0.96–1.92 mg L⁻¹) than at *En* sites (3.12–6.25 mg L⁻¹) or *Pel* sites (3.36–4.93 mg L⁻¹). Its percentage share ranged from about 18% to 67% of the total biomass of phytoplankton. In the second study year, the values of biomass of *L. cf. hieronymusii* did not differ significantly among habitats and ranged from 0.42 mg L⁻¹ to 1.90 mg L⁻¹, constituting from 64% to 92% of the cyanobacterial biomass and from 21% to 65% of the total phytoplankton biomass (Figure 3). Detailed taxonomic data are provided in Table S3.

**Figure 3.** Proportion of the biomass of taxonomic groups in total phytoplankton biomass at sampling sites in Probarskie Lake in August 2019 and 2020; *Pel* – pelagic sites, *noEn* – littoral sites without *E. nuttallii*, *En* – littoral sites dominated by *E. nuttallii* phytoocoenoses. Inset presents comparison of the distribution of total phytoplankton biomass in Probarskie Lake between two research years.
The PCA analysis on the distribution of phytoplankton in multidimensional space in relation to sites representing different habitat types (En, noEn, Pel) revealed phytoplankton similarity among sites of the same habitat type, i.e., sites tended to cluster within the same habitat types, however, they were not clearly separated (Figure 4). In both years of the research, the dominant cyanobacteria species L. cf. hieronymusii exhibited links with littoral sites dominated by E. nuttallii, whereas cryptophyte taxa belonging to the genera Cryptomonas and Rhodomonas and green algae from the order Volvocales (Pandorina, Eudorina) tended to accompany samples from pelagic sites and sites in littoral not inhabited by E. nuttallii (Figure 4).

The phytoplankton community exhibited significant fluctuations in abundance and diversity parameters over the last decade. Across the four seasons of research, the peak of phytoplankton abundance and total taxa number was stated in 2019 while the lowest total biomass together with the highest diversity (index H') in 2011 was observed (Table 2). The differences in the phytoplankton density, including the biomass of cyanobacteria, among years of the research, were reflected in ecological status assessment results (Table 2). High values of the biomass of cyanobacteria in 2019 resulted in a low EQR value of cyanobacterial metric EQR Y_{CY} = 0.02, which classified this waterbody as the one with bad ecological status. However, the phytoplankton metrics in 2019 did not correspond to the water quality parameters, mainly with relatively stable and high water transparency (Secchi disc visibility ranged between 2.4 and 2.9 m; mean 2.6 m; Table 1). In 2020, the total biomass of phytoplankton and the biomass of cyanobacteria were relatively low, at the level observed in the years before the appearance of E. nuttallii in Probarskie Lake (Table 2). A low density of cyanobacteria resulted in an increase in the metric value to 0.61, corresponding to good status, although closer to the boundary value between good and moderate status (Table S1).

Macrophytes characteristics

The total number of invertebrate individuals in both samples collected from Probarskie Lake in the season of 2019 was generally low. At sites (B)En and (B)noEn, 88 and 112 individuals belonging to 17 and 16 taxa were identified, respectively (Table S4). Overall, 11 taxa were found in both sites, while another 11 taxa were found only in one site.

At both sites, individuals of Diptera and Oligochaeta together constituted more than half of the benthic macrofauna community (Figure 5). In addition, individuals of Bivalvia (mainly Dreissenidae and Sphaeridae), Ephemeroptera (mainly Caenidae) and Trichoptera (mainly Leptoceridae) appeared at both sites. However, at (B)En site a much higher proportion of Oligochaeta (25%), Ephemeroptera (11%) and Odonata (9%) than at (B)noEn site was noted (9%, 6% and 2%, respectively). On the other hand, at the (B)noEn
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**Figure 4.** Distribution in the PCA ordination space of the phytoplankton taxa (total biomass) and sampling sites in Probarskie Lake in August 2019 and 2020. Only taxa, for which the first two axes explain at least 30% of the variability in the community structure, were included in the analysis; key to taxa name abbreviations is included in Table S3.
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Figure 5. Proportion of the taxonomic groups of macrozoobenthos at sampling sites in Probarskie Lake in September 2019; (B)En littoral site dominated by *E. nuttallii* phytoceoenoses; (B)noEn – littoral site without *E. nuttallii*.

Table 3. Values of the macrozoobenthic indices at two sampling sites in Probarskie Lake in September 2019; (B)En – site dominated by *Elodea nuttallii*, (B)noEn – site without *E. nuttallii*; index values normalized to the range [0–1], the higher the value the better the ecological conditions.

| Macrozoobenthic indicator | Sampling site |
|---------------------------|---------------|
|                           | (B)noEn | (B)En |
| EQR ASPT-PL               | 0.581    | 0.667 |
| EQR H’                    | 0.758    | 0.766 |
| EQR %Diptera              | 0.269    | 0.567 |
| EQR EPTCBO                | 0.455    | 0.364 |
| LMI_site                  | 0.489    | 0.599 |

**Site status class based on LMI**
- **(B)noEn** moderate
- **(B)En** good

A higher proportion of Heteroptera (7%) in the total invertebrate fauna was found than at the (B)En site (2%; Figure 5).

The macrozoobenthos samples from both sites were similar in terms of the number of individuals and taxonomic richness. Taxa that were present only at one of the two sites were those that were unabundant (1 to 5 individuals per sample) and their absence can be explained by their generally rare occurrence. The dominant taxa (Chironomidae, Oligochaeta, Ceratopogonidae, Caenidae) were recorded at both sites.

Despite the lack of clear differences between sites, at the (B)noEn site higher value of EQR EPTCBO, while at the (B)En site higher values of EQR ASPT-PL, EQR H’ and EQR %Diptera were noted. The value of the LMI ecological status index for the site (B)noEn was 0.489, which corresponds to a moderate ecological condition, while on the site (B)En it was 0.599, which corresponds to a good ecological condition, although closer to the good/moderate boundary (0.588) (Table 3).
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Figure 6. Distribution in the PCA ordination space of the macrozoobenthic taxa and sampling sites in Probarskie Lake in September 2017 and 2019; key to taxa name abbreviations is included in Table S4.

The LMI for the lake in 2019 was 0.544, which corresponds to moderate ecological status (Table 2). This was lower than that in the year 2017 (LMI = 0.630), when good ecological status was indicated. Comparing the compositional metric values between 2017 and 2019, the EQR ASPT-PL significantly increased, while EQR $H'$, EQR %Diptera and EQR EPTCBO decreased (Table 2). For the EQR %Diptera, the greatest decline, almost twofold, was observed. In consequence, the value of the overall LMI index for the lake decreased between the analysed years, indicating good ecological status in 2017 and moderate ecological status in 2019 (Table 2).

Based on the PCA, the macrozoobenthos samples from 2017 and 2019 were scattered along two main axes explaining 69.8% and 23.7% of variance, respectively (Figure 6). None of these axes reflected the presence of *E. nuttallii*. Samples from 2019 (with and without *E. nuttallii*) were located close to each other on the ordination plot, which indicates higher similarity within the year than between sites of a different location, regardless of *E. nuttallii* abundance in the sampling site. This may indicate that although between two analysed years the decrease in the ecological status class based on LMI was noticed, this deterioration cannot be directly
attributed to the increase in *E. nuttallii* abundance and its takeover of the dominance in the macrophyte community in the lake.

**Discussion**

Based on the physicochemical data, the waters of Probarskie Lake can be classified as highly alkaline and moderately fertile, slightly eutrophic (OECD 1982), with relatively high transparency and relatively stable quality over the last decade. The water quality of the lake corresponds to good ecological conditions as determined for this lake type, i.e., lowland, with highly alkaline, non-coloured waters and calcareous geology in the basin. The massive growth of *E. nuttallii* in Probarskie Lake indicates that the lake serves favourable conditions for the establishment and development of this alien species. In addition to *E. nuttallii*, the lake has been also inhabited by another alien hydrophyte, *Elodea canadensis*, although the contribution of this species to the total vegetated area was minor in all study years (no more than 6% of the total vegetated area). Both the American waterweeds, *Elodea canadensis* and *E. nuttallii*, are recognised to be highly adapted to a broad range of environmental conditions and can grow in waters of various nutrient availability, from mesotrophic to highly eutrophic (Pokorný et al. 1984; Madsen et al. 1991; Thiébaut et al. 1997; Barrat-Segretain 2001; Thiébaut 2005; Hérault et al. 2008), though in comparative studies by Greulich and Trémolieres (2006), Nuttall’s waterweed demonstrated preference towards more nutrient-rich waters than Canadian waterweed. Moreover, the growth of *E. nuttallii* is often stimulated by nitrogen fertilisation and the species benefits from an excess of ammonia (Best et al. 1996; Dendene et al. 1993) that is common in lakes located in an agricultural landscape. This may explain, at least to some extent, the invasion success of *E. nuttallii* in waters exposed to eutrophication pressure. The relatively high nitrogen concentrations in the littoral sites in 2019 may point to external loads from surface runoff and local nutrient enrichments. This was, however, not reflected in the pelagic waters with noticeably lower TN concentrations in 2019, nor confirmed in the second year of the study, when the nitrogen concentrations were relatively low in all sampled sites. During the last decade, no clear effects of the invasion of *E. nuttallii* on overall water quality were observed except for the increased TP concentration in 2020. This may suggest progressing eutrophication process and the risk of the ecosystem breakdown in the case of an abrupt breakdown of the *E. nuttallii* population (Sand-Jensen 2000; Di Nino et al. 2005). Decomposition of a large amount of biomass causes a release of significant quantities of biogenic substances and organic matter to the environment, oxygen depletion and a drop in pH of waters, resulting in disturbance in the nutrient balance and affecting food chains (Jones et al. 1996; Carey et al. 2016).
The most adverse effect of the invasion of *E. nuttallii* in the lake was the loss of biodiversity of aquatic vegetation. From the first appearance in 2017, the phytocoenoses of *E. nuttallii* within 2–3 seasons almost completely substituted the native hydrophytic flora, resulting in a significant decrease in hydrophyte diversity (decrease in $H'_\text{TOT}$ and, particularly, in $H'_\text{HYDR}$). Likewise, the results by Bubíková et al. (2021) showed that the presence of elodeas in Slovakian waters contributed to the unification of natural aquatic communities, decrease in species richness and beta diversity. However, studies from other regions provide contradictory results indicating the lack of clear effect of elodeas on macrophyte community characteristics (Greulich and Tremolieres 2006; Kolada and Kutyła 2016).

Despite significant changes in the macrophyte syntaxonomic composition and proportion among plant communities, the indicators describing the overall abundance and spatial structure of the vegetation (total vegetated area N, vegetation cover %COV, colonisation depth $C_{\max}$) remained essentially unchanged in the period before and after the invasion of Nuttall’s waterweed. The overall ecological status based on ESMI also showed no evidence of community deterioration, indicating macrophyte good ecological status in all analysed years. This indicates the lack of responsiveness of the ESMI method to the appearance of an alien species, despite its strong and decisive domination in the macrophyte community. The value of ESMI is determined primarily by the abundance components (N, %COV and $C_{\max}$), while the effect of phytocoenotic diversity indices is limited (Ciecierska and Kolada 2014). This means that as long as the phytolittoral zone remains extensive and densely vegetated, the ecological status of the lake will remain stable regardless of the changes in the taxonomic composition of the vegetation, and the presence of the alien species will not be reflected in the assessment. The broad tolerance of *E. nuttallii* to environmental conditions (Barrat-Segretain et al. 2002; Thiébaut 2005; Greulich and Trémolieres 2006), its ability to massive growth and colonising deeper parts of the littoral (here up to 5 m) contributed to the extensive and densely vegetated, though almost monospecific phytolittoral, and resulted in increased values of abundance metric. This was the main reason for the lack of clear changes in the ecological status class of the lake, which, despite fluctuations in the ESMI value, remained good during the last decade.

The appearance of *E. nuttallii* in Probarskie Lake and its dominance in the macrophyte community were not reflected in the assessment results based on other biological elements. In the phytoplankton community, considerable fluctuations in biotic parameters and assessment results over the last decade were observed and these changes could not be directly attributed to the appearance of alien plant species. Although in 2019 the exceptionally high phytoplankton biomass values at all the studied sites were observed, this increase was not confirmed in 2020, when the biomass values were several times lower than in 2019, at the level observed in the
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years before the appearance of *E. nuttallii* in the lake. Moreover, the quantitative change between 2019 and 2020 was not accompanied by taxonomic reconstruction and the proportion of taxa in the total biomass remained similar between those two years (similarity coefficient of taxonomic structure at the level of approx. 70%). The much higher algae biomass in 2019 could be, therefore, a response to temporary, short-term changes in nutrient concentration, which does not necessarily indicate a permanent increase in water trophy. The qualitative change of the phytoplankton requires a longer period of increased nutrient supply (Hörnström 1981), hence, the increase in nutrient concentration if temporal or momentary may not be reflected in the phytoplankton taxonomic structure.

The high density of the cyanobacteria in Probarskie Lake in 2019 resulted in the cyanobacterial metric $Y_{CY}$ value indicating a bad ecological status. This assessment result did not correspond to the relatively high water transparency (2.6 m) and the ESMI value (0.471) indicating a good status of the lake. One of the reasons for this inconsistency could be the dominance in phytoplankton of the cyanobacteria *Lyngbya* cf. *hieronymusii*, species rarely reported in Europe, but common in North America and tropical and subtropical countries (Komárek and Anagnostidis 1999), also rare and alien to Poland. Large filaments of this species significantly contributed to the total biomass but did not strongly affect water transparency (limited shading effect due to large body size). On the other hand, the cyanobacterial metric $Y_{CY}$ is calculated based on summer data only, when the probability of cyanobacterial growth peaks. The metric $Y_{CY}$ is one of three components of the phytoplankton assessment metric PMPL, and when used as the only phytoplankton indicator, it may be accidental and unreliably reflect the overall status of the algae community. The value of $Y_{CY}$ in 2020 was at the same level as in the pre-invasion period. In general, compared to the first half of the last decade, the characteristics of phytoplankton may indicate a deterioration of the lake conditions after the invasion of *E. nuttallii*. Nevertheless, the significant differences in phytoplankton characteristics between 2019 and 2020 and their fluctuations over the last 10 years make it impossible to indicate the directional changes in the phytoplankton community in Probarskie Lake.

In our study, we analysed the spatial diversity of the planktonic algae community due to either presence or absence of phytocoenoses of *E. nuttallii*. We assumed that the density and taxonomic structure of phytoplankton differs between pelagic and littoral sites as well as between the littoral sites with and without *E. nuttallii*. The premise of the above assumption is, *inter alia*, the allelopathic influence of this species reported by, e.g., Erhard and Gross (2006), Wu et al. (2009) and Vanderstukken et al. (2014). Contrary to our expectations and literature reports, in both study years the density of planktonic algae at sites with and without *E. nuttallii* was similar and statistically indifferent. Furthermore, although non-significant, the
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Phytoplankton biomass at sites dominated by *E. nuttallii* was usually the highest among all surveyed sites. It should be emphasized, however, that different phytoplankton species respond to allelopathic effects of submerged macrophytes differently. The systematic review by Maredová et al. (2021) revealed that most of the macrophyte species have shown a combination of inhibitory, stimulatory and neutral effects, which can be affected by environmental factors such as light, temperature and nutrient concentration. In Probarskie Lake, the total biomass of algae was determined by the overwhelming abundance of one macroscopic cyanobacterial species *Lyngbya cf. hieronymusii* and the sensitivity of this species to allelochemicals is not recognized yet.

Another potential reason for the lack of differences in physicochemical and phytoplankton properties among sites, at least the littoral ones, may be the presence of dense vegetation at all littoral sites, inhabited by either *E. nuttallii* or native species. Due to the feedback mechanism between phytoplankton and submerged macrophytes, the development of the latter contributes to the maintenance of a clear-water state through the reuse of nutrients from sediments, allelopathic impact on cyanobacteria, and competition with phytoplankton (van Donk and van de Bund 2002). In this aspect, the alien macrophyte species may act similarly to native ones. This has been observed, for example, in one of the Vistula floodplain lakes where the massive development of *E. nuttallii* between 2009 and 2015 has contributed to the improvement of water transparency from 0.5 to 1.6 m and the reduction of phytoplankton biomass from about 90 mg L\(^{-1}\) to the level of about 4 mg L\(^{-1}\) (Dembowska et al. 2021). Although never welcome outside its native range, in ecosystems exposed to eutrophication pressure with a high risk of algal blooms the presence of *E. nuttallii* could be more favourable than the phytoplankton-dominated state.

In the macrozoobenthos community, after the massive spread of *E. nuttallii* in Probarskie Lake, a decrease in the ecological status was observed from good in 2017 to moderate in 2019, though this decrease was relatively subtle and could be considered accidental (in both the study years, the LMI values were close to the good/moderate boundary 0.588; Table S1). Likewise, the effect of the presence or absence of *E. nuttallii* at two sampling sites surveyed in 2019 was not evident. The site with *E. nuttallii* was richer in sensitive taxa, resulting in higher ASPT-PL metric and had a lower proportion of Diptera, i.e., the taxa tolerant to lower oxygen concentration. Kelly et al. (2015) demonstrated that waters dominated by *E. nuttallii* were rich in oxygen and, therefore, favourable for macroinvertebrate species that require high oxygen conditions. The physicochemical measures in our study did not support this distinction, as oxygen concentrations at sites inhabited and not inhabited by *E. nuttallii* were indifferent, but these measurements reflected momentary conditions.
and no long-term effects have been observed in our study. It is unclear whether these slight differences in the taxonomic composition of uncommon taxa between invaded and uninvaded sites were related to the appearance of *E. nuttallii*, the natural variability of environmental conditions, or the effect of the sample size, or other factors.

At both sites, (B)En and (B)noEn, more than half of the benthic macrofauna community was composed of Diptera and Oligochaeta, taxa relatively tolerant to unfavourable oxygen conditions (Dumnicka 1994; Armitage et al. 1997). Apart from these two taxa, the fauna included mainly eurytopic taxa with broad environmental tolerance. Although the presence of caddisflies from the family Leptoceridae was recorded, i.e., a family that prefers clean waters (Morse 2009), representatives of relatively tolerant taxa, such as Asellidae, Lymneaidae, and Sialidae were also found. At the site with the dominance of *E. nuttallii*, a greater proportion of mayflies and dragonflies was observed, i.e., taxa sensitive to contamination and unfavourable oxygen conditions (Catling 2005; Menetrey et al. 2008). However, at the site without *E. nuttallii* the number of families from vulnerable groups, i.e., Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata was so high that the index EPTCBO reached a higher value determining a higher overall assessment of the site (B)noEn than it was for (B)En. It is worth emphasising that the invertebrate fauna in the littoral is heterogeneous and is subject to spatial variability in natural conditions and anthropogenic influences (Brauns et al. 2007; Porst et al. 2019). Thus, the differences in the index value of one class between the sites may result from many other factors than the presence of alien plant species, such as local contamination, shoreline modification, wind exposure, etc.

At both sampling sites, specimens of Dreissenidae were detected, specifically *Dreissena polymorpha* (Pallas 1771), which is considered an invasive species as well (CABI 2022). This species was noticed in relatively high abundance also in the previous study at all sampling sites. Both mesocosm experiments (Crane et al. 2020) and environmental research (Wegner et al. 2019) document that *Dreissena* mussels and *E. nuttallii* can facilitate each other’s growth during invasion establishment. As *D. polymorpha* is widespread in Polish lakes (Stańczykowska and Lewandowski 2012), this factor is worth considering in monitoring *E. nuttallii*’s spread in other locations in Poland.

The changes in the structure of biological assemblages and the values of ecological status indicators observed in the lake between 2011 and 2020 were relatively subtle (macrophytes, macrozoobenthos) or fluctuated (phytoplankton, water physicochemistry) and were not reflected in the results of the assessment of the ecological status of the lake. One of the reasons for such a situation may be the low sensitivity of the national methods for assessing the ecological status of lakes to the presence of alien
species, which have not been an ecological problem in Poland’s lakes so far. *Elodea nuttallii* is the first hydrophyte in Poland, which appeared highly invasive and aggressive to native flora. Another alien species of the genus *Elodea*, the Canadian waterweed, known in Poland for over 100 years, turned out to be non-invasive and non-aggressive, at least not in lake waterbodies monitored and assessed under WFD (Kolada and Kutyla 2016). Therefore, the insensitivity of the assessment method to its presence was not problematic from the perspective of water quality protection and management. The comprehensive overview by Kolada et al. (2018) suggests that in the nearest future, the problem of *E. nuttallii* invasiveness in Polish waters will increase. Therefore, the verification of ecological status assessment methods, especially the macrophyte method ESMI, should be considered in terms of increasing the diagnostic capacity to capture the problem of ecological invasions.

**Conclusions**

The appearance of Nuttall’s waterweed in the phytolittoral of Probarskie Lake and its relatively fast (within 2–3 seasons) taking over of the dominance of the macrophyte community was not reflected in the ecological status of the lake. In particular, the macrophyte method ESMI proved to be insensitive to the appearance of the invasive alien hydrophyte, although this species outcompeted native species and contributed to a significant decrease in syntaxonomic diversity of aquatic vegetation. The changes in the ecological status of two other analysed biological assemblages were either non-directional (phytoplankton) or too meaningless (macrozoobenthos) that they could be linked to the lake invasion by *E. nuttallii*. The increased total phosphorus concentration in 2020 may indicate the progressing eutrophication, though other physicochemical water quality parameters did not confirm this observation so far, being relatively stable over the entire analysed period both before and after the invasion.

Probarskie Lake appears to provide favourable conditions for the establishment and development of alien species. In addition to the massive development of *E. nuttallii*, the lake was inhabited also by *E. canadensis*. In the phytoplankton community the cyanobacteria *Lyngbya cf. hieronymusii* dominated, while in the macrozoobenthos the presence of *Dreissena polymorpha* systematically was noted. All these species are alien to Poland and require special attention from the ecological and management perspective.

The effective monitoring of alien invaders requires methods capable to detect the appearance of these species and their effect on the structure and functioning of native biota. Because national methods tested in this study appeared insufficiently responsive to this ecological problem, the urgent need for their verification emerged.
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**Authors’ contribution**

AK – research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, writing – original draft; AP – sample design and methodology, investigation and data collection, data analysis and interpretation, writing – review and editing; AB – investigation and data collection, data analysis and interpretation, writing – review and editing; SK – investigation and data collection, data analysis and interpretation, writing – review and editing.

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**Supplementary material**

The following supplementary material is available for this article:

**Table S1.** Lake classification system based on Ecological State Macrophyte Index (ESMI), phytoplankton metric “cyanobacteria biomass” recalculated to Ecological Quality Ratio (EQR) and Lake Macroinvertebrate Index (LMI).

**Table S2.** List of plant communities and their relative cover in the littoral area in the Lake Probarskie in subsequent years of the study; dominants within the macrophyte groups marked in bold.

**Table S3.** List of phytoplankton taxa and their biomass (mg L$^{-1}$) in the sampling sites in Probarskie Lake in two years of the study.

**Table S4.** List of macrozoobenthic taxa identified in the Lake Probarskie in 2017 and 2019. For 2019 the abundance (number of individuals) at two sampling sites in September 2019 is presented, separately; habitat types: (B)En – site dominated by *Elodea nuttallii*, (B)noEn – site without *E. nuttallii*. 

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