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Benthic diatoms in small Estonian lakes — primary niche substratum comparisons

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Comparisons of benthic diatom communities were carried out in 12 small Estonian lakes. We compared how substratum type (cobbles versus macrophytes) impact lake ecological status calculations (according to the Water Framework Directive). We hypothesized that in meso- to eutrophic lakes, both communities from macrophytes (epiphyton) and from cobbles (epilithon) would show similar modeling for lake ecological status. In general, epiphyton samples showed a slightly higher ecological status relative to epilithon samples. Comparing studied benthic diatom species assemblages, the number of species was in general higher in the epilithon, but fluctuated more in epiphyton samples. The primary species with abundances ≥ 10% was *Achnanthidium minutissimum sensu lato* prominently observed in 11 studied lakes. In addition *Epithemia sorex* dominated in Lake Tamula and *Sellaphora atomoides* in Lake Uljaste. Our results confirmed our hypothesis that in meso- to eutrophic lakes, the ecological status assessment results were similar using different substrata.

Introduction

According to the Water Framework Directive (WFD; European Union 2000), all EU countries need to monitor their freshwaters with the objective of achieving at least a "good" ecological status rating. According to WFD Annex V, both macrophytes and phytobenthos (including benthic diatoms) form one BQE (Biological Quality Element) of a lake's ecological quality assessment. Authors using ecological quality assessments have pointed out that different quality components should be evaluated separately (Kelly *et al.* 2015, Poikane *et al.* 2015). Discussion then appeared in the peer-reviewed literature, questioning if benthic diatoms are giving any additive information to the ecological status of lakes in addition to macrophytes (Kelly *et al.* 2016). Current research has shown that the ecological role of benthic diatoms in lakes depends on specific conditions (i.e. chemical conditions, size and type of lake, role of other biota, etc) (Stevenson 1997, Håkanson and Boulion 2004, Poulíčková *et al.* 2004, King *et al.* 2006).

Since benthic diatoms are included in lake ecological status assessments as one of the quality elements, an evaluation of the current knowledge was undertaken using the ISI Web of Science citation databases from years 2000 to 2020. Search terms used in June 2020 were

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for lake queries with the combination: *lake**, *diatom**, *index**, *NOT river**, *stream**; for the stream queries the combination was: *stream**, *river* diatom**, *NOT lake** and *index**. The results found 88 and 32 717 studies for lakes and streams, respectively (Table 1). This indicates that more studies are needed in lake assessments, in order to evaluate how benthic diatom communities are functioning and impacting ecological status assessments.

The literature shows that phytobenthos assemblages are affected by many different factors, especially substratum (e.g. Cox 1988, Michelutti *et al.* 2003, King *et al.* 2006, Passy 2007). Thus, if non-standardized methods are used in assessments, results can be shifted and ultimately not comparable. For instance, the structure and age of the macrophyte substratum can impact assessments; it has been shown, that diatoms colonizing younger plant parts have lower biomasses and communities are dominated by small-sized benthic diatoms (Poulíčková *et al.* 2004). This can eventually lead to the wrong opinion that phytobenthos metrics are not needed in lake's ecological status assessments next to phytoplankton and macrophytes.

In addition to macrophytes, inorganic substrata like pebbles and sand grains are populated by benthic diatoms (Krejci and Lowe 1986, Barnese and Lowe 1992). Both are analog substrata: they have rough surface offering many opportunities for diatoms to attach. Cobbles are less disturbed and a more stable substrata (Kahlert 2001). There are also differences in benthic diatoms populating on vertical and horizontal surfaces. Jones (1974) has shown higher benthic diatoms biomass on vertical microhabitats of cobbles. This phenomenon was likely caused by photoinhibition in shallow water, overshadowing by phytoplankton particles, and larger erosion events on horizontal microhabitats.

To standardize methodologies and minimize substratum impacts, artificial substrata with well-defined surface areas have been suggested

Table 1. Benthic diatoms on different substratum used in lake ecological status evaluations in different regions.

Substratum Stones	 Macrophytes	 Artificial	Region/country	Reference
x	x		Australia	Dela-Cruz, *et al.*, 2006
		x	North America	Sgro *et al.*, 2006
	x		Hungary	Stenger-Kovács *et al.*, 2007
	x		Turkey	Suvacu *et al.*, 2008
	x		Hungary	Hajnal *et al.*, 2009
x	x		Macedonia, Albania and Greece	Naumoski & Mitreski, 2010
	x	x	Hungary (L. Balaton)	Bolla *et al.*, 2010
	x		France	Cellamare *et al.*, 2012
x			Portugal	Novais *et al.*, 2012
x			Turkey	Sivaci *et al.*, 2013
	x		Hungary	Crossetti *et al.*, 2013
x		x	Ireland	Snell & Irvine., 2013
x	x		United Kingdom	Bennion *et al.*, 2014
x			Macedonia and Albania	Schneider *et al.*, 2014
x			Finland	Vilmi *et al.*, 2015
x	x		China	Ouyang *et al.*, 2016
x			Switzerland and France	Rimet *et al.*, 2016
x	x		Poland	Kolada *et al.*, 2016
x			France	Rivera *et al.*, 2018
	x		Hungary	Stenger-Kovács *et al.*, 2018
x			Finland	Vilmi *et al.*, 2019
x			Romania	Kelly *et al.*, 2019
x			Poland	Messyasz & Treska, 2019
	x		South Africa	Riato & Leira, 2020

(Biggs 1989, Potapova and Charles 2005). However, diatoms study results from artificial substrata are less representative of natural assemblages and populations have lower species diversity (Jones 1974). For this reason, artificial substrata were not used during this study, since the objective was to understand what are the natural impacts on benthic diatom communities in the sense of substratum type. As noted, studies have shown that macrophytes may not be the best substratum for sampling epiphytic/benthic diatom assemblages in lakes, as the macrophyte species can affect the final ecological status evaluation, which is dependent on lake type (Poulíčková *et al.* 2004). Earlier studies have concluded that samples collected from macrophytes in general show lower ecological status values, than samples collected from cobbles, but this phenomenon depends on lake's trophic conditions (Lalonde and Downing 1991, Kahlert 2001, King *et al.* 2006, Cejudo-Figueiras *et al.* 2010). In Estonia benthic diatoms, as the proxy for the phytobenthos, have been used in ecological assessments of streams for many years (Vilbaste 2004). The method used has been inter-calibrated and harmonized with pan-European diatom based ecological status assessments (Kelly *et al.* 2009, 2012, Kahlert *et al.* 2009, 2012, 2016).

The aim of the current study is to compare diatom communities of two prominent substrata types (cobbles and macrophytes) in Estonian lakes and analyze how results impact lake ecological status assessments, according to the Water Framework Directive. We hypothesized that in anthropogenic impacted and more eutrophic lakes conditions, differences between benthic assemblages on different substrates are less evident and hence lake ecological quality assessments are not affected by substratum type. With increasing anthropogenic stress and increasing eutrophication, substratum considerations can be less critical for the Water Framework Directive.

Material and methods

Study area

To study diatom assemblages of the two most common substrata types (cobbles and macrophytes), benthic diatoms samples from 12 lakes (Lake Lõõdla sampled both in 2014 and 2016) were collected and analyzed during 2014–2016 (Fig. 1). Sampled lakes were distributed throughout Estonia and covered a broad amplitude of hydrochemical conditions (*i.e.* nutrients, pH, total alkalinity (Tables 2 and 3)).

According to lake typology, based on WFD and Estonian Water Act (1994), Estonian lakes are divided into eight types. Differentiation principles are mainly size, stratification, water hardness, content of humic compounds, distance from the sea and content of dissolved chlorides. The larger lakes, Peipsi and Võrtsjärv, form separate classes S6 and S7, officially called "large lake types", while the other lakes are considered "small lakes," which form six lake types (Table 2) (Ott 2006). In the current study, all belong to Estonian lake types S1–S5 and S8 (Table 2), with surface area < 10 km². One exception is for type S8 (coastal lakes), which

Table 2. Estonian small lake types S1–S5 and S8 characterization according to the Water Framework Directive (Ott 2006).

Lake type	Type description	Total alkalinity (HCO_3– mgL^{-1})	Conductivity (μScm^{-1})	Chlorides (mgL^{-1})	Stratified	Colour (on Pt/Co scale)
S1	Alkalitrophic	> 240	> 400	≥ 25	No	—
S2	Shallow, light, medium alkalinity	80–240	165–400	≥ 25	No	—
S3	Deep, light, medium alkalinity	80–240	165–400	≥ 25	Yes	—
S4	Dark, soft water	< 80	< 165	≥ 25	No	≥ 100°
S5	Light, soft water	< 80	< 165	≥ 25	No	< 100°
S8	Not considered			≥ 25	Not considered	

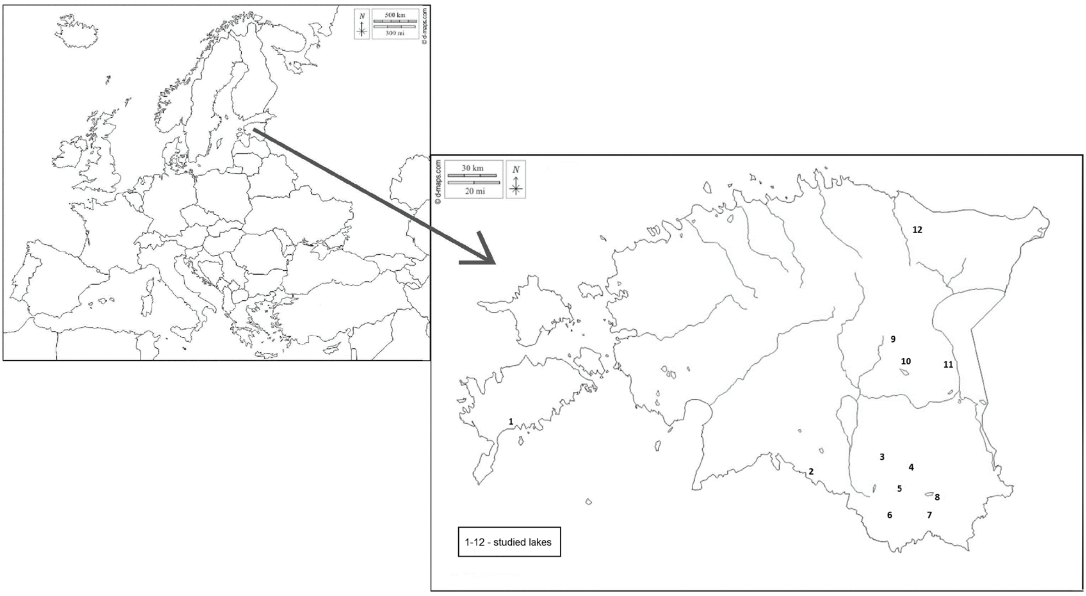

Fig. 1. Study area and position of 12 studied lakes in Estonia (1 — Suurlaht, 2 — Tündre, 3 — Pühajärv, 4 — Jõksi, 5 — Lõõdla, 6 — Ähijärv, 7 — Rõuge Suurjärv, 8 — Tamula, 9 — Kuremaa, 10 — Kaiavere, 11 — Lahepera, 12 — Uljaste).

represents lakes high in chlorides (≥ 25 mg L^{-1}) and located ≤ 5 km from the Baltic Sea (Estonian Water Act 1994).

When comparing studied lake types, the most sensitive to anthropogenic stress are lakes belonging to type S5 — light and soft water lakes, because of their low buffering (unable to maintain neutral pH) (Ott and Kõiv 1999). Lakes belonging to type S4 (dark and soft water) have high buffering, due to elevated humic acids and other humic compounds, that can bind nutrients. All the other lake types have higher buffering, located in carbonate-rich limestone areas where water is harder and pH in general higher (Ott and Kõiv 1999, Ott 2006).

All studied lakes had high pH (7.9–10.6) and lower conductivity (average 403 µS cm^{-1}), except for type S8 (coastal lakes), where conductivity was up to 1940 µS cm^{-1}. Nutrient content in all studied lakes during the summer (July–September) period was relatively high (average TP 0.03 mg L^{-1}, average TN 0.8 mg L^{-1}), and oxygen content was not limited (average O_2 9.7 mg L^{-1}). All lakes had relatively small surface areas, the smallest was lake Rõuge Suurjärv with a surface area 14.6 ha and largest was lake Suurlaht at 539 ha (Table 3). Average surface area for all studied lakes was 191 ha. The studied lakes were shallow with an average depth of 4.6 m. Lake Rõuge Suurjärv had the highest maximum depth (38 m), whereas lake Suurlaht was the shallowest (average depth 1.2 m). In comparison, eutrophicated lakes in decreasing order were Kaiavere (S2), Lahepera (S2), Kuremaa (S3), Tamula (S2), Lõõdla (S3) and Ähijärv (S3), while the most oligotrophic was lake Uljaste (S5) (Laarmaa *et al.* 2019).

All data were collected under the Estonian national hydrobiological monitoring program using small lakes and data stored in the Estonian environmental monitoring information system "KESE" database.

Sampling

Diatom assemblages were collected from littoral habitat during July–August from a 0.5 m depth in accordance with standard methods (CEN - EN 13946, 2014). From each lake at least 5 cobbles and 10 stems and leaves of

Nuphar/*Nymphaea* sp. or *Carex* sp. (whichever was present in sampling area), were collected. The biofilm was brushed off with a toothbrush and lake water. Collected samples were preserved in situ in 96% ethanol. These samples were then treated in the laboratory with peroxide (hot hydrogen peroxide oxidation) and mounted on microscope slides following CEN (2014). Naphrax® (refractive index = 1.74, Brunel Microscopes Ltd) was used as the mountant.

Since vascular plants age and morphological branching complexity are important factors for benthic diatoms community development (King *et al.* 2006), the epiphyton samples were collected from the same littoral zone and from the same macrophytes species within each lake.

Analyzing methods and statistical analysis

Identification of benthic diatoms was carried out using interference contrast microscopy (DIC) with a ZEISS AXIO Imager.A1 and 100× oil immersion objective (NA 1.3). At least 400 valves were counted and identified to the lowest taxonomical level using standard taxonomic literature (Hustedt 1985, Krammer and Lange-Bertalot 1986-1991, Krammer 1997a, 1997b, Lange-Bertalot 2001, Lange-Bertalot 2011). For consistency, all taxonomic identifications were checked and converted to current taxonomic assessments using the Algaebase online data system (Guiry & Guiry 2023).

Counted taxa were assembled into the OMNIDIA program and used to calculate three main indices (TDI — Trophic Diatom Index (Kelly and Whitton 1995), WAT — Watanabe index (Watanabe *et al.* 1986) and IPS — Indice Polluosensitivité Spécifique (Gemagref 1982). These indices were used to evaluate Estonia's small lakes ecological quality, according to WFD. TDI index values ranged from one (indicates oligotrophic conditions) to 100 (indicates highly eutrophic conditions) (Kelly and Whitton 1995). WAT index indicates water saprobity, whereas all benthic diatoms species, that are used to calculate index results, were divided into three classes: 1: saprophilic, 2: saprophobic, 3: indifferent (Watanabe *et al.* 1986). WAT index values ranged from 0 to 20, higher index value represent higher water quality (Watanabe *et al.* 1986). The results were used for ecological status assessments, following the Estonian rivers benthic diatoms ecological status evaluation methods (Timm and Vilbaste 2010). According to IPS, WAT and TDI results, all

Table 3. Lakes physical parameters, water chemistry data for three months (July, August, September), and number of diatom taxa on cobbles and macrophytes in Estonian lakes between 2014 and 2016.

Variable		Min.	Mean	Max.
Lake physical parameters	Lake area (ha)	14.6	191	539
	Catchment area (km^2)	1.1	56	92
	Lake depth (m)	1.2	4.6	38
Water chemistry	pH	7.9	8.9	10.6
	Water temperature (°C)	19.4	21.5	25.7
	O2 (mgL^{-1})	6.9	9.7	15.4
	O2%	82.0	110	188
	Conductivity (μScm^{-1})	23.0	403	1940
	NH4-N (mgL^{-1})	0.01	0.03	0.02
	BOD5 (mgL^{-1})	0.7	1.8	3.1
	PO4-P (mgL^{-1})	0.002	0.007	0.030
	NO3-N (mgL^{-1})	0.01	0.09	0.93
	TP (mgL^{-1})	0.01	0.03	0.07
	TN (mgL^{-1})	0.3	0.8	1.4
Number of diatom taxa on current substrate	Cobbles	20	33	48
	Macrophytes	8	21	38

studied lakes were divided into one of five ecological status classes: high, good, moderate, poor and bad (Table 4).

All calculated diatoms indices were compared with studied lakes overall ecological status scores in the same study year. It summarized results from all the other monitored biological quality elements (macroinvertebrates, macrophytes, fishes, phytoplankton, phytobenthos, zooplankton), hydrochemistry and hydromorphology (Ott 2006, Estonian Water Act 1994). IPS Sensibility (IPS-S) index was used to compare diatom communities that were in different ecological classes. The index showed whether or not diatom species tolerated higher eutrophication levels. Index scores varied from one (species tolerating highest eutrophication levels) to five (species tolerating lowest eutrophication levels). Dominant or most abundant benthic diatoms species of epilithon and epiphyton were then compared to find, if current species in general tolerated higher eutrophication levels, regardless of its lake ecological class.

The number of taxa enumerations and associated diversity indices (Pielou's index (Pielou 1966), Shannon's index (Shannon and Weaver 1949) and Simpson's index (Simpson 1949)) were used to compare benthic diatom assemblages of the epiphyton and epilithon. All three indices were calculated using software R ver. 4.2.1 package *vegan*. To reveal relations between calculated diversity indices and hydrochemical characteristics of the studied lakes, correlation analysis was carried out using Spearman's correlation with the statistical program R ver. 4.2.1 with the *vegan* package. For statistical analysis, all benthic diatom taxa with relative abundance less than five percent (less than 20 valves counted) were excluded.

Results

When compared, the number of diatoms taxa was higher in the epilithon (varied from 20 to 48) but differed more in the epiphyton (varied from 8 to 38) (Table 5). Only in Lakes Kaiavere and Lahepera were the number of diatom taxa higher in the epiphyton. In Lake Lõõdla both assemblages had a similar number of taxa (34 in epilithon and 35 in epiphyton) (Table 5). In general, 64 diatom taxa were found in the epiphyton, whereas in the epilithon assemblages this number was 102 (Table S1 in Supplementary Information). Comparing taxa with relative abundance of more than five percent, there were altogether 57 taxa inherent only for the epilithon and 19 only for the epiphyton. There were altogether 45 diatoms taxa that occurred both in epilithon and epiphyton samples. When comparing all taxa together (also including those with relative abundance less than five percent, 52 taxa only occurred in the epilithon and 24 species only in the epiphyton across the study lakes (Table S1 in Supplementary Information). Species diversity indices were slightly different between the epiphyton and epilithon samples, but all three indices – Pielou, Shannon's and Simpson's — showed higher scores in the epilithon samples, considering they had higher species number (Fig. 2).

The most abundant (appeared in ≥ 10% from all counted valves) or dominant (appeared in ≥ 25% of all counted valves) taxon in 11 of the studied lakes was *Achnanthidium minutissimum sensu lato* (*s.l.*). Only in Lake Tamula,

Table 4. Lake Index status using IPS, WAT and TDI data following the protocol of Timm & Vilbaste (2010).

Index	Interval	High	Good	Moderate	Poor	Bad
IPS	18.2–0	> 15.5	15.5 –> 12.0	12.0 –> 9.5	9.5–6.9	< 6.9
IPS EQR = IPS/18.2	1–0	> 0.85	0.85 –> 0.65	0.65 –> 0.52	0.52–0.34	< 0.34
WAT	18.7–0	> 15.9	15.9 –> 12.4	12.4 –> 9.7	9.7–7.1	< 7.1
WAT EQR = WAT/18.7	1–0	> 0.85	0.85 –> 0.66	0.66 –> 0.52	0.52–0.38	< 0.38
TDI	35–100	< 48	48 –< 61	61 –< 75	75 –< 87	87–100
100 - TDI	65–0	> 52	52 –> 39	39 –> 25	25–13	< 13
TDI EQR = (100 – TDI)/65	1–0	> 0.8	0.8 –> 0.6	0.6 –> 0.4	0.4–0.2	< 0.2

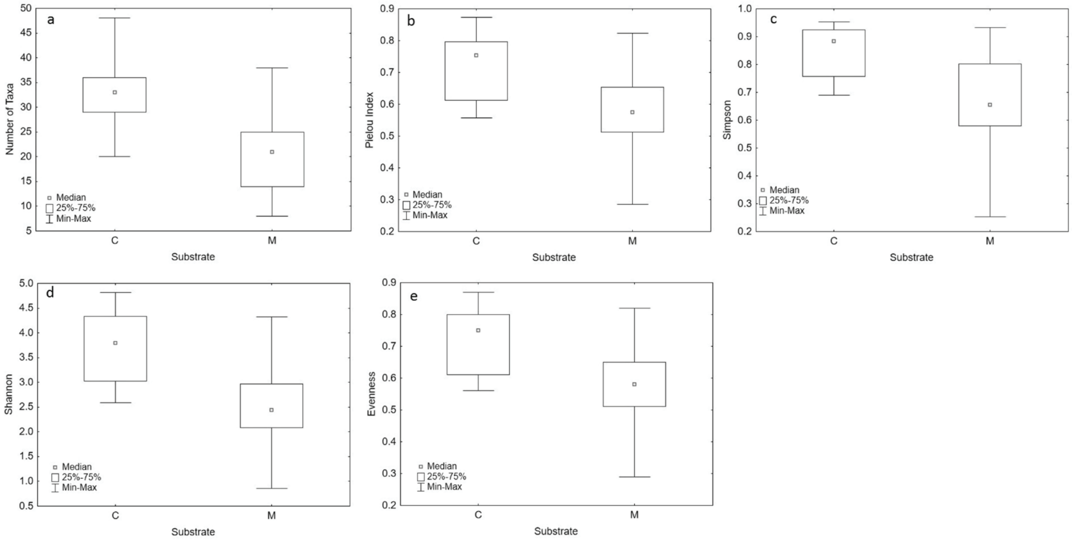

Fig. 2. Differences in number of (**a**) taxa, and (**b**) Pielou, (**c**) Simpson, (**d**) Shannon, and (**e**) Evenness indices of diatom communities on (**C**) cobbles and (**M**) macrophytes in Estonian small lakes.

Table 5. Most abundant (RA ≥ 10%) or dominant (RA ≥ 25%) taxa on cobbles and macrophytes, and their IPS-S indexes values (according to OMNIDIA program).

Lake/study year	Taxa on cobbles	IPS-S index value	Taxa on macrophytes	IPS-S index value	Species no. on cobbles/macrophytes
Kaiavere (2014)	*Achnanthidium minutissimum s.l.*	4.6	*Achnanthidium minutissimum s.l.*	4.6	20/23
Lahepera (2014)	*Achnanthidium minutissimum s.l.*	4.6	*Fragilariforma mesolepta*	5	26/38
Lõõdla (2014)	*Staurosira leptostauron*	4	*Achnanthidium minutissimum s.l.*	4.6	34/35
Rõuge Suurjärv (2015)	*Navicula cryptotenella*	4	*Achnanthidium minutissimum s.l.*	4.6	36/32
Uljaste (2016)	*Sellaphora atomoides*	2.2	*Achnanthidium pusillum*	5	25/12
Lõõdla (2016)	*Sellaphora atomoides*	2.2	*Achnanthidium minutissimum s.l.*	4.6	29/19
Pühajärv (2016)	*Staurosira venter*	3.8	*Cocconeis pediculus*	4	33/14
Kuremaa (2016)	*Achnanthidium minutissimum s.l.*	4.6	*Achnanthidium minutissimum s.l.*	4.6	29/11
Tamula (2016)	*Epithemia sorex*	4	*Epithemia sorex*	4	36/21
Ähijärv (2016)	*Achnanthidium minutissimum s.l.*	4.6	*Achnanthidium minutissimum s.l.*	4.6	46/25
Tündre (2016)	*Achnanthidium minutissimum s.l.*	4.6	*Achnanthidium minutissimum s.l.*	4.6	48/21
Jõksi (2016)	*Achnanthidium minutissimum s.l.*	4.6	*Achnanthidium minutissimum s.l.*	4.6	32/8
Suurlaht (2016)	*Nitzschia palea*	1	*Achnanthidium minutissimum s.l.*	4.6	41/25
		Mean 3.75		Mean 4.57	

we observed *Epithemia sorex* to be the most abundant both in the epilithon and epiphyton assemblages and in Lake Uljaste, *Sellaphora atomoides* was the dominant taxon in the epilithon. Although *A. minutissimum s.l.* was prominent in most samples, some exceptions occurred, depending on substratum type. In Lakes Lahepera and Kaiavere *A. minutissimum s.l.* was dominant in the epilithon, but not in the epiphyton, whereas in Lakes Suurlaht, Tündre, Ähijärv, Kuremaa, Pühajärv, Lõõdla and Rõuge Suurjärv it was reversed.

The ecological status of small lakes according to the benthic diatoms showed differences, depending on sampled substratum. Epiphyton samples in general scored a higher ecological status class — nine studied lakes were high (in Lake Lõõdla both 2014 and 2016) and three were scored as good. In the epilithon samples, only three lakes scored high, six good (in Lake Lõõdla both 2014 and 2016) and three with moderate ecological status scores (Table 6).

In three lakes both epilithon and epiphyton samples showed the same ecological status (Kaiavere (high), Jõksi (high) and Tamula (good)). Lakes Kaiavere and Tamula belonged to type S2, whereas Lake Jõksi is type S3. When compared, hydrochemical parameters in the eutrophic lake Tamula had higher average O_2% (saturated), PO_4-P (0.025 mg L^{-1}) and TP (0.072 mg L^{-1}). Lake Kaiavere had the highest average conductivity (422 µS cm^{-1}), but lowest average O_2 (6.9 mg L^{-1}).

In six lakes (Lahepera, Lõõdla (both 2014 and 2016), Rõuge Suurjärv, Kuremaa, Ähijärv, Tündre) the ecological status results in epilithon and epiphython samples differed by only one status class (Table 6). All lakes, except for

Table 6. Ecological status class according to diatom indices and summarized ecological status class (all quality elements together); C- substratum type cobbles, M- substratum type macrophytes.

Lake	Type	Year	IPS	WAT	100-TDI	Ecological Status Class	Summarized Ecological Status Class[1]
Kaiavere (C)	S2	2014	17.1	15.4	60.8	High	Moderate
Kaiavere (M)	S2	2014	17.2	13.7	70.2	High	
Lahepera (C)	S2	2014	15.5	16.4	55.1	High	Moderate
Lahepera (M)	S2	2014	13.9	13.7	45.3	Good	
Lõõdla (C)	S3	2014	13.8	11.0	46.6	Good	Good
Lõõdla (M)	S3	2014	15.8	14.9	55.1	High	
Rõuge Suurjärv (C)	S3	2015	15.5	12.6	47.3	Good	Good
Rõuge Suurjärv (M)	S3	2015	16.8	16.3	62.8	High	
Uljaste (C)	S5	2016	12.8	6.3	53.1	Moderate	Moderate
Uljaste (M)	S5	2016	19.7	12.1	29.3	Good	
Lõõdla (C)	S3	2016	13.6	10.2	40.9	Good	Good
Lõõdla (M)	S3	2016	16.1	17.5	52.3	High	
Pühajärv (C)	S3	2016	15.0	11.3	37.2	Moderate	Good
Pühajärv (M)	S3	2016	16.0	18.2	47.1	High	
Kuremaa (C)	S3	2016	15.1	13.5	45.9	Good	Moderate
Kuremaa (M)	S3	2016	17.9	15.6	69.0	High	
Tamula (C)	S2	2016	14.4	10.2	42.7	Good	Moderate
Tamula (M)	S2	2016	15.4	11.2	50.4	Good	
Ähijärv (C)	S3	2016	15.6	12.3	56.8	Good	Moderate
Ähijärv (M)	S3	2016	16.9	17.2	65.4	High	
Tündre (C)	S3	2016	15.2	11.1	44.2	Good	Good
Tündre (M)	S3	2016	18.8	14.6	64.9	High	
Jõksi (C)	S3	2016	16.0	15.3	65.1	High	Good
Jõksi (M)	S3	2016	17.6	18.8	70.9	High	
Suurlaht (C)	S8	2016	10.2	8.4	35.7	Moderate	High
Suurlaht (M)	S8	2016	15.7	16.2	63.2	High	

[1] According to Ott 2006

Lahepera belonged to type S3, whereas Lahepera belongs to type S2 (Table 6). Lake type S3 is also the only lake type among small lakes, that shows constant stratification (Table 2). All above mentioned lakes had medium TP ($\geq$ 0.03 mg L^{-1}) and TN ($\geq$ 0.88 mg L^{-1}) values, above medium was only Lake Lõõdla in 2016, when TN was 1.4 mg L^{-1} (Ott 2016, unpubl. data).

Differences in lake ecological status (two classes apart) were observed in Lakes Suurlaht and Pühajärv, where epiphyton samples showed high and epilithon samples moderate lake ecological scores. In Lake Uljaste, the epilithon samples showed moderate ecological status, but the epiphyton samples indicated a good score (Table 6). When compared, hydrochemical parameters from Lakes Suurlaht and Pühajärv, showed similarities. Both lakes had higher pH (10.6 in Lake Suurlaht and 8.94 in Lake Pühajärv) and low NO_3-N, which in both lakes was 0.02 mg L^{-1}. On the other hand, TN in Lake Pühajärv was much lower (0.55 mg L^{-1}), than in lake Suurlaht (1.2 mg L^{-1}), whereas TP was higher in lake Pühajärv (0.027 mg L^{-1}) and lower in Lake Suurlaht (0.017 mg L^{-1}).

Lakes Pühajärv and Suurlaht also had different diatoms species compositions which also differed from other studied lakes by most abundant/dominant species. In Lake Suurlaht, the dominant taxon on cobbles was *Nitzschia palea* and on macrophytes *Achnanthidium minutissimum s.l.*, whereas in Lake Pühajärv the most abundant species on cobbles was *Staurosira venter s.l.* and on macrophytes *Cocconeis pediculus* (Table 5). The above-mentioned species IPS-S index values, for Lake Pühajärv was high IPS-S (four), whereas in Lake Suurlaht differences between prominent taxa were observed. *Nitzschia palea* has an IPS-S index value of one (tolerates eutrophication), while *Achnanthidium minutissimum* has an IPS-S index value of 4.6 (tolerates low eutrophication levels) (Table 5).

The IPS-S index results from all sampled lakes for the most abundant or dominant taxa had average index scores approximating 3.75 in the epilithon and 4.57 in the epiphyton (Table 5). This showed that the most abundant diatom taxa in the epilithon tolerates more eutrophic conditions, compared to taxa in the epiphyton. In four lakes (Tündre, Uljaste, Rõuge Suurjärv, Lõõdla (both 2014 and 2016)) the epilithon sample scores indicated the same ecological status as the lake's summarized ecological status (Table 6). Only in Lake Suurlaht was the relationship reversed. In the other lakes (except from Jõksi), both substrates indicated a higher ecological status, compared with the lake's summarized ecological status. In contrast, the substrates in Lake Jõksi indicated a lower ecological status, relative to the lake's summarized ecological class (Table 6).

Statistical analysis showed significantly important ($p < 0.05$) negative correlations occurred between IPS index values and DO and DO% in the epilithon (Table 7). Whereas in the epiphyton, IPS correlated negatively with pH and summer medium water temperature. The WAT index showed negative correlations with BOD_5 in the epiphyton, whereas no correlations with hydrochemical parameters occurred in the epilithon. TDI showed no statistical correlations with any of the measured hydrochemistry parameters. In the epilithon, all three species diversity indices (Pielou's, Simpson's and Shannon's) showed significantly negative correlations ($p < 0.05$) with BOD_5 and TP. Also, species diversity and evenness in the epilithon correlated negatively with BOD_5 and TP, whereas number of taxa correlated negatively only with BOD_5 (Table 7). In the epiphyton only number of taxa correlated positively with summer average water temperature, with no other statistically important correlations observed.

Discussion

Benthic diatoms assemblages in lakes are determined by light conditions, climate, grazing, and hydrochemistry (Björk-Ramberg 1984, Lowe and Hunter 1988, Lalonde and Downing 1991, Kahlert 2001, Cejudo-Figueiras *et al.* 2010, Holomuzki *et al.* 2010, Cattaneo *et al.* 2011). In more eutrophic lakes the impacts and differences between diatom communities on different substrates are less evident (Wetzel 1983, Sand-Jensen and Borum 1991, Vadeboncoeur and Steinman 2002). Our results confirm the proposed hypothesis: in eutrophic Lakes Kaiavere and Tamula, there are no differences

Table 7. Spearman correlation ($p < 0.05$) between diatom indices and environmental variables on two substratum types (cobbles and macrophytes) in Estonian small lakes between 2014 and 2016; ns — not significant.

Substrate	Index	pH	Water temp.	O_2	O_2%	Conductivity	$NH_4^+ – N$	BOD_5	$PO_4^{3-} – P$	$NO_3^- – N$	TP	TN
Cobbles	IPS	ns	–0.54	–0.56	ns	ns	ns	ns	ns	ns	ns	ns
	WAT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	TDI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Number of taxa	ns	ns	ns	ns	ns	ns	–0.60	ns	ns	ns	ns
	Evenness	ns	ns	ns	ns	ns	ns	–0.57	ns	ns	–0.56	ns
	Shannon	ns	ns	ns	ns	ns	ns	–0.64	ns	ns	–0.58	ns
	Simpson	ns	ns	ns	ns	ns	ns	–0.56	ns	ns	–0.55	ns
	Pielou	ns	ns	ns	ns	ns	ns	–0.61	ns	ns	–0.59	ns
Macrophytes	IPS	–0.72	ns	ns	ns	ns	ns	ns	ns	–0.57	ns	ns
	WAT	ns	ns	ns	ns	ns	ns	–0.58	ns	ns	ns	ns
	TDI	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Number of taxa	0.53	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Evenness	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Shannon	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Simpson	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Pielou	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

in sample substratum ecological status results. Only minor ecological status differences (one status class apart) are evident in S3 type lakes, that are mesotrophic: Tündre, Ähijärv, Kuremaa, Lõõdla, Rõuge Suurjärv and as S2 mesotrophic Lake Lahepera. In contrast, Lake Uljaste (oligotrophic) had large differences in assessments between the epilithon and the epiphyton (Table 6).

Johnson *et al.* (2006) argued that lakes with a higher ecological status, when monitored for biological quality elements across substrata, should score a consistent unified ecological status. Whereas in lakes with higher anthropogenic impacts, larger differences between BQEs scores are evident. Our results did not show this relationship; in lakes with lower (i.e. Lake Ähijärv) and higher anthropogenic stress (i.e. Lake Tamula), the epiphyton and epilithon samples show a higher ecological status compared with the lakes overall ecological status evaluation (Table 6). Kolada *et al.* (2016) found that in general, lentic diatoms in lakes tend to give higher ecological status results, compared to other BQEs. Johnson *et al.* (2006) also argues that lentic diatom associations in lakes tend to be less precise in producing relevant lake ecological status assessments, compared to other biological metrics. Our results suggest also that in addition to anthropogenic stress, other factors including land use could play a significant role in impacting ecological status calculations according to benthic diatoms (Campos *et al.* 2021, Kennedy and Buckley 2021).

Bennion *et al.* (2014) show, that *Achnanthidium minutissimum s.l.* is commonly observed and associated with sensitive lentic diatom communities in both lakes and rivers. Our results confirm the same regularity, whereas *Achnanthium minutissimum s.l.* was dominant or most abundant in 10 of 12 studied lakes. According to many authors (e.g. Round *et al.* 1990, Johanson *et al.* 1997, Poulíčková *et al.* 2004), *A. minutissimum s.l.* is one of the first species, that starts forming a biofilm on macrophytes and cobbles both in lakes and rivers.

Biggs *et al.* (1998) found lentic diatom communities dominated by *Achnanthidium minutissimum s.l.* often indicate some (anthropogenic) disturbance or stress through grazing. In contrast, Hoagland (1982) suggest benthic diatom communities, where only small, rapidly growing species are present, represent the first stadium of so-called microsuccession. They argue that if the light intensity is high, while there are enough nutrients to consume, this stage of benthic diatoms community development will last for long periods. King *et al.* (2006) also pointed out multiple stages of "microsuccession" and agree, that in the first stage there's mostly small size and rapidly growing species (*r*-strategists). On the other hand, if the population density is high, larger species with selective niche requirements (*K*-strategists) will have advantage and develop (King *et al.* 2006).

Sellaphora atomoides was most abundant in epilithon of Lake Uljaste, where oligotrophic conditions are mainly present (Ott 2016, unpubl. data). The documented autecology of *S. atomoides* shows a preference for "pristine" conditions (Wetzel *et al.* 2015). However, reports of *S. atomoides sensu lato* today are widespread and found in waterbodies with anthropogenic impacts (Wetzel *et al.* 2015). The taxonomy status of *S. atomoides* needs to be verified in Lake Uljaste although some local anthropogenic impacts from public beaches have been documented (Ott 2016, unpubl. data).

Many authors (Schönfelder *et al.* 2002, Poulíčková *et al.* 2004, Leira *et al.* 2009) have argued that benthic diatom assemblage diversity does not reflect the overall ecological (trophic) state of a lake. In contrast, DeNicola and Kelly (2014) said, that in general, high periphyton diversity should indicate low levels of anthropogenic stress. However, the same authors also note that correlating species richness and other biodiversity indices values with anthropogenic stress indicators is difficult. Our results indicate the selected species diversity indices (Pielou, Simpson, Shannon) show higher scores for epilithon samples. In these samples, the calculated species diversity indices also correlate with BOD_5 and TP, whereas there is no statistically important connection between species diversity indices and measured hydrochemical parameters in the epiphyton. We conclude that epilithon samples tend to have (although loose) stronger connections with actual lentic hydrochemical conditions and hence a better ecological status scoring. Our lakes ecological status results also show slightly higher scores in the epiphyton samples. Those conclusions also agree with McCormic *et al.* (2019), that on soft substrates, diatoms growth may not be limited by nutrients, whereas samples collected from hard surfaces (i.e. cobbles) are affected by nutrient enrichment (Blumenshine *et al.* 1997, Vadeboncoeur *et al.* 2001, Nydick, *et al.* 2004).

Our results show that in most study lakes, IPS scores did not display any differences between substratum types and results weren't affected by the lakes overall ecological condition (Table 6). In contrast, the WAT index shows bigger differences depending on substratum type (Table 6). IPS has more correlations with lake's hydrochemical parameters in both the epilithon and the epiphyton, whereas WAT correlates with only one (BOD_5) hydrochemical parameter and only in the epiphyton samples (Table 7). The IPS index was made for use in lotic systems (Cemagref 1982), thus Bennion *et al.* (2014) developed a new index for ecological status assessment in lakes (LTDI, based on TDI index) according to benthic diatom assemblages. They showed samples collected from stones tend to have slightly higher LTDI scores, than samples collected from macrophytes. Since the mean difference was not significant, substratum type was hence not considered in model development. Our results in general confirm the same similarity with IPS index results (Table 6). Winter and Duthie (2000) have shown that in stream epilithon samples, even when pooled, the assemblages are still showing only local environmental conditions around the sampling place. Whereas samples collected from cobbles show environmental conditions representative of the whole waterbody. Kahlert and Gottschalk (2014) agree and show, that moving water over biofilms, attached to cobbles or macrophytes, may reflect more general environmental conditions compared to planktic lake communities. On the other hand, both in lentic and lotic systems, local anthropogenic pressures are well shown by phytobenthos assemblages with short response times, compared to macrophytes (Schneider *et al.* 2012). Many authors (Rothfritz *et al.* 1997, Kelly 2002, Lavoie *et al.* 2006, King *et al.* 2006) have also shown that diatom indices are relatively robust and therefore should reflect current conditions in a water body, despite spatial or temporal variation in the benthic diatom assemblage. We can therefore generalize, that although IPS is showing stronger connections with lake hydrochemical parameters, WAT should be used in Estonian small lakes for ecological quality assessments as the main benthic diatom index. More studies are needed to collaborate the current findings, since our dataset was small (only 12 sampled lakes) and didn't compare temporal variability (different year's) from the same lake.

Higher plants are considered "active substrates" and macrophyte species can strongly affect the benthic diatoms assemblage, especially in the community development stage (Rothfritz *et al.* 1997, Kelly 2002, Lavoie *et al.* 2006, King *et al.* 2006). Poulíčková *et al.* (2004) show younger plant parts with lower benthic diatom's biomasses and these communities are dominated by small-sized diatoms (i.e. *Achnanthidium minutissimum s.l.*). On older plant parts, the biomass of benthic diatoms is higher, since more nutrients are leaking and plants are covered by nutrient-high layers (polysaccharides), colonized by bacteria and rapidly growing benthic diatom species (King *et al.* 2006, Kelly *et al.* 2009). This age effect is more considerable in lakes with low nutrient content, where plants can be populated by benthic diatom species, which prefer higher nutrient conditions (i.e. *Nitzschia* spp.) (Lalonde and Downing 1991, Kahlert 2001, Cejudo-Figueiras *et al.* 2010). In lakes with higher nutrients content, the influence of nutrient leakage from vascular plants is low, compared to water column chemistry and therefore its effect on benthic diatom composition is marginal (Kahlert 2001, Cejudo-Figueiras *et al.* 2010). Our study results do not completely support this hypothesis (Table 5). The IPS-S index was used to compare differences in benthic diatoms assemblages reflecting taxon specific tolerances to anthropogenic stress and higher eutrophication levels (Kahlert and Rašić 2015). Dominant or most abundant benthic diatom species from the epilithon showed generally lower IPS-S values compared to the epiphython (Table 5). Hence, we can conclude epilithon assemblages are reflecting actual lake ecological condition, whereas assemblages from the epiphyton have a loose connection to their actual ecological condition.

Conclusions

Our results confirm the proposed hypothesis: in eutrophic Lakes Kaiavere and Tamula, there was no differences in sampled substratum ecological status results. Only minor ecological status differences (one status class apart) were evident in S3 type lakes, that were mesotrophic: Tündre, Ähijärv, Kuremaa, Lõõdla, Rõuge Suurjärv and as S2 mesotrophic Lake Lahepera. We suggest using WAT as the main diatom index in Estonian small lakes ecological status assessment. Since anthropogenic stress didn't show a clear connection with studied lakes benthic diatoms assemblages, we suggest that other factors, including lakes catchment's land use and soil types, are affecting benthic diatom's assemblages in Estonian small lakes. Further studies are needed to verify this hypothesis.

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References

Barnese L.E. & Lowe R.L. 1992. Effects of substrate, light, and benthic invertebrates on algal drift in small streams. *Joirnal of the North American Benthological Society* 11: 49–59.

Bennion H., Kelly M.G., Juggins S., Yallop M.L., Burgess A., Jamieson J. & Krokowski J. 2014. Assessment of ecological status in UK lakes using benthic diatoms. *Freshwater Science* 33: 639–654.

Biggs B.J.F. 1989. Biomonitoring of organic pollution using periphyton, South Branch, Canterbury, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 23: 263–274.

Biggs B.J.F, Stevenson R. J. & Lowe R.L. 1998. A habitat matrix conceptual model for stream periphyton. *Archif für Hydrobiologie* 143: 21–56.

Björk-Ramberg S. 1984. Production of epipelic algae before and during lake fertilization in a subarctic lake. *Holarctic Ecology* 6: 349–355.

Blumenshine S.C., Vadeboncoeur Y. & Lodge D.M. 1997. Benthic-Pelagic Links: Responses of Benthos to Water-Column Nutrient Enrichment. *Journal of the North American Benthological Society* 16: 466–479.

Bolla B., Borics G., Kiss K.T., Reskóné N.M., Várbíró G. & Ács É. 2010. Recommendations for ecological status assessment of Lake Balaton (largest shallow lake of Central Europe), based on benthic diatom communities. *Vie et Milieu - Life and Environment* 60(3): 197–208.

Campos C. A., Kennard M. J. & Gonçalves Jr J. F. 2021. Diatom and macroinvertebrate assemblages to inform management of Brazilian savanna's watersheds. *Ecological Indicators* 128: 107834, DOI: https://doi.org/10.1016/j.ecolind.2021.107834.

Cattaneo A., Couillard Y. & Fortin C. 2011. Littoral diatoms as indicators of recent water and sediment contamination by metals in lakes. *Journal of Environmental Monitoring* 3: 572–582.

Cejudo-Figueiras C., Álvarez-Blanco I., Bécares E. & Blanco S. 2010. Epiphytic diatoms and water quality in shallow lakes: the neutral substrate hypothesis revisited. *Marine and Freshwater Research* 61: 1457–1467.

Cellamare M., Morin S., Coste M. & Haury J. 2012. Ecological assessment of French Atlantic lakes based on phytoplankton, phytobenthos and macrophytes. *Environmental Monitoring and Assessment* 184: 4685–4708.

Cemagref, 1982. *Etude des méthodes biologiques quantitatives d'appréciation de la qualité des eaux*. Rapport Division Qualité des Eaux Lyon — Agence de l'Eau Rhône — Méditerranée — Corse, Pierre — Bénite.

Cox E.J. 1988. Taxonomic studies on the diatom genus *Navicula* V. The establishment of *Paribellus* gen. nov. for some members of *Navicula* sect. *Microstigmaticae. Diatom Research* 3: 9–38.

Crossetti L.O., Stenger- Kovács C. & Padisák J. 2013. Coherence of phytoplankton and attached diatom-based ecological status assessment in Lake Balaton. *Hydrobiologia* 716: 87–101.

Dela-Cruz J., Pritchard T., Geoffrey G. & Ajani P. 2006. The use of periphytic diatoms as a means of assessing impacts of point source inorganic nutrient pollution in south-eastern Australia. *Freshwater Biology* 51: 951–972.

DeNicola D. M. & Kelly M. G. 2014. Role of periphyton in ecological assessment of lakes. *Freshwater Science* 33: 619–638.

Estonian Water Act 1994. Riigi Teataja (22.02.2019, 1) (available at: https://www.riigiteataja.ee/en/eli/ee/Riigikogu/act/530062022002/consolide)

Guiry, M.D. & Guiry, G.M. 2023. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. (available at: https://www.algaebase.org; searched on May 22, 2023)

Hajnal É., Stenger–Kovács C., Ács É., & Padisák J. 2009.

DILSTORE software for ecological status assessment of lakes based on benthic diatoms. *Fottea* 9(2): 351–354.

Håkanson L. & Boulion V.V. 2004. Empirical and dynamical models of production and biomass of benthic algae in lakes. *Hydrobiologia* 522: 75–97

Hoagland K.D. 1982. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae) *American Journal of Botany* 69: 188–213.

Holomuzki J.R., Feminella J.W. & Power M.E. 2010. Biotic interactions in freshwater benthic habitats. *Journal of the North American Benthological Society* 29: 220–244.

Hustedt F. 1985. *The Pennate Diatoms* (a translation of Hustedt`s "*Die Kieselalgen, 2. Teil*") N.G. Koeltz Scientific Books, Koenigstein.

Johnson R.K., Hering D., Furse M.T. & Clarke R.T. 2006. Detection of ecological change using multiple organism groups: metrics and uncertainty. *Hydrobiologia* 566: 115–137.

Jones J.G. 1974. A method for observation and enumeration of epilithic algae directly on the surface of stones. *Oecologia* 16: 1–8.

Kahlert, M. 2001. *Biomass and nutrient status of benthic algae in lakes*. *Acta Universitatis Upsaliensis*. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology, PhD Thesis.

Kahlert M. & Gottschalk S. 2014. Differences in benthic diatom assemblages between streams and lakes in Sweden and implications for ecological assessment. *Freshwater Science* 33: 655–669.

Kahlert, M. & Rašić I. 2015. Similar small-scale variation of diatom assemblages on different substrates in a mesotrophic stream. *Acta Botanica Croatica*. 74: 363–376.

Kahlert M., Ács É., Almeida S.F.P., Blanco S., Dreßler M., Ector L., Karjalainen S.M., Liess A., Mertens A., van der Wal J., Vilbaste S. & Werner P. 2016. Quality assurance of diatom counts in Europe: towards harmonized datasets. *Hydrobiologia* 772: 1–14.

Kahlert M., Kelly M.G., Albert R.-L., Almeida S.F.P., Bešta T., Blanco S., Coste M., Denys L., Ector L., Fránková M., Hlúbiková D., Ivanov P., Kennedy B., Marvan P., Mertens A., Miettinen J., Picinska-Falłtynowicz J., Rosebery J., Tornés E., Vilbaste S. & Vogel A. 2012. Identification versus counting protocols as sources of uncertainty in diatom-based ecological status assessments. *Hydrobiologia* 695: 109–124.

Kahlert M., Albert R.-L., Antilla E.-L., Bengtsson R., Bigler C., Eskola T., Gälman V., Gottschalk S., Herlitz E., Jarl-man A., Kasperoviciene J., Kokociński M., Luup H., Miettinen J., Paunksnyte I., Piirsoo K., Quintana I., Raunio J., Sandell B., Simola H., Sundberg I., Vilbaste S. & Weckström J. 2009. Harmonization is more important than experience – results of the first Nordic-Baltic diatom intercalibration exercise 2007 (stream monitoring). *Journal of Applied Phycology* 21: 471–482.

Kelly, M.G., 2002. Role of benthic diatoms in the implementation of the Urban Wastewater Treatment Directive in the River Wear, North–East England. *Journal of Applied Phycology* 14: 9–18.

Kelly M.G. & Whitton B.A., 1995. A new diatom index for monitoring eutrophication in rivers. *Journal of Applied Phycology* 7: 433–444

Kelly M.G., Krokowski J. & Harding J.P.C. 2016. RAPPER: A new method for rapid assessment of macroalgae as a complement to diatom-based assessments of ecological status. *Science of The Total Environment* 568: 536–545.

Kelly M.G., King L. & Bernadette W. 2009. The conceptual basis of ecological status assessments using diatoms. *Biology and Environment-proceedings of The Royal Irish Academy - Biology Environment* 109: 175–189.

Kelly M.G., Trobajo R., Rovira L. & Mann D.G. 2015. Characterizing the niches of two very similar *Nitzschia* species and implications for ecological assessment. *Diatom Research* 30: 27–33.

Kelly M.G., Chiriac G., Soare-Minea A., Hamchevici C. & Juggins S. 2019. Use of phytobenthos to evaluate ecological status in lowland Romanian lakes. *Limnologica* 77: 125682, DOI: https://doi.org/10.1016/j.limno.2019.125682

Kelly M.G., Gómez-Rodríguez C., Kahlert M., Almeida S.F.P., Bennett C., Bottin M., Delmas F., Descy J.-P., Dörflinger G., Kennedy B., Marvan P., Opatrilova L., Pardo I., Pfister P., Rosebery J., Schneider S. & Vilbaste S. 2012. Establishing expectations for pan-European diatom based ecological status assessments. *Ecological Indicators* 20: 177–186.

Kennedy B. & Buckley Y. M. 2021. Use of seasonal epilithic diatom assemblages to evaluate ecological status in Irish lakes. *Ecological Indicators* 129: 107853, DOI: https://doi.org/10.1016/j.ecolind.2021.107853.

King L., Clarke G., Bennion H., Kelly M.G. & Yallop M. 2006. Recommendations for sampling littoral diatoms in lakes for ecological status assessments. *Journal Applied Phycology* 18: 15–25.

Kolada A., Pasztaleniec A., Bielczyńska A. & Soszka H. 2016. Phytoplankton, macrophytes and benthic diatoms in lake classification: Consistent, congruent, redundant? Lessons learnt from WFD-compliant monitoring in Poland. *Limnologica - Ecology and Management of Inland Waters* 59: 44–52.

Krammer K.1997a. *Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 1. Allgemeines und Encyonema part*. Bibliotheca Diatomologica Band 36. J. Cramer, Stuttgart.

Krammer K.1997b. *Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 2. Encyonema part., Encyonopsis und Cymbellopsis.* Bibliotheca Diatomologica Band 37. J. Cramer, Stuttgart.

Krammer K. & Lange-Bertalot H. 1986-1991. *Bacillariophyceae. Teil 1-4. Süßwasserflora von Mitteleuropa.* VEB Gustav Fischer Verlag, Jena, Stuttgard, New York.

Krejci M.E. & Lowe R.L. 1986. Importance of sand grain mineralogy and topography in determining micro-spatial distribution of epipsammic diatoms. *Journal of the North American Benthological Society* 5: 211–220.

Laarmaa R., Ott I., Timm H., Maileht K., Sepp M., Mäemets H., Palm A., Krause T. & Saar K. 2019. *Eesti järved [Estonian lakes]*. Varrak, Tallinn [In Estonian]

Lalonde S. & Downing J.A. 1991. Epiphyton biomass is related to lake trophic status, depth and macrophyte architecture. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 2285–2291.

Lange-Bertalot H. 2001. *Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 2. Navicula sensu stricto. 10 Genera Separated from Navicula sensu lato. Frustulia.* A.R.G. Gantner Verlag K.G, Ruggell.

Lange-Bertalot H., Bak M. & Witkowski A. 2011. *Diatoms of Europe. Vol.6. Eunotia and some related genera.* A.R.G. Gantner Verlag K.G, Ruggell.

Lavoie I., Campeau S., Fallu, M.-A. & Peter D. 2006. Diatoms and biomonitoring: Should cell size be accounted for? *Hydrobiologia* 573: 1–16.

Leira M., Chen G., Dalton C., Irvine K. &Taylor D. 2009. Patterns in freshwater diatom taxonomic distinctness along an eutrophication gradient. *Freshwater Biology* 54: 1–14.

Lowe R.L. & Hunter R.D. 1988. Effect of grazing by *Physa integra* on periphyton community structure. *Journal of the North American Benthological Society* 7: 29–36.

Messyasz B. & Treska E. 2019. Benthic diatoms as valuable indicators of anthropogenic eutrophication in biomonitoring of Ribbon Lake. *Ecological Chemistry and Engineering S*. 26(4): 709–726.

Naumoski A. & Mitreski K. 2010. Classifying diatoms into trophic state index classes with novel classification algorithm. *Procedia Environmental Sciences* 2: 1124–1138.

Novais M.H., Blanco S., Delgado C., Morais M., Hoffmann L. & Ector L. 2012. Ecological assessment of Portuguese reservoirs based on littoral epilithic diatoms. *Hydrobiologia* 695: 265–279.

Nydick K.R., Lafrancois B.M., Baron J.S. & Johnson B.M. 2004. Nitrogen regulation of algal biomass, productivity, and composition in shallow mountain lakes, Snowy Range, Wyoming, USA. *Canadian Journal of Fisheries and Aquatic Sciences* 61: 1256–1268.

Ott I. 2006. Some principles of ecological quality classification in Estonian lakes. In: H. de Wit, & B. L. Skjelkvale (Eds.). Proceedings of the 21th meeting of the ICP waters programme Task Force in Tallinn, Estonia, October 17-19. 2005 (8−14). Norwegian University of Science and Technology.

Ott I. & Kõiv T. 1999. *Eesti väikejärvede eripära ja muutused. Estonian small lakes: Special features and changes.* EV Keskkonnaministeeriumi Info- ja Tehnokeskus, Eesti Teaduste Akadeemia, Eesti Põllumajandusülikooli Zooloogia ja Botaanika Instituut, Tallinn. [In Estonian]

Ouyang L., Pan Y., Huang C., Tang Y., Du J., & Xiao W. 2016. Water quality assessment of benthic diatom communities for water quality in the subalpine karstic lakes of Jiuzhaigou, a world heritage site in China. *Journal of Mountain Science* 13: 1632–1644.

Passy S.I. 2007. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany* 86: 171–178.

Pielou E. 1966. The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* 13: 131–144.

Poikane S., Birk S., Böhmer J., Carvalho L., de Hoyos C., Gassner H., Hellsten S., Kelly M.G., Solheim A.L., Olin M., Pall K., Phillips G., Portielje R., Ritterbusch D., Sandin L., Schartau A.-K., Solimini A.G., van den Berg M., Wolfram G. & van de Bund W. 2015. A hitchhiker's guide to European lake ecological assessment and intercalibration. *Ecological Indicators* 52: 533–544.

Potapova M. & Charles D.F. 2005. Choice of substrate in algae-based water-quality assessment. *Journal of the North American Benthological Society* 24: 415–427.

Poulíčková A., Duchoslav M. & Dokulil M. 2004. Littoral diatom assemblages as bioindicators of lake trophic status: A case study from perialpine lakes in Austria. *European Journal of Phycology* 39: 143–152.

Riato L. & Leira M. 2020. Heterogeneity of epiphytic diatoms in shallow lakes: Implications for lake monitoring. *Ecological Indicators* 111: 105988, DOI: doi.org/10.1016/j.ecolind.2019.105988

Rimet F., Bouchez A. & Tapolczai K. 2016. Spatial heterogeneity of littoral benthic diatoms in a large lake: monitoring implications. *Hydrobiologia* 771: 179–193.

Rivera S.F., Vasselon V., Jacquet S., Bouchez A., Ariztegui D. & Rimet F. 2018. Metabarcoding of lake benthic diatoms: from structure assemblages to ecological assessment. *Hydrobiologia* 807: 37–51.

Rothfritz H., Juttner I., Suren A.M. & Ormerod S.J. 1997. Epiphytic and epilithic diatom communities along environmental gradients in the Nepalese Himalaya: Implications for the assessment of biodiversity and water quality. *Archiv für Hydrobiologie* 138: 465–482.

Round F.E., Crawford R.M. & Mann D.G. 1990. *The Diatoms. Biology and Morphology of the Genera*. Cambridge University Press, Cambridge.

Sand-Jensen K. & Borum J. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. *Aquatic Botany* 41: 137–175.

Schneider S.C., Lawniczak A.E., Picińska-Faltynowicz J. & Szoszkiewicz K. 2012. Do macrophytes, diatoms and non-diatom benthic algae give redundant information? Results from a case study in Poland. *Limnologica* 42: 204–211.

Schneider S.C., Cara M., Eriksen T.E., Budzakoska Goreska B., Imeri A., Kupe L., Lokoska T., Patceva S., Trajanovska S., Trajanovski S., Talevska M. & Veljanoska Sarafiloska, E. 2014. Eutrophication impacts littoral biota in Lake Ohrid while water phosphorus concentrations are low. *Limnologica* 44: 90–97.

Schönfelder I., Gelbrecht J., Schönfelder J. & Steinberg C. 2002. Relationships between littoral diatoms and their chemical environment in Northeastern German lakes and rivers. *Journal of Phycology* 38: 66–89.

Sgro G.V., Ketterer M.E. & Johansen J.R. 2006. Ecology and assessment of the benthic diatom communities of four Lake Erie estuaries using Lange-Bertalot tolerance values. *Hydrobiologia* 561: 239–249.

Shannon C.E. & Weaver W. 1949. *The Mathematical Theory of Communication*. Urbana, University of Illinois Press.

Simpson E.H. 1949. Measurement of Diversity. *Nature* 163: 688.

SIST EN 13946:2014 - Water quality - Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes. European Standards.

Sivaci R.E., Barinova S., Solak C.N. & Çobanoglu K. 2013. Ecological assessment of Great Lota Lake (Turkey) on the base of diatom communities. *African Journal of Biotechnology* 12: 453–464.

Snell M.A. & Irvine K.A. 2013. Importance of scalar and riparian habitat effects for assessment of ecological status using littoral diatoms. *Ecological Indicators* 25: 149–155.

Stenger-Kovács C., Buczkó K., Hajnal É. & Padisák J. 2007. Epiphytic, littoral diatoms as bioindicators of shallow lake trophic status: Trophic Diatom Index for Lakes (TDIL) developed in Hungary. *Hydrobiologia* 589:141–154.

Stenger-Kovács C., Körmendi K., Lengyel E., Abonyi A., Hajnal É., Szabó B., Buczkó K. & Padisák J. 2018. Expanding the trait-based concept of benthic diatoms: Development of traitand species-based indices for conductivity as the master variable of ecological status in continental saline lakes. *Ecological Indicators* 95: 63–74.

Stevenson R.J. 1997. Scale-dependent determinants and consequences of benthic algal heterogeneity. *Journal of the North American Benthological Society* 16: 248–262.

Suvacu E.R., Ēankaya E., Kulunē S. & Dere Ș. 2008. Seasonal assessment of epiphytic diatom distribution and diversity in relation to environmental factors in a karstic lake (Central Turkey). *Nova Hedwigia* 86: 215–230.

Timm H. & Vilbaste S. 2010. Pinnavete ökoloogilise seisundi hindamise metoodika bioloogiliste kvaliteedielementide alusel. Bentiliste ränivetikate kooslus jões. Suurselgrootute põhjaloomade kooslus jões ja järves. Lepingu 4 – 1.1/166 aruanne. [In Estonian]

Vadeboncoeur Y. & Steinman A.D. 2002. Periphyton function in lake ecosystems. *The Scientific World Journal* 2: 1449–1468.

Vadeboncoeur Y., Lodge D.M. & Carpenter S.R. 2001. Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. *Ecology* 82: 1065–1077.

Vilbaste S. 2004. Application of diatom indices in the evaluation of the water quality in Estonian streams. Proceedings of the Estonian Academy of Sciences. *Biology, Ecology* 53: 37–55.

Vilmi A., Karjalainen S.M., Landeiro V.L. & Heino J. 2015. Freshwater diatoms as environmental indicators: evaluating the effects of eutrophication using species morphology and biological indices. *Environmental Monitoring and Assessment* 187: 243, DOI: 10.1007/s10661-015-4485-7.

Vilmi A. Tolonen K.T., Karjalainen S.M. & Heino J. 2019. Niche position drives interspecific variation in occupancy and abundance in a highly-connected lake system. *Ecological Indicators* 99: 159–166.

Watanabe T., Asai K. & Houki A. 1986. Numerical estimation of organic pollution of flowing waters by using the epilithic diatom assemblage – Diatom Assemblage Index (DIApo). *Science of The Total Environment* 55: 209–218.

Water Framework Directive: European Commission. 2000. Directive 2000/60/EC of the European Parliament and the Council establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities: European Union*

Wetzel R. [Ed.] 1983. *Periphyton of freshwater ecosystems*. Develop. Hydrobiol. 17. Dr. W. Junk BY Publ., The Hague.

Wetzel C.E., Ector L., Van de Vijver B., Compère P. & Mann D.G. 2015. Morphology, typification and critical analysis of some ecologically important small naviculoid species (Bacillariophyta). *Fottea, Olomouc* 15: 203–234.

Winter J. & Duthie H.C. 2000. Epilithic diatoms as indicators of stream total N and total P concentration. *Journal of the North American Benthological Society* 19: 32–49.