

# Heterogeneity of epiphytic diatoms in shallow lakes: implications for lake monitoring

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## Highlights

- Epiphytic diatoms were sampled from the littoral and pelagic zone for one year.
- Littoral diatoms and littoral physico-chemistry showed high temporal variability.
- Pelagic diatoms correlated better with physico-chemistry than littoral diatoms.
- Pelagic diatoms had relatively low variability and may be more efficient bioindicators.

## Abstract

Current diatom assessments of shallow lakes typically use littoral epiphytic diatoms as bioindicators for eutrophication pressure. However, challenges in establishing robust ecological assessments still exist because of the natural pressures that shallow lakes are subjected to which are difficult to quantify (e.g., water-level fluctuations or wave action).

Information on the spatial and temporal variability of epiphytic communities within a shallow lake is limited although important for the development of robust bioassessment protocols. We compared epiphytic diatom taxa and functional group compositions between the littoral and pelagic zone of three least-disturbed shallow lakes in the northeastern part of South Africa along a temporal gradient. In comparison to the pelagic zone, the littoral zone was

characterized by almost double in species richness. Temporal variability was significantly higher for littoral diatom communities and littoral water physico-chemistry than those of the pelagic. Overall, water depth was a good predictor of the epiphytic diatom taxa and functional group composition. We also found that pelagic diatom taxa and functional groups correlated better with pelagic physico-chemistry than littoral diatoms to littoral physico-chemistry. This could be attributed to un-modeled factors related to natural physical pressures that will have more of an impact on littoral habitats than less exposed pelagic habitats. Given these findings, using pelagic epiphytic diatoms as a bioindicator in shallow lake monitoring programs could be more efficient than littoral diatoms at providing robust ecological assessments due to their relatively low variability.

Keywords: Algae, Functional group, Littoral zone, Pelagic, Temporal variability, Bioassessment, South Africa

## **1. Introduction**

In Europe and the US, diatoms are widely used for routine assessments of freshwater lake condition in compliance with water legislation statutory requirements, such as the EU Water Framework Directive (WFD) and US Environmental Monitoring and Assessment Program - Surface Waters (EMAP-SW). Efforts to develop diatom lake assessment tools have focused on large, deep lakes, but in the last two decades smaller, shallower lakes have received increasing research attention (Moss et al., 2003; Pouličková et al., 2004; Quintana et al., 2015). Shallow lakes are the most abundant lentic habitat in the world and are also among the most threatened ecosystems (Downing et al., 2006). The cumulative loss of shallow waterbodies from increasing land transformation and environmental change can alter the

natural hydrological and chemical processes that occur in catchments, with a significant impact on biodiversity (Leibowitz, 2003), and natural ecosystem function (Riley et al., 2018).

According to the WFD, the ecological status of waterbodies must be assessed using standardised bioassessment methods. A number of diatom-based indices for use in deep lake monitoring programmes have led to the development of standard assessment methodologies (King et al., 2006). For shallow lakes, however, difficulties still exist in the development of bioassessment methods. Shallow lakes are subjected to multiple stressors, both natural and anthropogenic, and extracting anthropogenic impacts from natural sources of variation (e.g. eutrophication vs water-level fluctuations) is a common challenge in establishing reliable bioassessment programmes (Bruce et al., 2013). The need for a standard assessment protocol for shallow lakes is especially important in regions rich in shallow lentic waterbodies such as the Mediterranean (Poikane et al., 2015), or further afield in the Mpumalanga Province of South Africa, where increasing mining development and intensive agricultural practices has contributed to substantial loss and degradation of shallow waterbodies (Riatio et al., 2018).

Many diatom studies in shallow lakes have focused on inter-lake comparisons of epiphytic diatom communities (attached to plants) sampled from the littoral zone (e.g., Blanco et al., 2004; Kitner and Poulícková, 2003; Stenger-Kovács et al., 2007), since epiphytic habitats represent a significant component of shallow lakes, and the littoral zone is a major source of total lake primary productivity (Gaiser et al., 2006; Wetzel and Allen, 1972). Most shallow lakes have a sizeable littoral zone compared to the pelagic area (Wetzel, 2001), in which sections of the littoral zone support aquatic macrophytes, resulting in a range of patchily distributed habitats, and subsequently, large habitat complexity (Trigal et al., 2006). In contrast, the pelagic zone harbors less macrophytes and therefore, is structurally less complex (Rennie and Jackson, 2005). Moreover, the littoral zone, particularly in temporarily inundated lentic systems, may be subjected to strong hydrological variations

which can directly affect sedimentation, resuspension, and biogeochemical dynamics (Niemistö et al., 2008). Unlike the deeper pelagic zone, which provides more stable physico-chemical conditions (Cantonati and Lowe, 2014). The natural variability in physical and chemical conditions within different areas of the lake may cause great spatial variations in the diatom species composition (Cantonati et al., 2014; Stevenson et al., 2013). Thus, understanding the natural variation of the diatom communities is necessary for developing reliable assessment methodologies for shallow lakes since it may mask the effects caused by anthropogenic disturbances. The influence of natural variation, both temporal and spatial, on diatom community structure within a shallow lake is, however, understudied.

Research on spatial distributions and temporal patterns of epiphytic diatom communities in shallow lentic waterbodies have focused more on the effects of wetland hydroperiod on diatom assemblages across the Florida Everglades (e.g., Gottlieb et al., 2006; Lee et al., 2013). Intra-lake variability of epiphytic diatom communities, in the context of biomonitoring shallow lakes, is yet to be examined.

The objective of this study is to compare epiphytic diatom communities between the littoral zone and the pelagic zone of three least-disturbed shallow lakes. We assessed monthly variation in diatom taxon and functional group abundances within each zone of each lake. Shallow lake studies typically sample one site in the littoral zone (e.g., Kelly et al., 2007). Assuming the littoral zone has large habitat complexity, we predicted this would induce heterogeneity into littoral diatom communities. For this reason, we sampled four sites within the littoral zone of each lake for each sampling event.

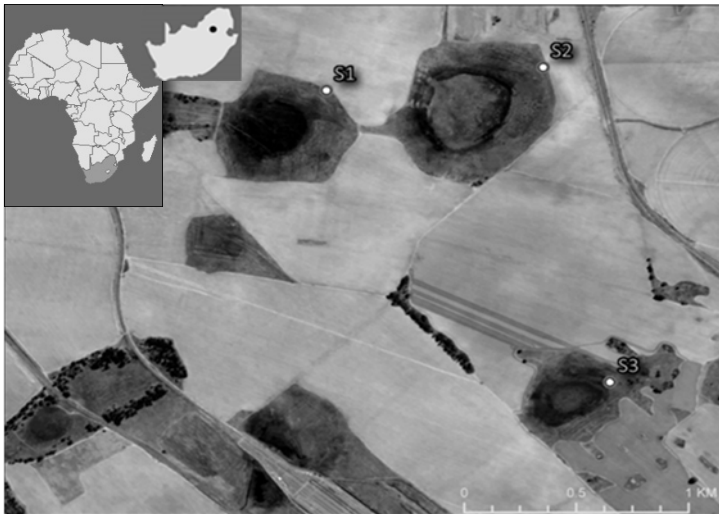
We aimed to: (1) examine the temporal variability in taxon and functional group community structure between the littoral and pelagic zone and establish whether there are major differences in community richness and composition between the two zones; (2) determine potential ecological drivers of community change in each zone by relating

environmental variables to taxonomic and functional group abundances; (3) identify indicator taxa and indicator functional groups for each zone potentially useful for bioindicator purposes, and (4) assess the implications for monitoring epiphytic diatoms from the littoral and pelagic zone for shallow lake assessments.

## 2. Materials and methods

### 2.1. Study area

Three temporary shallow lakes (average water depth < 2 m) in the Bapsfontein region of South Africa were selected as they were a good representative sample of this wetland type in the Mpumalanga Highveld, and had similar macrophytes, conductivity, surrounding land-use and were in close proximity to one another (radius of 1 km) (Fig. 1). The study lakes (S1, S2 and S3) were sampled once a month from March 2011 to February 2012. The physical and chemical characteristics of each study lake are in Table 1; and description of each lake including surface size and depth, as well as meteorological features of the region are provided in Riato et al. (2017a).



**Fig. 1.** Map of study area showing location of each study lake (S1, S2 and S3).

Inset maps from left to right show map of Africa with South Africa at the southern tip of the continent, and the study area location in South Africa.

**Table 1** Mean values,  $\pm$  standard deviation and ranges of the physical and chemical variables of the littoral and pelagic zones for sites S1, S2 and S3 used in the analyses, where n=13 for each site.

	S1						S2						S3					
	Littoral			Pelagic			Littoral			Pelagic			Littoral			Pelagic		
Distance between pelagic and littoral sampling points (m)	54						63						37					
Physical-chemical variables	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
ETo (mm)	3.4 $\pm$ 1.0	2.2	4.8	3.4 $\pm$ 1.0	2.2	4.8	3.4 $\pm$ 1.0	2.2	4.8	3.4 $\pm$ 1.0	2.2	4.8	3.4 $\pm$ 1.0	2.2	4.8	3.4 $\pm$ 1.0	2.2	4.8
Depth (cm)	29.9 $\pm$ 15	10.7	44.9	61.8 $\pm$ 16.4	36.8	84.3	46.3 $\pm$ 29	5.9	74.1	88.4 $\pm$ 18.2	62.1	114.4	35.5 $\pm$ 15.4	3.0	62.1	79.2 $\pm$ 15.6	52.1	99.8
Temperature ( $^{\circ}$ C)	15.8 $\pm$ 6.3	5.2	28.4	17.3 $\pm$ 6.8	6.9	27.6	18.5 $\pm$ 6.9	7.2	30.5	21.7 $\pm$ 6.3	11.8	30.1	21.9 $\pm$ 7.4	7.5	33.7	22.4 $\pm$ 7.0	10.8	32.1
DO (ppm)	3.7 $\pm$ 4.7	0	16.9	3.0 $\pm$ 2.7	1.0	9.3	6.1 $\pm$ 8.2	0	39.8	3.6 $\pm$ 1.0	1.2	5.2	8.5 $\pm$ 13.8	0	85.1	4.8 $\pm$ 0.9	3.1	7.0
pH	7.2 $\pm$ 0.7	6.0	9.4	7.3 $\pm$ 0.6	6.5	8.6	7.2 $\pm$ 0.6	6.4	8.5	7.5 $\pm$ 0.5	6.9	8.6	7.3 $\pm$ 0.9	6.0	10.1	7.5 $\pm$ 0.6	6.7	8.7
Conductivity ( $\mu$ Scm-1)	129.5 $\pm$ 65.1	44.0	315.0	110.8 $\pm$ 31.5	59.0	176.0	93.3 $\pm$ 41.3	31.0	211.0	105 $\pm$ 26.5	50.0	146.0	99.5 $\pm$ 48.6	29.0	320.0	114.8 $\pm$ 29.2	76.0	157.0

**Table 2** Details of sampling design.

Sampling design feature	Lake study sites		
	S1	S2	S3
No. of sampling events	13	13	13
No. of samples collected in pelagic zone per event	1	1	1
No. of samples collected in littoral zone per event	4	4	4
Total no. of samples collected in pelagic zone during study	13	13	13
Total no. of samples collected in littoral zone during study	52	52	52
Total no. of samples from macrophyte, <i>Leersia hexandra</i> in pelagic zone	13	13	13
Total no. of samples from macrophyte, <i>Leersia hexandra</i> in littoral zone	38	7	7
Total no. of samples from macrophyte, <i>Hemarthria altissima</i> in pelagic zone	0	0	0
Total no. of samples from macrophyte, <i>Hemarthria altissima</i> in littoral zone	20	8	24
Total no. of samples from macrophyte, <i>Setaria sphacelata</i> in pelagic zone	0	0	0
Total no. of samples from macrophyte, <i>Setaria sphacelata</i> in littoral zone	29	17	6

## 2.2. Field sampling and laboratory procedures

Details of the sampling design are provided in Table 2. For each sampling event, one sample of epiphytic diatoms was collected from dominant macrophyte, *Leersia hexandra* Sw., at the deepest point in the basin centre (pelagic zone) of each study lake. Littoral epiphytic diatoms were sampled from one of the following dominant macrophyte species: *Leersia hexandra*, *Hemarthria altissima* (Poir.) Stapf & C.E.Hubb or *Setaria sphacelata* (Schumach.) Stapf & C.E. Hubb. ex M.B. Moss, at four random sites within the littoral zone of each lake. Littoral sites were randomly selected prior to each sampling event using ArcGIS 10.1 (ESRI, 2011).

Following the preferred method for sampling diatoms in shallow lakes (Blanco and Bécares, 2006), five healthy established submersed macrophyte stems from different individuals of the same species were pooled, avoiding dead material and new shoots. Stem sections were snipped at 5–20 cm below the water surface and placed into a zip lock bag with a small amount of distilled water. Epiphytes were dislodged from all stems by shaking the samples for 2 min following Zimba and Hopson (1997). Diatom samples were returned to the laboratory where they were acid cleaned and mounted on microscope slides. 400 frustules were counted and identified to the lowest feasible taxonomic level using standard European diatom floras (e.g., Krammer and Lange-Bertalot, 1986-1991; Lange-Bertalot et al., 2001; Lange-Bertalot, 2000-2002), several papers on the Southern African flora by Cholnoky, Schoeman and Archibald (e.g., Schoeman and Archibald, 1976-1980), and books and recent papers on *Gomphonema* taxonomy (e.g., Reichardt, 2015; Reichardt and Lange-Bertalot, 1999; Rose and Cox, 2014). We included planktonic taxa in the count, which is consistent with other studies that were performed in shallow lakes (Borrego-Ramos et al., 2019). The inclusion of planktonic species in the analyses could provide important insight into the diatom distribution patterns in these ecosystems and provide signs of underlying ecological

processes (e.g., competition for space and light). We named a taxon as species affinis (abbreviated: spec. aff.) when its identification suggested it was related to a taxon (or group of these) already described yet showing certain differences with the accepted description of that taxon.

For all sampling sites, chemical and physical variables were measured at the same time and location as the diatom sampling. For littoral and pelagic sites, conductivity, dissolved oxygen (DO), pH and temperature were measured using a Hach HQ40D Dual-Input Multi-Parameter meter; water depth was recorded at the same location. We did not include nutrients because of: 1) budget constraints, and 2) results from a study by Riato et al. (2017a) that sampled the pelagic zone of the same study lakes for 1.5 years (from high inundation to drying out period) indicated that nutrients did not have a strong influence on the diatom communities.

We included total relative evapotranspiration (ETo) data in the analysis as a proxy for the seasonal (winter-summer) gradient, to determine if ETo had an influence on taxonomic and functional group composition. For each sampling month, we used the monthly ETo, calculated as the mean of the daily ETo values for the sampling month.

## ***2.3 Data Analysis***

### ***2.3.1 Richness***

Sampling effort differed between the littoral zone (52 samples) vs the pelagic zone (13 samples) for each study lake. To allow for comparisons of taxa richness between zone types at each study lake, richness was standardised for sampling effort using six non-parametric richness estimators calculated in EstimateS 9.1.0 (Colwell and Elsensohn, 2014; Gotelli and Colwell, 2001). This included four abundance-based estimators [Chao1, abundance-based coverage estimator (ACE), Jack1, and Jack2] and two incidence-based estimators [Chao2 and



incidence-based coverage estimator (ICE)]. Based on the lowest and highest of the six estimators, we calculated the range of the portion of predicted richness that was actually observed. We applied the 95% confidence interval (CI) of Chao1 and Chao2 to establish whether richness differed between zone types (Colwell, 2013).

For the following analyses, we included only those taxa that reached relative abundance of at least 1% occurring in at least two samples in our dataset as analyses may be skewed by rare taxa (Potapova and Charles, 2003). The ‘complete dataset’ contained 128 diatom taxa and the ‘reduced dataset’ had 96 taxa.

### *2.3.2 Indicator Species Analysis*

We used Indicator Species Analysis (IndVal; Primer 6; Dufrene and Legendre, 1997) to (1) identify taxa characteristic of the littoral and the pelagic zones within each study lake (PC-ORD, version 5.10; McCune and Mefford, 2006); and (2) determine whether taxa characteristic of littoral and pelagic zones was shared or exclusive among study lakes.

### *2.3.3 Temporal variation in diatom communities and physico-chemical variables*

We assessed the temporal variability of the littoral and pelagic diatom communities in each lake. We used non-metric multidimensional scaling (NMDS; PC-ORD) to visualise differences in community composition between the littoral and pelagic zone of each lake throughout the study. We used square-root-transformed abundance to reduce the influence of abundant species on ordination scores.

We then calculated separately for the littoral sites and the pelagic site, Euclidean distances between successive monthly samples in ordination space using the two NMDS axes in the final solution. The larger the Euclidean distance between monthly samples, the greater the temporal variability. For littoral sites, we calculated the mean Euclidean distance for each

monthly transition. We used a one-way repeated measures ANOVA to compare Euclidean distance mean values between the two zone types.

Temporal variability of the physico-chemical composition was also assessed. For this we calculated principal component analyses (PCA; PC-ORD) for each study lake separately using variables, water depth, conductivity, DO, pH and temperature. Euclidean distances between successive monthly samples in ordination space were calculated using the scores of the first and second principal component axes. We then calculated Euclidean distances for the physico-chemical composition of the littoral sites and used one-way repeated measures ANOVA to determine differences between zone types. All variables, except water depth and temperature, were log transformed to reduce skewness and normalise distributions.

For the analyses of the functional group data, we used the 'complete dataset'. We assigned diatom taxa at the species or genus level to two types of functional groups a) life-forms and b) ecological guilds, in accordance with Passy (2007a, 2007b), and Rimet and Bouchez, (2012) (Table 3). A taxon can have more than one life-form group since certain taxa can have multiple life-forms throughout its life-cycle (Rimet and Bouchez, 2012). We adapted the functional groups to accommodate taxa identified in our study which were not mentioned in (Rimet and Bouchez, 2012). We then re-calculated IndVal, NMDS, and Euclidean distances between successive monthly samples, as described above.

**Table 3** Assignment of taxa to life-forms and ecological guilds following Passy (2007a,b), and Rimet and Bouchez (2012). Refer to Krammer and Lange-Bertalot (1986-1991) for genus/species authorities.

Functional groups	Definition of functional group classification	Taxa Assigned
<i>Life-forms</i>		
Mobile	Free moving e.g. some species vertically migrate into the sediments to acquire nutrients	<i>Achnanthes</i> , <i>Achnantheidium</i> , <i>Amphora montana</i> , <i>Brachysira</i> , <i>Caloneis</i> , <i>Cocconeis placentula</i> var. <i>lineata</i> , <i>Craticula molestiformis</i> , <i>Diademsis</i> , <i>Encyonema</i> , <i>Encyonopsis</i> , <i>Eolimna</i> , <i>Eunotia</i> , <i>Frustulia</i> , <i>Gomphonema</i> , <i>Hantzschia amphioxys</i> , <i>Luticola</i> , <i>Mayamaea</i> , <i>Navicula</i> , <i>Nitzschia</i> , <i>Pinnularia</i> , <i>Rhopalodia</i> , <i>Sellaphora</i> , <i>Stauroneis</i> , <i>Stenopterobia curvula</i>
Pioneer	Species colonise bare substrates faster than other species	<i>Achnantheidium minutissima</i> var. <i>minutissima</i> , <i>A. minutissima</i> var. <i>affinis</i> , <i>A. saprophilum</i>
Tube-forming	Species live in mucous substance within which they can move freely	<i>Frustulia</i> , <i>Encyonema mesianum</i>
Rosette colony	Species attached to substrate by a short stalk at one pole; colonies look fan-shaped	<i>Ulnaria acus</i>
Ribbon colony	Species attached to one another either by interlocking spines or by a layer of mucous on their valve face, forming long, ribbon-like colonies	<i>Eunotia bilunaris</i> , <i>Eunotia minor</i> , <i>Eunotia pectinalis</i>
Pad	Species grows upright to substrate, attached by a mucilage pad	<i>Encyonopsis</i> , <i>Fragilaria</i> , <i>Ulnaria</i>
Stalk	Species grows upright to substrate, attached by a stalk	<i>Achnanthes</i> , <i>Achnantheidium</i> , <i>Gomphonema</i>
Adnate	Species grows parallel to substrate, attached by their valve face	<i>Amphora montana</i> , <i>Cocconeis placentula</i> var. <i>lineata</i> , <i>Rhopalodia gibba</i>
<i>Ecological guilds</i>		
High-profile	Species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, pedunculate, and colonial centrics	<i>Diademsis</i> , <i>Encyonema mesianum</i> , <i>Eunotia</i> , <i>Fragilaria</i> , <i>Ulnaria</i> , <i>Gomphonema</i>
Low-profile	Species of short stature, including prostrate, adnate, small erect, solitary centrics, slow-moving species	<i>Achnanthes</i> , <i>Achnantheidium</i> , <i>Amphora montana</i> , <i>Brachysira</i> , <i>Cocconeis placentula</i> var. <i>lineata</i> , <i>Encyonopsis</i>
Motile	Fast-moving species	<i>Caloneis</i> , <i>Craticula molestiformis</i> , <i>Eolimna</i> , <i>Hantzschia amphioxys</i> , <i>Luticola</i> , <i>Mayamaea</i> , <i>Navicula</i> , <i>Nitzschia</i> , <i>Pinnularia</i> , <i>Rhopalodia</i> , <i>Sellaphora</i> , <i>Stauroneis</i> , <i>Stenopterobia curvula</i>
Planktonic	Solitary or colonial centrics	<i>Cyclotella meneghiniana</i> , <i>Discostella pseudostelligera</i> , <i>Fragilaria tenera</i> , <i>Ulnaria acus</i> , <i>Nitzschia acicularis</i>

### 2.3.4 Environmental predictors

We used Primer 6's RELATE (Spearman's Rank Correlation method, Clarke and Ainsworth, 1993) to evaluate correlations between the physico-chemical data and taxon abundance and functional group abundance data for each zone type at each study lake. Plant substrate type was included in physico-chemical data for the littoral zone analyses, but not for the pelagic zone analyses since one plant type was sampled in the pelagic zone throughout the study. Distance-based linear models (DistLM; Anderson et al., 2008; Anderson, 2001) were then used to determine: (1) which zone type explained more of the variation in both taxonomic

and functional group composition; (2) which physico-chemical variable accounted for significant portions of the observed variability in taxonomic and functional group composition within each zone type at each study lake; and (3) if the taxonomic composition and functional group composition explained by each variable was shared or exclusive between zone types at each study lake and between the three sites. Models were run to identify the most significant set of physico-chemical variables and to evaluate the cumulative effect of the variables explaining community composition when fitted sequentially (sequential tests), using the stepwise selection procedure and Akaike corrected information criterion (AICc) (Akaike, 1987). Each model was re-run using only the variables selected for this model and distance-based redundancy analysis (dbRDA) was performed to visualize the influence of predictor variables identified by DistLM.

To ensure model robustness, we used data of which correlations of physico-chemical data and abundance data were significant ( $P < 0.05$ ) from the RELATE analyses. To reduce skewness and normalise distributions for data analyses, all physico-chemical variables were log transformed when necessary, and all variables were normalised. To avoid multicollinearity, significant co-linear relationships were identified between environmental variables (Spearman's Rank Correlation coefficient  $r \geq 0.7$ ) and the variable with a higher overall mean correlation coefficient with multiple metrics was omitted prior to analyses (Hering et al., 2006a, 2006b).

### **3. Results**

#### ***3.1 Richness, indicator taxa and indicator functional groups***

The percentage of predicted richness actually observed was higher in the littoral zone (S1: 77-94%, S2: 75-92%, S3: 87-97%) than in the pelagic zone (S1: 64-86%, S2: 71-89%, S3: 66-92%, Table 4). Richness estimators suggested higher richness in the littoral zone and

lower richness in the pelagic zone, and in most cases, were almost double in richness (Table 4). Similar trends were also found with observed species richness.

**Table 4** Observed species richness (Species obs.), abundance- and incidence-based richness estimators, and percentage of estimated richness actually observed of epiphytic diatoms within the littoral and pelagic zone of each study lake (S1, S2 and S3). ACE, abundance-based coverage estimator; CI, confidence interval; ICE, incidence-based coverage estimator.

Site/zone	Species obs.	Abundance-based estimators				Incidence-based estimators		Percentage observed (range)
		Chao1 (95% CI)	ACE	Jack1	Jack2	Chao2 (95% CI)	ICE	
S1 Littoral	103	109.1 (104.5-127.73)	109.0	124.6	133.5	119.2 (108.8-148.4)	120.9	77-94%
S1 Pelagic	51	59.1 (52.8-86.6)	58.6	68.5	80.0	78.8 (59.8-138.9)	73.1	64-86%
S2 Littoral	79	86.2 (80.9-105.9)	91.6	98.6	105.6	94.1 (84.2-122.8)	100.5	75-92%
S2 Pelagic	41	53.2 (43.2-109.2)	46.3	52.1	57.6	52.1 (43.9-83.1)	53.5	71-89%
S3 Littoral	85	88.0 (85.5-101.5)	88.7	97.8	97.1	90.1 (86.3-104.4)	92.3	87-97%
S3 Pelagic	51	55.6 (52.0-72.4)	56.8	66.7	77.2	77.7 (58.9-141.3)	68.8	66-92%

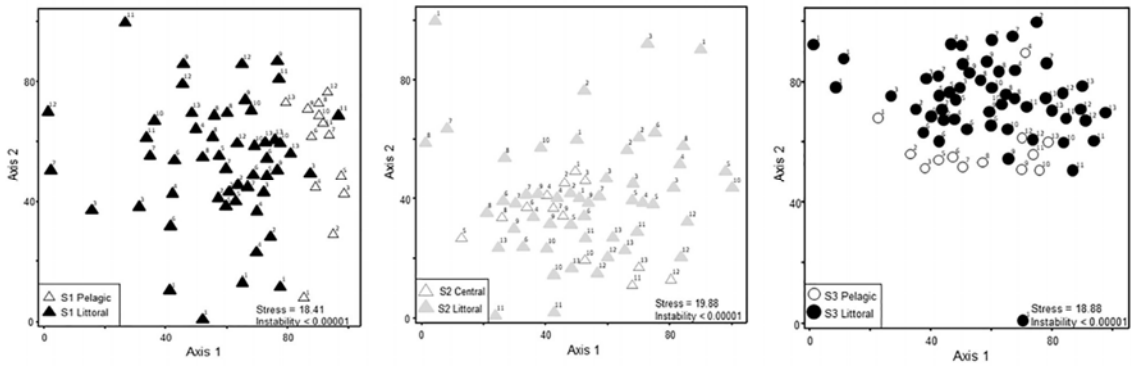
IndVal identified 14 indicator taxa representative of the littoral zone ( $P < 0.05$ , Table 5) and 12 indicator taxa characteristic of the pelagic zone. Of these, only a few indicator taxa for each zone type were shared among study lakes. However, sites did share a common genus that was characteristic of each zone. In the littoral zone, indicator taxa were mostly of the *Nitzschia* genus. Indicator taxa *Nitzschia palea* var. *palea*, *Pinnularia subcapitata* var. *elongata* and *Sellaphora joubaudii* were representative of littoral zones at both sites, S1 and S3. While in the pelagic zone, indicator taxa belonged, for the most part, to the *Gomphonema* genus, in which *Gomphonema* spec aff. *angustatum* was characteristic of the pelagic for S2 and S3. Indicator functional groups of the pelagic zone were tube-forming life-forms, represented by indicator taxon *Encyonema mesianum*; stalk life-forms, represented by indicator taxa of the *Gomphonema* genus; and high-profile guilds, characterised by indicator taxa of the *Gomphonema* genus and *Encyonema mesianum*. In the littoral zone, there was only one indicator functional group, the motile guild, represented by indicator taxa of the *Nitzschia* genus and *Pinnularia subcapitata* var. *elongata* at study lakes, S1 and S3.

**Table 5** Indicator taxa and indicator functional groups identified for the littoral and pelagic zones at each study lake using Indicator Species Analysis (IndVal,  $P < 0.05$ ). Refer to Krammer and Lange-Bertalot (1986-1991) for species authorities.

	Littoral		Pelagic	
	Taxa	Functional group	Taxa	Functional group
S1	<i>Gomphonema exilissimum</i>	Motile	<i>Encyonema mesianum</i>	Tube-forming
	<i>Navicula tridentula</i>		<i>Pinnularia subgibba</i> var. <i>subgibba</i>	High profile
	<i>Nitzschia gracilis</i>		<i>Gomphonema parvulum</i> sensu lato	
	<i>Nitzschia palea</i> var. <i>debilis</i>		<i>Gomphonema spiculoides</i>	
	<i>Nitzschia palea</i> var. <i>palea</i>		<i>Brachysira neoexilis</i>	
	<i>Pinnularia subcapitata</i> var. <i>elongata</i>			
	<i>Sellaphora joubaudii</i>			
S2	<i>Stenopterobia curvula</i>		<i>Gomphonema parvulus</i>	
	<i>Gomphonema gracile</i>		<i>Gomphonema</i> spec aff. <i>angustatum</i>	
	<i>Pinnularia subgibba</i> var. <i>subgibba</i>			
S3	<i>Nitzschia acidoclinata</i>	Motile	<i>Achnanthydium minutissimum</i>	Stalk
	<i>Pinnularia subcapitata</i> var. <i>elongata</i>		<i>Nitzschia acicularis</i>	
	<i>Sellaphora joubaudii</i>		<i>Gomphonema parvulum</i>	
	<i>Nitzschia palea</i> var. <i>palea</i>		<i>Gomphonema auritum</i>	
			<i>Gomphonema</i> spec aff. <i>angustatum</i>	

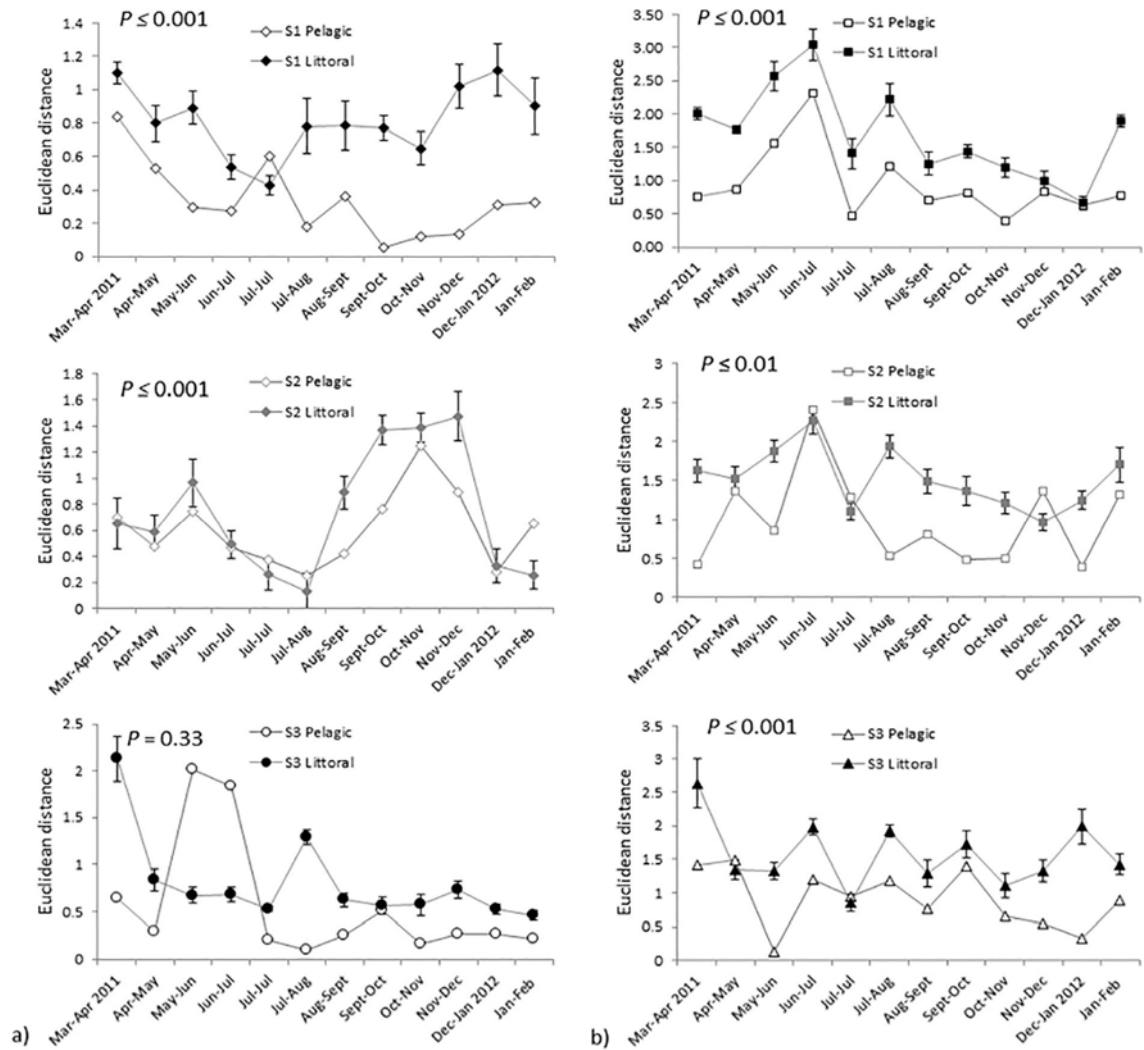
### 3.2 Temporal variation in community and physico-chemical composition

NMDS ordinations of taxon abundances showed a separation of littoral sites from pelagic sites for lakes S1 and S3, but not for S2 (Fig. 2). For all three study lakes, the littoral sites were strongly separated in ordination space. In contrast, the pelagic sites were mostly arranged along one axis and were not as strongly separated as the littoral sites, indicating these sites were more similar to each other relative to the littoral sites. NMDS ordinations of functional group abundances illustrated the same patterns (Appendix A).



**Fig. 2.** Non-metric multidimensional scaling (NMDS) ordinations of monthly taxon abundances from the 4 random littoral sites and the pelagic site during each month (n=13) at each study lake, S1, S2 and S3. Numbers refer to successive monthly samples from March 2011 to February 2012.

Within each lake, Euclidean distances of taxon abundance and physico-chemical composition showed similar trends between the littoral and pelagic sites throughout the study (Fig. 3a, 3b). Overall, Euclidean distances were significantly higher in the littoral sites than in the pelagic sites, implying that littoral diatom communities and littoral physico-chemical compositions had significantly higher temporal variability than pelagic diatom communities and pelagic physico-chemical compositions.



**Fig. 3.** Euclidean distance between a) each consecutive sampling month of diatom taxon abundances in NMDS ordination space for the 4 littoral sites (mean  $\pm$ SE) and the pelagic site, and b) each consecutive sampling month of the physico-chemical composition in PCA ordination space for the 4 littoral sites (mean  $\pm$ SE) and the pelagic site.  $P$  values are for comparisons between littoral and pelagic sites (one-way repeated measures ANOVA,  $P < 0.05$ ).

### 3.3 Relationships of taxonomic and functional group composition to physico-chemical variables

Correlation analyses (RELATE) between the taxonomic and functional group composition of the pelagic zone and the physico-chemical variables were significant for all three study lakes ( $P \leq 0.05$ ; Table 6). Among the littoral communities, the taxonomic and functional group composition of sites S1 and S2 showed no significant correlations with the physico-chemical



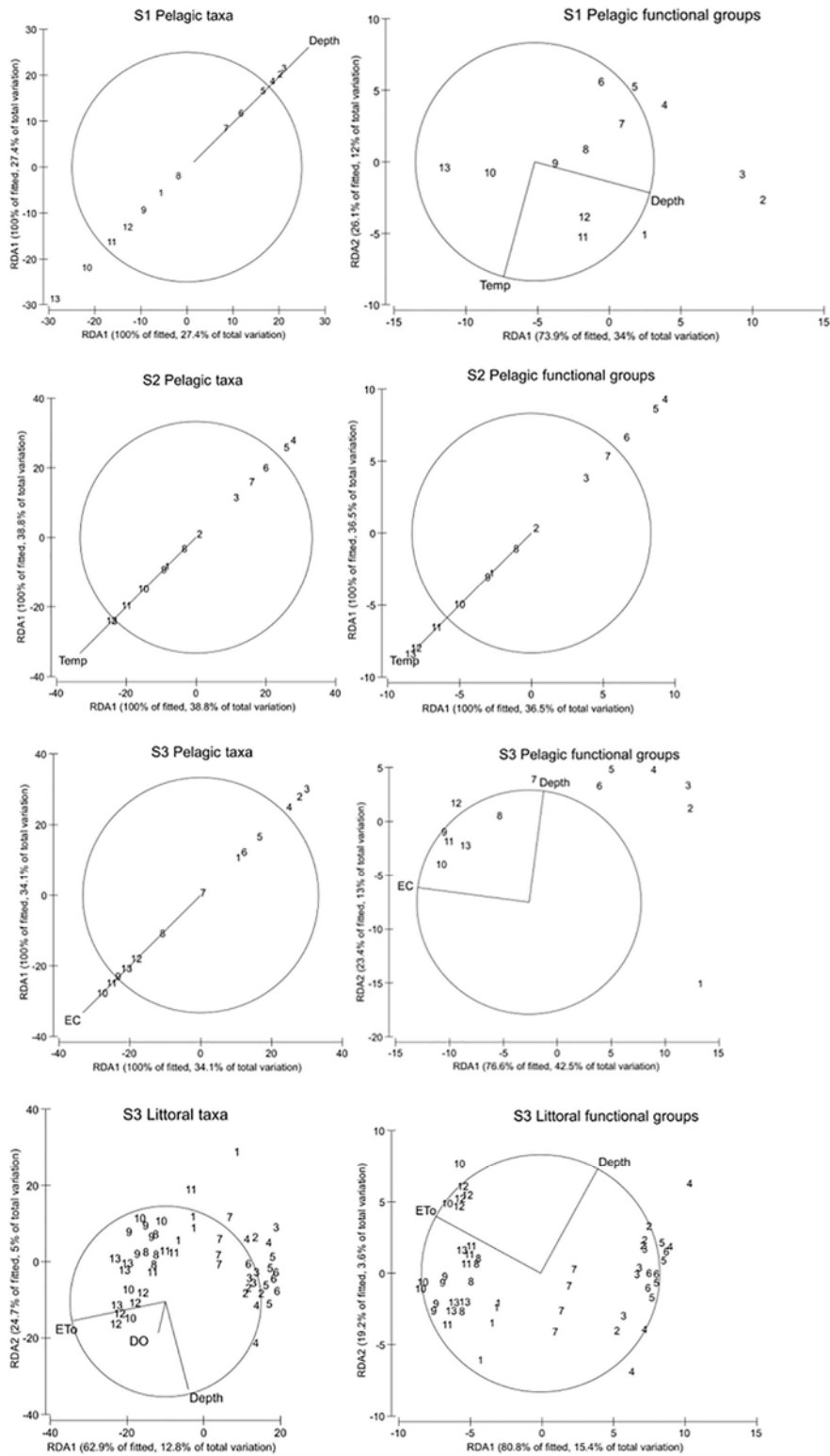
variables, whereas site S3 showed highly significant correlations ( $P \leq 0.001$ ). In order to ensure a more robust regression model, DistLM was run for taxon abundance, functional group abundance and physico-chemical variables for the pelagic zone at each of the three study lakes, and for the littoral zone at study lake S3 only. Models for all three pelagic zones explained more of the variation in both taxonomic and functional group composition (range, 27.44 – 55.47 Cum.%) than models based on the taxonomic and functional group composition for S3 littoral zone (range, 19.03 – 20.38 Cum.%, Table 7). Overall, the importance of physico-chemical variables was similar between the taxonomic and functional group composition for each zone type at each study lake, but variables differed somewhat between study lakes and between zone types (pelagic zone sites vs S3 littoral zone) (Table 7, Fig. 4). Significant variables across all DistLM models included water depth, temperature, ETo, EC, and DO, in which water depth was the most common predictor variable.

**Table 6** RELATE results for correlations of taxon abundance and functional group abundance data to physico-chemical data for each zone type at each study lake. *Rho* is the Spearman's correlation and significance of each correlation is indicated by \* $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ .

		Taxon abundance data	Functional group abundance data
		<i>Rho</i>	<i>Rho</i>
S1	Littoral	0.12	0.06
	Pelagic	0.46***	0.32*
S2	Littoral	0.07	0.06
	Pelagic	0.43**	0.42**
S3	Littoral	0.31***	0.27***
	Pelagic	0.38**	0.41**

**Table 7** Distance-based linear model (DistLM) results between, taxon abundance, functional group abundance and physico-chemical variables for the pelagic zone at each of the three study lakes, and for the littoral zone at site S3 only. A step-wise selection procedure and Akaike’s corrected information criterion (AICc) was applied to select the group of variables that accounted for the greatest proportion of taxonomic/functional group composition. Cumulative percentage of variance explained (Cum. (%)) represents explained variation attributable to each variable added to the model when fitted sequentially. Significance is indicated by \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ .

		Taxon abundance data				Functional group abundance data					
		Variables selected (Best model)	AICc	Pseudo- F	<i>P</i>	Cum. (%)	Variables selected (Best model)				
							AICc	Pseudo- F	<i>P</i>	Cum. (%)	
S1	Pelagic	Depth	90.71	4.16	0.005**	27.44	Depth	60.92	4.42	0.01**	28.66
							Temp.	60.77	3.21	0.02*	45.98
S2	Pelagic	Temp.	86.11	6.99	0.002**	38.85	Temp.	58.81	6.31	0.01**	36.47
S3	Pelagic	EC	92.56	5.70	0.001***	34.10	EC	67.53	7.32	0.001***	39.96
							Depth	67.11	3.48	0.108	55.47
S3	Littoral	ETo	382.28	6.88	0.001***	12.10	ETo	286.35	8.91	0.001***	15.12
		Depth	381.76	2.69	0.004**	16.67	Depth	286.15	2.36	0.05*	19.03
		DO	381.74	2.24	0.011*	20.38					



**Fig. 4.** Distance-based redundancy analysis (dbrRDA) ordination plots of taxon abundance and functional group abundance for the pelagic zone at each of the three study lakes, and for the littoral zone at site S3 only. Plots are overlaid with normalised taxa physico-chemical variables (based on distLM analyses in Table 7).

## 4. Discussion

### 4.1 *Heterogeneity of epiphytic diatom communities*

In comparison to the pelagic zone, the littoral zone was characterized by almost double in species richness within each lake. We hypothesize that enhanced species richness is related to the inherent complexity or heterogeneity of habitats in the littoral of lakes. Positive relationships between species richness and habitat heterogeneity in lakes are well documented for fish (e.g., Benson and Magnuson, 1992; Plaska and Rechulicz, 2008) and macroinvertebrates (e.g., Rennie and Jackson, 2005; Trigal et al., 2006; White and Irvine, 2003). While the heterogeneity of algal communities within a lake (e.g., pelagic vs. littoral communities) is under researched, a few studies have associated higher taxonomic richness in algae with a high degree of habitat complexity (Cattaneo et al., 1998; Muylaert et al., 2010).

Research exploring the use of littoral phyto-benthos for monitoring the largest European lake, Lake Balaton, showed littoral benthic diatoms to be heterogeneous, in spite of similar water physico-chemical conditions (Bolla et al., 2010). Multiple biotic and abiotic factors can influence the diatom assemblage structure and their heterogeneity in lakes. Differences in structural complexity produced by macrophytes may considerably influence the structure of epiphytic assemblages as a result of differential shading, grazing pressure and nutrient availability (Letáková et al., 2018; Millie and Lowe, 1981; Søndergaard et al., 2010). Concentrations of nutrients may be higher in the littoral zone than in the pelagic since the littoral zone harbors more plant material, and thus, will have more decomposition of dead plant material, as was previously shown in an African shallow lake (Howard-Williams, 1972). Moreover, disturbance factors are more significant in the shallow zone compared to the deeper pelagic zone and play a major role in the structuring of the algal communities in lakes (Cantonati and Lowe, 2014). Hydraulic pressures such as water-level fluctuations, and wind and wave action (Leira and Cantonati, 2008; Rimet et al., 2015) can have important

impacts on littoral diatom communities. In shallow waters, waves and sediment resuspension have an important impact on abiotic factors. Thus, light availability, water energy, temperature and nutrients can vary greatly across the littoral zone, and, affect the occurrence and composition of diatom assemblages (Cantonati and Lowe, 2014; Lowe, 1996). In contrast, the deeper pelagic zone is a more stable environment. This corroborates our findings when measuring the temporal variability of diatom communities and physico-chemical compositions of littoral and pelagic sites.

Overall, pelagic diatom communities and pelagic physico-chemical composition showed significantly lower temporal variability than those in the littoral zone. Short-term extreme weather events like strong winds or heavy rainfall will have more of an impact on nearshore littoral waters than offshore pelagic waters. For example, incoming water from the catchment can cause acute changes in littoral water chemistry (Howard-Williams and Lenton, 1975). This can lead to a higher variability of littoral communities and littoral chemistry. Indeed, a multitude of environmental factors play an important role in structuring the diatom communities within a lake, producing distinct assemblages for littoral and pelagic habitats, as was reported in previous studies (Cantonati et al., 2009, 2014; Celewicz-Goldyn et al., 2010; Vadeboncoeur et al., 2014). However, the importance of individual factors on the interplay between biotic and abiotic features for the epiphytic diatom community in shallow lakes is still unclear.

#### ***4.2 Distinct taxa and functional groups of the littoral zone and pelagic zone***

Overall, taxa characteristic of zone types differed among sites; only a few indicator taxa were common among sites. Understanding the main drivers causing differences in the variation in species is difficult due to the numerous biotic and abiotic factors that can influence community composition (e.g., regional species pool, connectivity between systems, species

interactions and dispersal constraints) (Cornell and Harrison, 2014; Srivastava, 1999). We did, however, find commonality of littoral and pelagic indicator taxa among sites at the genus-level. The *Nitzschia* genus, which was comprised of motile diatoms, characterized the littoral zone. Motile species are often found higher-nutrient habitats (Passy, 2007a; Yallop and Kelly, 2006). Since the littoral zone is often more enriched than the pelagic, even in shallow lakes (Howard-Williams, 1972; Howard-Williams and Lenton, 1975), this may explain why motiles are characteristic of littoral waters. However, *N. acidoclinata*, a taxon characteristic of the littoral zone of lake S3, is known to favour oligotrophic waters, suggesting that other environmental factors could have an important role in the growth of motile diatoms. For example, the exposed littoral zone is more prone to wind-induced water turbulence than the more wind protected pelagic area, which may result in higher sediment resuspension and lower irradiance (Bolla et al., 2010). In this regard, wind disturbances could be promoting motile diatoms in littoral habitats because of their ability to migrate to the surface layer of the biofilm and maintain access to light (Stenger-Kovács et al., 2013).

In contrast, long stalked high profile diatoms of the *Gomphonema* genus characterized the pelagic zone. Several authors have identified the high profile guild as the most sensitive group to disturbances. For example, water-level fluctuations (Leira et al., 2015), flooding and elevated ionic content (B-Béres et al., 2014; Riato et al., 2017b; Stenger-Kovács et al., 2013), and high grazing pressure, since they are positioned high within the biofilm which is more prone to grazing (Luttenton et al., 1986; Rimet et al., 2009). Since these disturbances are more common to the littoral zone than to the more stable pelagic zone, this could explain the high profile preference for pelagic waters here. Another explanation for high profile preference for pelagic habitats could be because of their high surface ratio, which makes them strong competitors for nutrients (Rimet et al., 2015) in lower-nutrient pelagic waters.

### ***4.3 Environmental predictors of littoral and pelagic diatom communities***

Overall, water depth was a good predictor of the epiphytic diatom taxa and functional group composition. Water depth has already been identified as a key factor influencing epiphytic diatom communities in shallow lakes (Riato et al., 2017a,b; Wantzen et al., 2008). We also found that pelagic diatom taxa and functional groups correlated better with pelagic physico-chemistry than littoral diatoms to littoral physico-chemistry. Rimet et al. (2015) also found weak correlations between lake littoral benthic diatoms and local littoral chemistry (specifically nutrients parameters), and demonstrated that the impact of wind on littoral diatoms was weakening the correlation to littoral nutrient concentrations because of an increase of diatoms adapted to turbulent environments. This is especially important for littoral diatom assessments of trophic status in European shallow lakes, where eutrophication is the leading cause of lake degradation. In our study lakes, it is conceivable that un-modeled factors related to wind-induced water turbulence or water-level fluctuations inherent in shallow lakes, were preventing littoral diatom communities from correlating with the littoral chemistry. This has important implications for selecting a reliable biomonitoring site for the routine assessment of temporary shallow lakes; to effectively monitor the ecological status of a lake, epiphytic diatoms should be sampled in the pelagic zone.

## **5. Conclusions**

Results from this study demonstrate that littoral epiphytic diatom assemblages are more heterogeneous than pelagic epiphytic assemblages. Comparison of our results with previous findings on the heterogeneity of epiphytic diatoms in shallow lakes is difficult due to the lack of studies on this topic. Nevertheless, our results are comparable with earlier studies describing the heterogeneity of littoral benthic diatoms in large lakes which was explained by differing environmental conditions (Bolla et al., 2010; Rimet et al., 2015).

We also found that pelagic epiphytic diatom taxa and functional groups had better capacity to assess environmental changes than littoral taxa and functional groups. While epiphytic littoral diatoms have been regarded as promising biological indicators for shallow lake assessments (Blanco et al., 2014), difficulties still exist in establishing reliable pressure–response relationships in a multiple stressor environment (Bruce et al., 2013). The littoral zone of lakes is most affected by multiple physical pressures (Cantonati and Lowe, 2014). That, combined with the heterogeneity of habitats in the littoral of lakes, will produce a more heterogeneous diatom community and increase inherent “noise” in biological assessments. Based on our findings, we propose that shallow lake biomonitoring programmes are designed to collect epiphytic diatoms where there is less extremes, somewhere between the pelagic zone and the littoral zone, to optimize accuracy of assessments. This would reduce the background noise by natural variability found in the littoral zone and avoid possible underestimation of lake ecological status if the pelagic community response to pollutants entering the lake is too slow. Our study is limited, however, to three shallow lakes, so the generality of our findings needs to be tested.

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