INTRODUCTION

Sediment traps deployed in lakes allow quantification and compositional analysis of biological assemblages over extended periods of time (weeks, months, years), and can be used to monitor changes in ecosystems responding to environmental and climate variables, such as periods of ice-cover, water column mixing, nutrient input or stratification (Kamenik and Schmidt, 2005; Pla-Rabes and Catalan, 2011). The study of these biological assemblages from sediment traps, together with their environmental and climatic preferences during a year, constitutes a training set. These data are then developed into a calibration model (called transfer functions) that correlate the biological and environmental data, which is used to reconstruct quantitatively an environmental variable of interest (Birks et al., 2012). Biological communities in lakes are structured by local and regional factors. The training set must adequately capture the regional variations of physical, chemical and climatic factors that are relevant to biological indicators (Reavie and Juggins, 2011). Optimally, large training sets are required to maximize the likelihood of high similarity between modern and fossil assemblages, adequate characterization of environmental variables, and to capture the gradient of interest that is subject to reconstruction. However, unusual environmental conditions compared to the inter-decadal variability occurring during the sampling period (usually one year) can hamper the identification of some of the gradients in the training set. Climatic variables in particular show strong inter-annual variability. These are more susceptible to be misrepresented in cases where the amplitude of the climate...
gradient within the region of study (sampling sites) is lower than the gradients of other environmental variables. The inter-annual variability of environmental and climatic variables in lakes of mid-latitudes can be very high, which in turn is partly responsible for the differences observed from year-to-year in biotic communities living in the water column (Catalan and Fee, 1994). Thus, longer time series of sediment trap studies may help to better identify the source of inter-annual variability in biological communities (Goldman et al., 1989). Geographical, morphological and some environmental sources of variation are attenuated, so the processes generating variability should be fewer than in studies covering only one year.

Sediment trap studies covering more than one year on multiple lakes are very rare because they are time consuming and cost-intensive. Limnologists working in the field of conservation and management have used lake monitoring for several years to study the recovery of heavily human impacted lakes (De Hoyos and Comín, 1999; Battarbee et al., 2014), or the causes of shifts in planktonic communities (Goldman et al., 1989; George, 2000). However, studies of this length have rarely been used in the context of developing training sets, although they would help to better understand some aspects related to its design, and potential for application in transfer functions.

Chrysophytes (Chrysophyceae and Synurophyceae groups) are unicellular, golden brown algae. They constitute a major component of phytoplankton communities in freshwater systems, having preference for cold and oligotrophic lakes (Eloranta et al., 1995; Siver et al., 1995). Chrysophytes produce siliceous resting stages known as stomaticysts (or just cysts) that are commonly preserved in lake sediments (Zeeb and Smol, 2001). The composition of chrysophyte communities in lakes is related to specific ecological parameters of the water masses they live (e.g., temperature, ice-cover and nutrient availability), and hence they are often used as paleoenvironmental and paleoclimatic proxies (Adam and Mahood, 1981; Charles and Smol, 1988; Smol, 1995; Duff et al., 1997). Chrysophytes exhibit marked seasonality, making it possible to identify assemblages dependent on specific lake conditions (i.e., duration of ice-cover, spring and autumn overturn, or summer stratification) (Sandgren, 1988; Pla-Rabes and Catalan, 2011).

Recent studies have shown that cysts are potential proxies for spring and autumn temperatures and mixing regimes using sediment traps (Brown et al., 1997; Kamenik and Schmidt, 2005; Pla and Anderson, 2005; Pla and Catalan, 2005; De Jong et al., 2013). However, chrysophytes studies (vegetative or cysts) from sediment traps spanning a year or longer are scarce (Kamenik and Schmidt, 2005), or limited to one lake (Kristiansen, 1988; Siver and Hamer, 1992; Agbeti and Smol, 1995; Kamenik et al., 2001; Pla-Rabes and Catalan, 2011). Siver and Hamer (1992) studied a three year period of sediment trap chrysophyte assemblages in a small lake in New England in order to study their response to seasonal climate and environmental gradients. One of the main conclusions of the study was that knowing the distributions of chrysophyte taxa along a seasonal gradient allowed for the division of sediment assemblages into warm and cold-water floras. Likewise, Pla-Rabes and Catalan (2011) found a close link between many chrysophyte types sampled monthly in a Pyrenean lake and spring and summer temperatures.

During the CLIMPOL project (Climate of northern Poland during the last 1000 years: Constraining the future with the past), 50 lakes of northern Poland were instrumented with sediment traps and thermistors, and regular field surveys were carried out to analyse a broad range of environmental variables. The goal of this study was to determine the composition of chrysophyte assemblages in order to develop a training set. This research yielded important results, including the demonstration that chrysophyte assemblages have a strong relationship with water chemistry, and the number of days below 4°C (DB4°C), which is a variable related to cold-season air temperatures (Hernández-Almeida et al., 2015a, 2015b). After one year of exposure, 37 sediment traps and corresponding environmental information were recovered. Sediment traps were again deployed, and 14 of them were recovered in November 2013. The aim of the present study was to investigate the potential of multi-year training set sampling to identify underlying environmental variables that influence shifts in biological assemblages. With the hypothesis that inter-annual variability of biotic assemblages should respond mainly to temperature gradients, we sampled during two consecutive years. The first year showed more oceanic weather conditions (mild temperatures), while the other showed rather continental ones (more extreme temperatures). During both years, other environmental variables were very similar in terms of trophic state and water chemistry. Using sediment traps, limnological and water chemistry measurements, we studied the response of chrysophyte assemblages to environmental and climatic factors in 14 lakes in northern Poland over the two years. Analyses of multi-year biological assemblages from the sediment trap allow strong comparison to the environmental data, and increase our knowledge on inter-annual variability of biological assemblages and taxon autoecology, with the aim of improving calibration models for their later application in paleolimnological reconstructions.

**METHODS**

**Field methods and regional settings**

The chrysophyte cyst dataset consists of 14 sediment trap samples from lakes in northern Poland, located across a W-E gradient along the Great Poland, Pomeranian and
Masurian lake districts (Fig. 1; Tab. 1). A simple Bloesch-type sediment trap (Bloesch and Burns, 1980; PVC-liners with a length of 80 cm and a diameter of 9 cm, 2 tubes per trap) was used, with the openings of the traps approximately 1.5 m above the lake bottom. The first exposure period (TS1) covered November 2011-October 2012, and the second (TS2) was between November 2012-November 2013. For TS1, field surveys to take water chemistry samples and to make limnological measurements were taken at 3-months intervals, while for TS2 a single field survey was made in November 2013 to recover the sediment traps and make the limnological measurements.

Tab. 1. Code, geographical coordinates, inflow, mixing regime and description of the fourteen lakes included in this study.

<table>
<thead>
<tr>
<th>Lake name</th>
<th>Label</th>
<th>Latitude (North)</th>
<th>Longitude (East)</th>
<th>Depth (m)</th>
<th>Inflow</th>
<th>Mixing</th>
<th>Description of catchment and impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Błotno</td>
<td>BLO</td>
<td>53°19’35”</td>
<td>15°29’42.”</td>
<td>10.9</td>
<td>No</td>
<td>D</td>
<td>Mostly agriculture fields. Eutrophic lake</td>
</tr>
<tr>
<td>Suminko</td>
<td>SMK</td>
<td>54°11’12”</td>
<td>17°48’</td>
<td>17.2</td>
<td>Yes</td>
<td>M</td>
<td>Limited human impact, mostly forest. Highest nutrient concentrations in bottom waters.</td>
</tr>
<tr>
<td>Archidiakonka</td>
<td>ARC</td>
<td>53°11’57”</td>
<td>18°36’32”</td>
<td>6.9</td>
<td>Yes</td>
<td>P</td>
<td>Agriculture fields and settlement. Heavily impacted by nutrients.</td>
</tr>
<tr>
<td>Płużnie Duże</td>
<td>PLI</td>
<td>53°36’</td>
<td>19°01’24”</td>
<td>21.6</td>
<td>No</td>
<td>D</td>
<td>Agriculture fields.</td>
</tr>
<tr>
<td>Czarne</td>
<td>CAR</td>
<td>53°17’06”</td>
<td>19°15’42”</td>
<td>13.5</td>
<td>Yes (minor)</td>
<td>D</td>
<td>Agriculture fields.</td>
</tr>
<tr>
<td>Priamy</td>
<td>PRI</td>
<td>53°32’10”</td>
<td>20°36’55”</td>
<td>20.5</td>
<td>No</td>
<td>M</td>
<td>No human impact. Highest nutrient concentrations in bottom waters.</td>
</tr>
<tr>
<td>Brożówka</td>
<td>BRO</td>
<td>54°05’34”</td>
<td>21°57’</td>
<td>8.7</td>
<td>No</td>
<td>P</td>
<td>Mostly forest but large farm nearby.</td>
</tr>
<tr>
<td>Łazduny</td>
<td>LAZ</td>
<td>53°51’24”</td>
<td>21°57’30”</td>
<td>22.5</td>
<td>No</td>
<td>D</td>
<td>No human impact.</td>
</tr>
<tr>
<td>Żabińskie</td>
<td>ZAB</td>
<td>54°07’54”</td>
<td>21°58’59”</td>
<td>41.5</td>
<td>Yes (minor)</td>
<td>D</td>
<td>Limited human impact.</td>
</tr>
<tr>
<td>Łękuk Wielki</td>
<td>LEK</td>
<td>54°07’32”</td>
<td>22°01’41”</td>
<td>12.8</td>
<td>Yes</td>
<td>D</td>
<td>No human impact.</td>
</tr>
<tr>
<td>Kępno</td>
<td>KEP</td>
<td>53°46’12”</td>
<td>22°06’36”</td>
<td>18.0</td>
<td>Yes</td>
<td>D</td>
<td>No human impact.</td>
</tr>
<tr>
<td>Dobrzyń</td>
<td>DOB</td>
<td>53°62’41”</td>
<td>22°08’02”</td>
<td>20.9</td>
<td>Yes</td>
<td>D</td>
<td>Large farm nearby. Lake may be impacted by nutrient transport.</td>
</tr>
<tr>
<td>Szóstak</td>
<td>SZOS</td>
<td>53°58’34”</td>
<td>22°09’09”</td>
<td>24.5</td>
<td>Yes (minor)</td>
<td>D</td>
<td>Agriculture fields and settlement. However, lake is not eutrophic, and water transparency is high.</td>
</tr>
<tr>
<td>Szurpily</td>
<td>SUR</td>
<td>54°13’48”</td>
<td>22°53’53”</td>
<td>42.0</td>
<td>Yes (minor)</td>
<td>D</td>
<td>Catchment deforested but mainly meadows (no intensive agriculture). Human impact limited.</td>
</tr>
</tbody>
</table>

D, dimictic; P, polymictic; M, meromictic.

Fig. 1. Location of the 14 studied lakes in northern Poland.
Measurements of limnological variables (conductivity, dissolved oxygen, pH and turbidity) were taken at different depths using a YSI 6820 meter (Yellow Spring Instruments, USA). As chrysophytes live in the upper waters, environmental data at 2 m below the lake surface corresponding to November 2012 and 2013 were used for all the analyses. High correlation between annual environmental data from TS1 and November 2012 indicates that autumn data are representative for annual means (Tab. 2). More details about the selection of the lakes and physical, chemical and morphological parameters are provided in Hernández-Almeida (2015a, 2015b).

Lakelands of northern Poland are typical components of the lowland postglacial landscape formed mainly during the Last Glacial Maximum (~21-14.5 ka BP) (Marks, 2002). This region is a mosaic and heterogeneous in respect to land forms (postglacial outwash, moraine hills, and fluviatile material) and land use (arable plots, wood lots, meadows and wetlands) (Hillbricht-Ilkowska, 1993). The spatial extent of the study area is large (52°31’- 54°19’ N, 14°37’-22°53’ E), covering more than 700 km from the westernmost to the easternmost lake. The fourteen lakes included in this study are generally small (<20 ha), below 260 m asl, between 6 and 44 m deep and circumneutral to alkaline (pH ranging 7.4-8.5) (Tab. 3), mostly dimictic, and with mod-

### Tab. 2. Linear correlation between annual (November 2011-November 2012) and November 2012 (TS1) water chemistry and limnological measurements.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mg L⁻¹)</td>
<td>2.9 (0.6)</td>
<td>2.1 (0.6)</td>
<td>2.1 (0.6)</td>
</tr>
<tr>
<td>K⁺ (mg L⁻¹)</td>
<td>0.6 (0.3)</td>
<td>0.6 (0.3)</td>
<td>0.6 (0.3)</td>
</tr>
<tr>
<td>Mg²⁺ (mg L⁻¹)</td>
<td>3.5 (1.7)</td>
<td>2.3 (1.7)</td>
<td>2.3 (1.7)</td>
</tr>
<tr>
<td>Ca²⁺ (mg L⁻¹)</td>
<td>44.3 (27.6)</td>
<td>34.0 (27.6)</td>
<td>34.0 (27.6)</td>
</tr>
<tr>
<td>HCO₃⁻ (mg L⁻¹)</td>
<td>132.7</td>
<td>128.1</td>
<td>128.1</td>
</tr>
<tr>
<td>SO₄²⁻ (mg L⁻¹)</td>
<td>5.6 (2.7)</td>
<td>7.4 (2.7)</td>
<td>7.4 (2.7)</td>
</tr>
<tr>
<td>Fluorides (mg L⁻¹)</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>Cl⁻ (mg L⁻¹)</td>
<td>2.6 (1.5)</td>
<td>1.1 (1.5)</td>
<td>1.1 (1.5)</td>
</tr>
<tr>
<td>Total N (µg L⁻¹)</td>
<td>0.6 (0.3)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>Total P (µg L⁻¹)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>pH</td>
<td>7.9</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>1.3</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Chlorophyll-a (µg L⁻¹)</td>
<td>2.5</td>
<td>5.8</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Tab. 3. Minimum, maximum and mean values for the climatic (in bold), water chemistry and limnological variables measured during the sampling periods TS1, TS2, and merged.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual temperature (°C)</td>
<td>6.8 (5.4)</td>
<td>6.3 (5.4)</td>
<td>6.3 (5.4)</td>
</tr>
<tr>
<td>MAM temperature (°C)</td>
<td>7.6 (5.4)</td>
<td>5.3 (5.4)</td>
<td>5.3 (5.4)</td>
</tr>
<tr>
<td>DJF temperature (°C)</td>
<td>-3.6 (-6.0)</td>
<td>-4.4 (-6.0)</td>
<td>-4.4 (-6.0)</td>
</tr>
<tr>
<td>JJA temperature (°C)</td>
<td>16.4 (18.0)</td>
<td>17.3 (18.0)</td>
<td>17.3 (18.0)</td>
</tr>
<tr>
<td>SON temperature (°C)</td>
<td>8.0 (8.6)</td>
<td>8.2 (8.6)</td>
<td>8.2 (8.6)</td>
</tr>
<tr>
<td>Warm-cold (°C)</td>
<td>16.5 (18.0)</td>
<td>21.4 (23.8)</td>
<td>21.4 (23.8)</td>
</tr>
<tr>
<td>Annual ppt. (mm)</td>
<td>43.0 (61.0)</td>
<td>41.7 (60.0)</td>
<td>41.7 (60.0)</td>
</tr>
<tr>
<td>DB4°C</td>
<td>94.0 (116.0)</td>
<td>85.0 (139.0)</td>
<td>85.0 (139.0)</td>
</tr>
</tbody>
</table>

MAM, March April May; DJF, December January February; JJA, June July August; SON, September October November.
erate agricultural activity and/or forestry in the catchments (Tab. 1). The climate of the area is temperate, lying in the transition between oceanic and continental air masses and declining air temperature towards the east, particularly in winter and spring (Przybylak, 2010). Several studies have highlighted the importance of the longitudinal climatic gradient in northern Poland on biotic and abiotic factors in the natural environment, such as tree ring-growth of pines (Zielski et al., 2010) or ice-cover duration in lakes (Wrześniowski et al., 2013). The studied lakes are frozen normally between December and April with a mean W-E gradient in duration of ice-cover equal to ca. 45 days (Marszelewski and Skowron, 2006, 2009).

Meteorological data

The meteorological data consisted of monthly air temperature and atmospheric precipitation. All data adjusted for inhomogeneities, were obtained from the Institute of Meteorology and Water Management National Research Institute of Poland database. The meteorological stations for air temperature and precipitation were closer than 50 km, and 20 km from the lake, respectively. The instrumental data were interpolated for the position of the lakes following Alexandersson (1986) and Alexandersson and Moberg (1997). The ‘warm-cold’ variable refers to the difference in air temperature between summer-autumn, characterized by ice-free conditions, and winter-spring seasons, in which ice-cover typically develops.

Laboratory methods

Major ion concentration (Ca²⁺, Mg²⁺, Na⁺, K⁺, SO₄²⁻, F⁻, Cl⁻) were determined by ion chromatography (ICS 1100, Dionex, USA) equipped with an IonPack AS22 column for anions and an IonPack CS16 column for cations. Nutrient (total P -TP- and total N -TN-) analyses were performed using the colorimetric method and a Spectroquant NOVA 400 spectrophotometer (Merck).

Chrysophyte cyst analyses

Analyses of chrysophyte cysts from sediment traps from TS1 (Hernández-Almeida et al., 2015a, 2015b) and TS2 (this study) were made following the standard diatom procedures with HCl and H₂O₂ digestion (Battarbee, 1986), and repeated washing with distilled water. Samples were sieved with Milipore nylon-filters (60 µm) to remove particles outside the potential size range of cysts. Cysts were analysed using a scanning electron microscope (Carl-Zeiss EVO40). A minimum of 300 modern cysts were counted per sample. The cyst identification criteria followed PEARL (Paleoecological Environmental Assessment and Research Laboratory), Duff et al. (1995), Kamenik et al. (2001), Pla (2001), Wilkinson et al. (2001) and Huber et al. (2009). The new cysts found in the Polish lakes were assigned a new number using the prefix ‘TSP’ (Training Set Poland).

Numerical methods

Density plots comparing the mean values in several climatological and environmental variables between 1961-2010, with the respective mean values for TS1 and TS2, were used to evaluate whether the sampling years represented normal or unusual conditions at the lakes. Paired t-tests were used to test if there were significant differences between environmental and climatic conditions during TS1 and TS2 (Tab. 4). Prior to statistical analyses, environmental variables were explored for normal distribution. Water chemistry variables (except pH) were log transformed. Chrysophyte cyst data were expressed in percentages. Species abundances were squared root transformed to stabilize their variances. All species present in at least 2 training set lakes with abundance >1% were retained in the numerical analyses. Main patterns in the environmental and climatic variables and the corresponding lakes were explored by principal component analyses (PCA) (Aitchison, 1983). The significance of the PCA axes was assessed by the broken stick model.

Non-metric multidimensional scaling (NMDS) based on a Bray-Curtis similarity matrix (Minchin, 1987) was used to examine the unconstrained variation of chrysophyte cyst assemblages among sites from the two different years. Rare species were down-weighted to reduce their influence in the ordination. Vectors of environmental variables were then plotted passively onto the NMDS to assess which ones are correlated with the main variability of species assemblages using the ‘envfit’ function. This

Tab. 4. Results of the paired t-tests of differences of the climatic variables and water chemistry and nutrients between TS1 and TS2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAM temperature (°C)</td>
<td>41.51</td>
<td>3.31e-15</td>
</tr>
<tr>
<td>DJF temperature (°C)</td>
<td>24.78</td>
<td>2.49e-12</td>
</tr>
<tr>
<td>JJA temperature (°C)</td>
<td>-17.91</td>
<td>1.51e-10</td>
</tr>
<tr>
<td>SON temperature (°C)</td>
<td>-0.13</td>
<td>0.89</td>
</tr>
<tr>
<td>Warm-cold (°C)</td>
<td>-47.75</td>
<td>5.42e-16</td>
</tr>
<tr>
<td>Annual ppt. (mL)</td>
<td>-1.00</td>
<td>0.33</td>
</tr>
<tr>
<td>Fluorides (mg L⁻¹)</td>
<td>-9.52</td>
<td>3.15e-07</td>
</tr>
<tr>
<td>Total N (µg L⁻¹)</td>
<td>-0.88</td>
<td>0.38</td>
</tr>
<tr>
<td>Total P (µg L⁻¹)</td>
<td>1.95</td>
<td>0.07</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻¹)</td>
<td>10.22</td>
<td>1.39e-07</td>
</tr>
<tr>
<td>DOC (mg L⁻¹)</td>
<td>-0.23</td>
<td>0.81</td>
</tr>
<tr>
<td>pH</td>
<td>1.00</td>
<td>0.33</td>
</tr>
<tr>
<td>Chlorophyll-a (µg L⁻¹)</td>
<td>-0.32</td>
<td>0.74</td>
</tr>
</tbody>
</table>

MAM, March April May; DJF, December January February; JJA, June July August; SON, September October November.
RESULTS

The winter temperatures during TS2 values were colder (>1SD) than the mean of the last 50 years, whereas TS1 values were close to the mean (Fig. 2a). In spring, TS1 was warmer (>3SD) and TS2 is colder (2SD) than the 1961-2010 mean temperatures (Fig. 2b). In summer, warmer conditions than the last 50 years occurred during TS1 (>1SD) and TS2 (>4SD), as well as in autumn (>3SD for TS1 and TS2) (Fig. 2c,d). Finally, mean ‘warm-cold’ temperature contrast of TS2 shows the greatest difference relative to the mean from 1961-2010 (>6SD), while TS1 value was close to the mean (Fig. 2e).

Average values for each lake of selected biologically important chemical and climatic variables during TS1 and TS2 are shown in Fig. 3. Temperature-related variables and conductivity show marked and consistent changes between both years. Winter and spring seasons were markedly colder and the summer was warmer during TS2. It is noticeable that, for winter and spring, there is also a W-E temperature gradient in both years, which is also visible in the ‘warm-cold’ variable (Fig. 3b). Conductivity also shows higher values during TS2, although there is no longitudinal gradient. Results of paired t-test reveal that only F-, conductivity and seasonal temperature variables, except SON (September October November) temperatures, show statistically significant differences between the two studied periods (Tab. 4).

The two first axis of the PCA (significant according to the broken stick model) explained 53.7% of the variance (Fig. 4). Most chemical parameters were independent of climatic parameters, especially of winter, spring, annual temperatures and the seasonal ‘warm-cold’ contrast. Water chemistry variables and nutrients (TN and TP) are weakly correlated to depth and annual precipitation. The lakes from the two different years show consistent relative positions along PCA1, which correlate with the water chemistry variables (Fig. 4). Lake ARC is the most eutrophic lake (highest TN and TP), with highest conductivity and ion content. This can be attributed to its location close to agricultural fields (Tab. 1). The low trophic state and conductivity in Lake SMK is explained by the lack of important human activities in its catchment. Lakes show a marked shift along climate variables (correlated with PCA2) during both years (Fig. 4). The position of the lakes in the ordination space shifted mainly along temperature related variables [DJF (December January February), MAM (March April May) and ‘warm-cold’]. Air temperatures at Lake BLO, located in western Poland, are among the warmest during both years, while those from Lake SUR, located in eastern Poland, are among the coldest in both years (Fig. 1).

Using SEM, 130 cyst types were identified for TS1 and 83 for TS2. After removal of the least common cyst types (<1% abundance, and present in less than 2 samples), 45 types remained in samples from TS1 and 52 in TS2 (61 types in total when both years were merged). Some cyst types show clear differences in the abundances between both periods. Cyst types PEARL 41, 31, 114/115, 164 and 210 are more common and show higher percentages in during TS2, while PEARL 1, 114/115, 164 and 210 are more common and show higher abundance during TS1 (Fig. 5).

RDA and stepwise selection was used to identify which variables were most important for the chrysophyte data during TS1 and TS2, and both years. Non-significant variables were entered as passive variables onto species ordination to avoid overfitting (grey arrows in Figs. 6 and 7). The gradient lengths of the DCA1 for TS1 and TS2 are short, 1.21 and 1.46 SD, respectively. During both individual years, water chemistry variables were the most
important ones; with the only difference that pH was only significant during the first year. In TS1, TN, TP and pH explain together 14.9% of the variance (Fig. 6a) while, in TS2 (Fig. 6b), TN and TP explain 9.1%. Temperature-related variables (seasonal and ‘warm-cold’ temperature) are not significant in any sampling period. However, when both datasets are merged, the lengths of the gradients and significant variables are different from the individual

Fig. 2. Density plots showing the distribution of seasonal temperature variables in the 14 lakes from northern Poland for the period 1961-2010. a) DJF, December January February; b) MAM, March April May; c) JJA, June July August; d) SON, September October November; e) ‘warm-cold’ temperatures. The vertical color lines represent the mean temperature value for the period 1961-2010 (red), mean temperature value for Training set 1-year (TS1, yellow), and mean temperature value for Training set 2-year (TS2, blue). The horizontal bar represents ±1 SD from the mean temperature value for each variable for the period 1961-2010.
Fig. 3. Barplots showing the W-E distribution of some variables relevant for the study during TS1 (yellow) and TS2 (blue). a) pH; b) ‘warm-cold’ season; c) total nitrogen; d) annual precipitation; e) total phosphorous; f) conductivity. X-axis represents lake codes which are shown in Tab. 1.
years (TS1 and TS2 separately). Temperature-related variables [JJA (June July August), MAM and ‘warm-cold’ contrast] arise as important variables due to the large temperature contrast between both years (Fig. 7). Variance partitioning shows that the explained variance by the subset of climatic variables (JJA, MAM and ‘warm-cold’ contrast) is 8.5%, while the explained variance by the water chemistry variables that were significant during the individual years (TN, TP and pH) is 10.3%. Partial RDAs revealed that 2% of variance in the cyst dataset was shared between the climatic and the water chemistry variables.

The NMDS ordination shows a clear distribution of TS1 and TS2 along NMDS1 (Fig. 8). When environmental variables are passively plotted on the NMDS ordination space, we observed that there is a general trend for temperature related variable to be more strongly correlated with NMDS1. The NMDS plot with fitted contour lines of ‘warm-cold’ temperatures (Fig. 8) shows the distribution of TS1 and TS2 sites along this variable.

DISCUSSION

The comparison between the individual RDAs of two different years (Fig. 6 a,b) depicts interesting results: during TS1 and TS2, nutrients (TN, TP) and pH (only during TS1) were significant and explained the highest amount of variance in the chrysophyte dataset. Air temperatures were generally less important. It is worth noting that pH was found to be important during TS1 despite the short pH gradient (7.9-8.3), reflecting the importance of this factor in determining the composition of cyst assemblages in lakes, as it has been demonstrated by several studies the lakes from Central European and Canada (Rybak et al., 1991; Siver et al., 1995; Facher and Schmidt, 1996; Pla et al., 2003). The low inter-annual variability in pH indicates that lakes are well buffered.

![Fig. 4. Main ordination axes of a principal component analyses of the merged variable dataset (TS1 and TS2).](image-url)

![Fig. 5. Relative abundance of most common (present in at least two sites, maximum abundance >5%) cyst types in the 14 study lakes in northern Poland (TS1, yellow; TS2, blue).](image-url)
Nutrient status (TN and TP) influenced the chrysophyte assemblages during both sampling periods (Fig. 6). Several studies have reported shifts in chrysophyte assemblages along TP and TN gradients (Sandgren, 1988; Pla et al., 2003; Pla and Anderson, 2005). Interestingly, the nutrient status of the lakes (defined by their relative positions along NMDS2), did not change notably between the two years (Fig. 8). Differences in nutrient content between the fourteen studied lakes are mainly related to land-use in the catchment area (Tab. 1). Nutrient concentrations in northern Polish lakes are correlated with TN and TP loads emerging from the catchment (Pasztaleniec and Kutyla, 2015). Human activity (especially agriculture) in the catchment, and flushed forest detritus are key drivers of nutrient loadings to northern Polish lakes (Hillbricht-Illkowska, 1993, 1999; Klimaszyk and Rzymski, 2011).

This situation changes when both years are combined in the RDA, where JJA, MAM and ‘warm-cold’ temperatures become significant variables (Fig. 7). This is explained by the stronger inter-annual variability of the climate-related variables (Tab. 3). The indirect gradient analysis illustrates this situation well (Fig. 8). Although some changes in the cyst assemblages along the NMDS2 are attributed to nutrient levels in the lakes, a major shift in the cyst assemblage composition between years occurs along the NMDS1, which is related to climate related variables, such as the seasonal changes between the cold and the warm season air temperatures. This is in agreement with previous research on chrysophyte-based transfer functions (Kamenik and Schmidt, 2005; Pla and Catalan, 2005; De Jong and Kamenik, 2011; Pla-Rabes and Catalan, 2011; De Jong et al., 2016; Hernández-Almeida et al., 2015b) where temperature related variables such as the length of winter, the length of the ice-cover period and the timing of spring mixing were found to be important variables explaining the variance of chrysophyte cyst assemblages in lake sediments.

It has to be noted that the amount of variance explained by climatic (JJA, MAM and ‘warm-cold’ contrast: 8.5%) and the water chemistry variables (TN, TP and pH: 10.3%), although not very high, is in the same range than other studies using chrysophyte cyst assemblages, such as Pla et al. (2001, 2003), and Kamenik and Schmidt (2005). This is likely due to the inclusion in the training set of all kinds of lakes with very different characteristics, without limiting sources of variability.

Over the two years of monitoring, seasonal air temperatures exhibited strong variations, but also relative to the mean temperatures from 1961-2010 (Fig. 2). The greatest difference is observed in the ‘warm-cold’ contrast in TS2, whose difference from the mean is > 6SD, while TS1 is close to the mean of the last 50 years (Fig. 2e). This indicates that the two years represent quite different climate conditions over the region of study. During TS1 conditions were typical for oceanic climate, with few extremes, a mild cold season and a moderately warm season during the year 2011-2012 (low ‘warm-cold’ contrast, Fig. 2e). In contrast, TS2 was characterized by more continental climate, with more extreme temperatures (high ‘warm-cold’ contrast, Fig. 2e). During TS2, the summer

Fig. 6. Redundancy analyses (RDA) ordination triplot of sediment trap chrysophyte cyst assemblages from northern Poland, for (a) TS1 and (b) TS2. Statistical significant variables (reduced model) used in the constrained ordination are in black, while the grey ones are non-significant variables which were entered passively onto the ordination. Sites are displayed as colored dots, species as white dots.
was warmer, and hence the period of thermal water column stratification was longer. This inter-annual and inter-seasonal temperature variability was possibly related to the North Atlantic Oscillation (NAO). TS1 was characterized by positive NAO conditions in winter and negative NAO conditions in summer, while TS2 experienced a very different pattern, with slightly positive NAO conditions in winter, and positive NAO in summer (NAO index data from Climatic Research Unit, University of East Anglia, http://www.cru.uea.ac.uk/~timo/datapages/naoi.htm). The strong influence of the NAO on winter Polish temperatures has been shown by Luterbacher et al. (2010). In summer, although lower than in winter, there is still significant positive correlation between summer NAO in northern Europe and the eastern Baltic (Folland et al., 2009). The shift from oceanic to continental climate would affect the length of ice-cover in northern Poland (Wrzesiński et al., 2013), the duration of the summer stratification and also the timing of lake mixing phases (Skowron, 2012). Consequently changes in the NAO would affect the length and intensity of spring and autumn overturn periods, which are affecting chrysophytes cyst assemblages (Pla-Rabes and Catalan, 2011).

The link between chrysophyte cysts and climate likely occurs indirectly, via changes in thermal structure that determine the annual water column mixing cycle, and nutrient and light availability (Sandgren, 1988). Chrysophyte cyst assemblages (Fig. 7) were characterized by the dominant genera *Bosmina*, *Daphnia*, and *Lepidodora*, which are known for their ability to thrive in warmer conditions. The redundancy analysis (RDA) ordination triplot (Fig. 7) showed that the winter NAO conditions were positively correlated with the cyst assemblages from TS1, while TS2 showed a different pattern with a slight positive NAO in winter and positive NAO in summer. The non-metric multidimensional scaling (NMDS) ordination plot (Fig. 8) further confirmed these trends, with the variable “warm-cold” showing a strong correlation with the assemblages from TS1. These results suggest that changes in the NAO can significantly influence the distribution and abundance of chrysophyte cysts in sediment traps in Poland.
cysts show a large seasonal replacement, and different species flourish under different environmental conditions through the year (Sandgren, 1988; Eloranta et al., 1995; Siver et al., 1995; Pla-Rabes and Catalan, 2011). Fig. 5 shows the composition and replacement of the cyst assemblages during the two periods of study. Some cyst species found typically under temperate conditions show higher percentages and/or are present in more lakes during TS1. Cyst type PEARL 79 is produced by a chrysophyte species (Dynobrion sociale var. americanum; Betts-Piper et al., 2004) which lives mainly during the spring season (Brook and Woodward, 1956). Other cyst types, like PEARL 1 and 180 were found in low elevation lakes in the Pyrenees (coded as ST001 and ST180, respectively) (Pla, 2001; Pla and Catalan, 2005), and have been found positively correlated to October-March temperatures in sediments of an alpine lake in Switzerland (Kamenik et al., 2010). In contrast, cyst types living in colder winter and spring conditions have been found more abundant during TS2. PEARL 31 has been related to chrysophyte species tolerant to cold waters (Adam and Mahood, 1981; Duff and Smol, 1989). Similarly, PEARL 41, 164 and 210 have been found at high elevation lakes in the Pyrenees, related to colder temperatures in winter and spring (Pla, 2001; Pla and Catalan, 2005). PEARL 114/115 and 178 have been previously recorded as typical of arctic and mountain lakes, characterized by oligotrophic and/or cold waters (Stewart et al., 2000; Betts-Piper et al., 2004; Sórorczi et al., 2014). Hence, different mixing regimes would change the structure of the community (Sandgren, 1988; Reynolds, 2006) and, consequently, the final structure of the stomatocyst assemblage (Pla-Rabes and Catalan, 2011). Kamenik and Schmidt (2005) hypothesized, and Pla-Rabes and Catalan (2011) explained, a possible limnological mechanism related to resources availability. According to their working hypothesis, chrysophytes might thus be affected by the timing of spring ice-off, which determines the length and intensity of the spring overturn period and, consequently, the release of nutrients from the hypolimnion into the photic zone for the main growing season.

Organic matter remineralisation in the hypolimnion is higher during cold and long winters with development of ice-cover, increasing the dissolved nutrient content in deep waters (Catalan et al., 2002). However, it also implies a late and shorter spring mixing; consequently, nutrients are not completely recycled and could remain available for the autumn overturn. In contrast, if conditions were more oceanic (as occurs with the NAO+ during winter 2012), spring overturn would start early and would be longer (until summer stratification could be set), which would enhance the release of nutrients (in-lake recycling) into the water column and set quite different environmental conditions for algal growth (i.e., light environment and nutrients). A stronger summer stratification would also imply different environmental conditions such as nutrient depletion and hydrodynamics in the epilimnion during the summer season, presumably affecting chrysophyte composition and encystment patterns during the warm season (Agbeti and Smol, 1995; Pla-Rabes and Catalan, 2011).

Our study confirms what has been found by other sediment trap monitoring studies of planktonic communities in lakes across Europe, which observed similar inter-annual ecological shifts as a consequence of climate variability. De Hoyos and Comín (1999) demonstrated that inter-annual changes of the relative abundance of different phytoplanktonic groups (e.g., diatoms, chrysophytes, dinoflagellates, cyanobacteria) in Lake Sanabria (Spain), were largely due to the differences in the water turnover rate which, in turn, is caused by climatic factors. Similar results were found by Forsström et al. (2005) in a lake in the Finnish Lapland, and described the inter-annual differences in phytoplankton community structures as the result of changes in the length of the mixing cycle and the water temperature. A relationship of phytoplankton communities in lakes distributed across Europe with an extreme shift in winter conditions driven by the NAO was also found by Weyhenmeyer et al. (2003).

Here, we have shown that although regional variability in water chemistry variables and nutrients is very important for cysts in northern Poland, inter-annual shift in cyst assemblages responds mainly to large differences in seasonal meteorological conditions between two sampling years. Our results provide important constraints for transfer function studies. In regions with limited climatic gradients across the region of the training set, the study of biological assemblages using sediment trap samples from different lakes over repeated years may increase the climate gradient of interest taking advantage of the inter-annual climate variability. This sampling technique can be extended to other planktonic groups (e.g., diatoms, Cladocera) in order to obtain a modern dataset that can reflect regional-scale variables (climate), and not just variables that are more sensitive to local conditions (nutrients). These results, therefore, have also important implications for the interpretation of the fossil record in lake sediments, as they provide detailed information of the effects of regional climatic variability on biological communities.

**CONCLUSIONS**

Our study of inter-annual chrysophyte cyst assemblages from 14 lakes in northern Poland shows that while water chemistry and nutrients are the most important variables for the cyst distribution in this region, climatic variables are mainly responsible for the inter-annual shift in cyst assemblages. During individual years, environmental variables related to nutrient content were the most signif-
icant ones for the chrysophyte cyst assemblages, because the climate gradients were short. However, when datasets of two different years were merged, air temperature variables became important. The shift from oceanic to continental climate across both years drove the length of ice-cover, the timing of lake mixing phases, and the period of summer stratification; phenomena that control nutrient input and light in the epilimnion, which have a strong influence on the succession of cyst species throughout the year. This new approach of sampling during two years with very different meteorological conditions produced a valuable training set sensitive to climate variables, which otherwise would have remained not significant compared to the larger spatial variability of other limnological variables. More studies using long sediment trap monitoring of lakes in a training-set will provide important information in order to understand better the palaeo-records and improving transfer functions.

ACKNOWLEDGMENTS

We would like to thank CLIMPOL project members for their support. Thanks also to Martin Schmidt for laboratory and SEM assistance. We are very grateful to Oliver Heiri and André F. Lotter for the fruitful discussions about the general concept of the paper. We also thank John P. Smol, an anonymous referee and Aldo Marchetto (Editor) for comments on this manuscript. This research was supported by a grant from Switzerland through Swiss Contribution to the enlarged European Union (CLIMPOL PSPB-086/2010).

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