Chrysophyte resting stages: a tool for reconstructing winter/spring climate from Alpine lake sediments

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Chrysophyte algae produce siliceous resting stages (stomatocysts) that are indicators of past environmental conditions. The objective of this study was to assess their strength for climate reconstructions. Stomatocysts were collected using sediment traps exposed in 45 mountain lakes (1502–2309 m a.s.l., Austrian Alps). Bihourly water-temperature measurements were used to determine dates of freezing and break-up, spring and autumn mixing. Canonical correspondence analyses revealed that the stomatocyst assemblages were related to the dates of ice break-up and spring mixing. The two dates are controlled by winter/spring air temperature. We developed a weighted averaging–partial least squares (WA-PLS) stomatocyst/date-of-spring-mixing regression and calibration model ($R^2_{\text{boot}} = 0.85$), and reconstructed 'dates of spring mixing' for Jezero v Ledvici (1824 m a.s.l., Slovenian Alps) from AD 1842 to 1996. Sample-specific standard errors of prediction corresponded to 0.6°C – 1.0°C. Despite dating uncertainties and poor fits of fossil assemblages with the training set, reconstructed 'dates of spring mixing' were significantly correlated with the mean March–April air temperature, which is known to drive break-up dates. Furthermore, the record was in agreement with glacier advances during the Little Ice Age.

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Global warming is one of the major issues confronting mankind and has vital ecological and economic consequences. Climate data beyond the instrumental period are essential if we are to understand the climate system and to forecast future climate (Bradley 2000). Mountain lakes are sensitive to climate changes and information about past climate is stored in their sediments (Psenner & Schmidt 1992; Battarbee *et al.* 2002; Catalan *et al.* 2002b). Sedimentary records, such as chironomid and diatom remains, have been used to reconstruct climate throughout the Holocene (Heiri *et al.* 2003; Schmidt *et al.* 2004a). However, multiple records are needed for more reliable and more detailed (e.g. seasonal) climate reconstructions (Battarbee 2000).

Chrysophytes, the 'golden algae' (classes Chrysophyceae and Synurophyceae), are an essential component of mountain lakes (Rott 1988; Fott *et al.* 1999). They produce siliceous resting stages (stomatocysts or simply cysts) that are abundant and diverse in mountain-lake sediments (Pla 2001). Alpine and subalpine cyst assemblages have been shown to respond to changes in water chemistry, such as the acid-base balance, conductivity and nutrients (Facher & Schmidt 1996; Lotter *et al.* 1997; Pla *et al.* 2003; Kamenik *et al.* in press b). Because water chemistry is linked to catchment characteristics (Kamenik *et al.* 2001b), these changes are often related to deforestation, pasturing or erosion (Kamenik *et al.* 2001a; Brancelj *et al.* 2002; Kamenik *et al.* in press b). In addition, environmental stressors, such as atmospheric pollution, can affect cyst assemblages (Betts-Piper *et al.* 2004; Kamenik *et al.* 2005, in press a).

Cysts are potential indicators of past climate change because of their distinct seasonality (Smol & Cumming 2000; Kamenik *et al.* 2001a; Catalan *et al.* 2002b). Changes in sedimentary cyst assemblages were shown to correlate with trends in past air temperature (Kamenik *et al.* 2001a; Koinig *et al.* 2002; Catalan *et al.* 2002a; Šporka *et al.* 2002). Pla & Catalan (2005) used cyst assemblages for tracking past changes in the 'altitude anomaly' of a Pyrenean lake, which they assumed to be a proxy for winter/spring climate.

In this article, we examine the potential of cyst assemblages for climate reconstruction in sensitive Alpine lakes. We (i) study the response of cyst assemblages produced during one year to climate-related variables such as water temperature and freeze/break-up dates, (ii) develop a quantitative regression and calibration model for the climate-sensitive variable 'date of spring mixing', (iii) present a cyst-based climate reconstruction for Jezero v Ledvici (Lake Ledvica, NW Slovenia), and (iv) validate the

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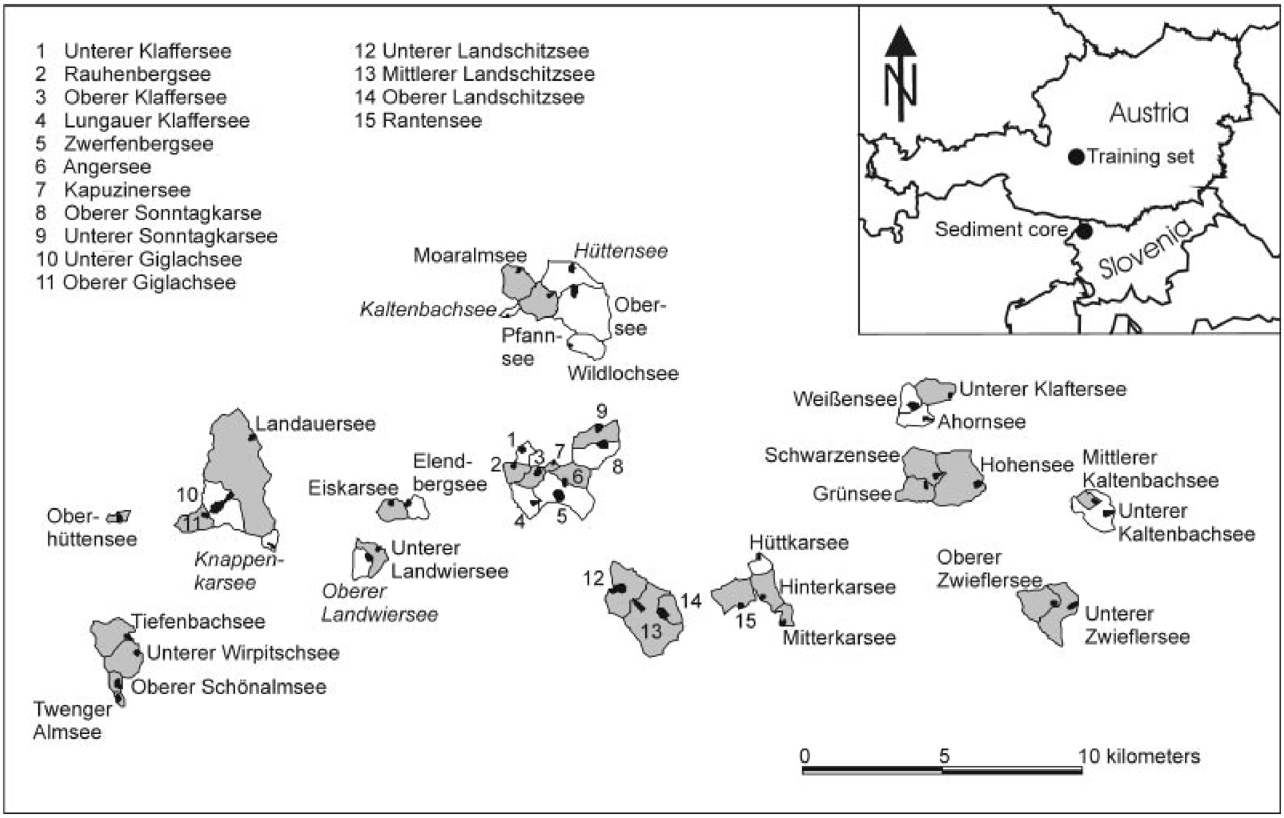

Fig. 1. Location of the training set and of the coring site Jezero v Ledvici, which is located 100 km to the south (inset). The 45 training-set lakes and their catchments are shown in detail. After 1 year of exposure, we recovered 73% of the sediment traps. Our cyst-based 'date of spring mixing' inference model is based on 29 sediment-trap samples from lakes indicated by grey catchments. Lake names given in italics indicate model-specific outliers.

reconstruction with meteorological data and known glacier fluctuations. Autecological information on specific cyst types can be found in Kamenik *et al.* (in press b).

Study area

Training set: Niedere Tauern

We selected 45 lakes along an altitude gradient (1502–2309 m a.s.l.) which corresponds to a change in air temperature of *c.* 4.6°C (Agustí-Panareda & Thompson 2002). The lakes stretch from the sub-alpine forested belt up to the mid-alpine zone. They are located in the Central Alps (Niedere (Schladminger) Tauern; 47°13′ – 47°21′ N, 13°36′ – 14°04′ E; Fig. 1) and were chosen to be as pristine as possible, while encompassing a wide spread of geographic settings, water chemistries and basin morphologies. Their watersheds of predominantly crystalline bedrock, schists and metamorphic carbonates lack glaciers. The oligo- to ultra-oligotrophic lakes are not acidified. They combine four major environmental gradients related to climate, trophic status, catchment characteristics and lake morphology (Table 1). Their water chemistry is mainly determined by changes in bedrock, from silicate to carbonate minerals, affecting pH, alkalinity, conductivity, calcium and magnesium, and by trophic variables such as total phosphorus, dissolved organic carbon, potassium, total inorganic nitrogen and total dissolved reactive silica (Kamenik *et al.* 2001b).

Climate reconstruction: Jezero v Ledvici

Lake Ledvica is located in the treeline ecotone (1824 m a.s.l.) of the karstic limestone Valley of Seven Triglav Lakes (46°20′30″ N, 13°47′20″ E; Fig. 1) in the centre of the Triglav National Park (Slovenia), about 100 km south of the training-set lakes. In contrast to the training-set lakes, Jezero v Ledvici experiences a Mediterranean-type of climate (Agustí-Panareda & Thompson 2002). Monthly mean air temperatures, measured 104 m below the lake, lie slightly below 0°C during about 3 to 4 months of the year; however, daily mean air temperatures only rarely remain below 0°C for longer than a few consecutive days (Brancelj *et al.* 2000). Precipitation is high,

Table 1. Summary statistics and principal components (PC) analysis of 28 environmental variables measured at 32 sites. Lake Pfannsee was excluded because it lacks chemical measurements. PC axes 1–4 represent climate, trophic status, catchment characteristics (bedrock mineralogy and terrestrial vegetation) and water depth, respectively. The four PC axes are significant according to the broken stick model (Jackson 1993). PC scores ≥ 0.7 are presented in boldface.

		Summary statistics						Principal components analysis			
	Units	Mean	Min	P$_{25}$	Median	P$_{75}$	Max	Axis 1	Axis 2	Axis 3	Axis 4
Water chemistry											
pH		7.3	6.9	7.1	7.2	7.4	8.2	−0.36	0.62	−0.62	−0.18
Alkalinity	μeq L^{-1}	233.9	22.0	82.0	137.0	238.0	1364.0	−0.53	0.42	−0.68	−0.14
Conductivity	μS$_{25}$ cm^{-1}	34.9	11.9	20.5	28.3	37.1	137.3	−0.58	0.37	***−0.71***	−0.02
Ca^{2+}	μeq L^{-1}	265.5	70.1	140.8	216.3	275.5	1058.8	−0.52	0.32	***−0.76***	−0.05
Mg^{2+}	μeq L^{-1}	49.5	10.5	16.6	23.9	45.3	303.8	−0.61	0.57	−0.41	0.01
Na^{+}	μeq L^{-1}	18.3	9.7	14.3	18.4	23.2	27.1	−0.48	−0.55	−0.31	0.24
K^{+}	μeq L^{-1}	7.4	3.4	5.4	7.7	9.2	11.6	0.03	***−0.76***	0.19	0.17
SO$_4^{2-}$	μeq L^{-1}	76.2	28.7	59.3	71.6	96.3	175.0	−0.31	−0.17	−0.56	0.63
Cl^{-}	μeq L^{-1}	3.0	1.7	2.3	2.8	3.6	4.8	−0.66	−0.24	0.12	−0.09
TIN	μeqL^{-1}	7.9	0.1	5.3	8.2	10.3	18.1	0.06	***−0.78***	−0.27	−0.09
DN	μg L^{-1}	227	82	182	227	271	395	0.04	***−0.75***	−0.32	−0.01
P$_{tot}$	μg L^{-1}	3.2	1.1	2.2	3.1	4.0	7.8	0.01	0.56	0.36	−0.36
P$_{dis}$	μg L^{-1}	1.5	0.2	1.1	1.4	1.7	3.0	−0.53	0.37	−0.05	−0.40
DOC	mg L^{-1}	0.78	0.40	0.54	0.66	0.94	2.06	−0.21	0.28	***0.77***	−0.21
DRSi	μg L^{-1}	851	279	562	750	1083	1685	−0.32	***−0.79***	−0.18	0.07
Climate-related variables											
Altitude	m a.s.l.	1957	1502	1859	1940	2099	2309	***0.81***	0.37	−0.03	0.19
Ice cover	Days	211	163	199	206	220	277	***0.87***	0.23	−0.19	−0.06
Freezing	Julian days	305	286	303	305	308	326	−0.68	0.09	0.18	0.40
Break-up	Julian days	151	123	142	146	159	198	***0.84***	0.31	−0.18	0.07
A$_{mix}$	Julian days	292	262	286	294	298	302	***−0.84***	0.12	0.16	0.37
S$_{mix}$	Julian days	162	133	147	156	177	206	***0.89***	0.34	−0.12	0.09
T$_{June}$	°C	4.6	0.0	1.3	5.6	7.1	9.1	***−0.86***	−0.31	0.19	−0.16
T$_{July}$	°C	8.7	1.7	7.7	9.2	10.6	12.4	***−0.89***	0.10	0.33	−0.01
T$_{Aug}$	°C	10.9	6.1	10.0	11.0	12.0	13.7	−0.56	0.45	0.46	0.11
T$_{Sept}$	°C	7.7	5.5	6.6	7.7	8.5	9.5	***−0.70***	0.32	0.36	0.13
T$_{Oct}$	°C	4.6	1.6	3.8	4.9	5.4	6.2	***−0.88***	0.12	0.20	0.19
Lake morphology											
Max. depth	m	14.1	5.7	8.0	11.4	18.8	33.6	0.18	0.43	0.22	***0.76***
Rel. depth†	m	7.0	3.0	4.0	6.0	8.4	16.9	0.48	0.47	0.05	0.49

†Ratio of lake-surface area to maximum water depth.

averaging 2500–3000 mm yr^{-1} (Brancelj *et al.* 2000). Ice cover lasts from the end of November until the end of May (Brancelj *et al.* 2002). Jezero v Ledvici has non-permanent inflows and a sinkhole-outflow. The dimictic, neutral to slightly basic (mean pH = 7.8), well-buffered (mean alkalinity = 1400 μeq L^{-1}), oligotrophic (P-PO$_4$ = 2 μg L^{-1}, chlorophyll-*a* <1 μg L^{-1}) hard-water (conductivity = 150 μS$_{25\,cm}$$^{-1}$) lake is situated in a geologically active region. Earthquakes triggered landslides from the steep eastern lakeshore causing irregular sedimentation rates, allochthonous nutrient input, sediment re-suspension and periods of eutrophication (Brancelj *et al.* 2000, 2002; Catalan *et al.* 2002b). However, we selected Jezero v Ledvici because (i) it is located within the altitude range of the training set, (ii) Agustí-Panareda & Thompson (2002) retrodicted mean monthly air temperature from AD 1781 to 1997 for this site, and (iii) from AD 1944–1956 it had a high mean sediment accumulation rate of 0.022 g cm^{-1} yr^{-1} (Brancelj *et al.* 2002). Parts of the sediment core could therefore be sampled at sub-annual resolution, thus matching the high temporal resolution of the sediment-trap based training set.

Material and methods

Stomatocyst analysis

Modern cyst assemblages were collected from summer 1998 to summer 1999 using sediment traps (Bloesch & Burns 1980; Kamenik *et al.* 2001a) which were exposed in the deepest area of the lakes with the tube openings approximately 1.5 m above the sediment surface. We exposed and recovered the traps in between the two main periods of cyst production, i.e. freezing and thawing of the lakes (Agbeti & Smol 1995; Pla 1999; Kamenik *et al.* 2001a). After one year of sediment-trap exposure, we found and recovered 73% of the traps.

Subfossil cyst assemblages were collected from a 30-cm-long sediment core (LEDV5) taken with a modified Kajak gravity corer and sampled in 2 mm intervals (Brancelj *et al*. 2002). The samples were dated with ^{210}Pb, ^{226}Ra and ^{137}Cs using a piecewise CRS (constant rate of ^{210}Pb supply) model (Appleby 2000).

Samples were cleaned using standard diatom procedures (Battarbee 1986: HCl and H_2O_2, and repeated washing in distilled water). Cysts were analysed using a scanning electron microscope (Jeol JSM-35) equipped with an image analysing system (QUANTEL Crystal), enabling detailed cyst identification and counting (Kamenik & Schmidt in press). A minimum of 500 modern and 200 sediment-core cysts were counted per sample. Corroded sediment-core samples with less than 200 cysts per SEM stub were skipped without further analysis. Identification of cysts followed Kamenik *et al*. (in press a, b).

Environmental variables

Kamenik *et al*. (2001b) and Schmidt *et al*. (2004a, b) set out the details of the training set measurements. The survey involved water temperature, lake morphology and water chemistry (Table 1) measured during autumn mixing (18th/19th October 1999), when chemical characteristics were similar throughout the water column. Epilimnion water temperatures were measured at 2-hourly intervals during sediment-trap exposure using two 8-bit MINILOG-TR thermistors (Vemco Ltd) per lake. After one year of thermistor exposure, we found and recovered 73% of the thermistors. The temperature readings were averaged for the months June to October (T_{Jun} to T_{Oct}). Schmidt *et al*. (2004a, b) estimated freeze and break-up dates for each lake by visual examination of individual temperature logs. The reference dates 'date of spring mixing' (S_{mix}) and 'date of autumn mixing' (A_{mix}) were defined as the first day after thawing or summer stratification when the mean daily water temperature was 4°C (Schmidt *et al*. 2004a, b).

Numerical methods

Correlations of cysts with environmental variables. – A preliminary detrended correspondence analysis (DCA, detrending by segments) on the cyst assemblages resulted in a gradient length of 2.2 SD units, suggesting unimodal cyst responses (ter Braak 1987; Birks 1995). Canonical correspondence analysis (CCA) was used to analyse the relationship between cyst assemblages and environmental variables, and to identify outlying samples with unusual values of environmental variables or unusual cyst assemblages (ter Braak 1987). A series of detrended CCAs (DCCA) constrained to a single environmental variable at a time were run to check the influence of each variable on the assemblages (marginal effects), to assess the gradient length of the environmental variable in SD units, and to determine the strength of each variable by its ability to maximize the dispersion of the taxon scores (ter Braak & Juggins 1993). The last was expressed as a ratio of the first constrained DCCA eigenvalue to the second unconstrained DCA eigenvalue (λ_1/λ_2). We used weighted correlations and variance inflation factors (VIF) to identify intercorrelated explanatory variables which were deleted from subsequent analysis; these variables have unstable canonical coefficients and do not merit interpretation (ter Braak & Šmilauer 2002). The minimum set of environmental variables was determined by manual forward selection (ter Braak & Šmilauer 2002). Partial CCAs were run to estimate independent and shared effects of environmental variables on the cyst assemblages (Borcard *et al*. 1992; Lepš & Šmilauer 2003). The significance of explanatory variables, individual CCA and DCCA axes was tested using 9999 unrestricted Monte Carlo permutations (ter Braak & Šmilauer 2002). Probability values were adjusted for multiple testing (P_{adj}) using a Bonferroni-type test procedure (Hochberg 1988).

Cyst abundances were square-root transformed stabilizing their variances. Rare species were down-weighted because the square-root transformation increases their weight and they can have undue influence on the ordination. Cyst types with a maximum abundance $<1\%$ or occurrences in only one lake were disregarded. All environmental variables, except pH (i.e. $-\log_{10}[H^+]$), were $\log_{10}$ transformed to avoid skewed distributions. Linear relationships between $[H^+]$ and other ions were thus retained (Stumm & Morgan 1996). Lake Pfannsee was excluded from all multivariate analyses because it lacked chemical measurements. Ordinations were performed using the program CANOCO 4.5 (ter Braak & Šmilauer 2002).

Transfer functions. – To establish transfer functions for a climate-sensitive environmental variable, the following models were tested: Weighted averaging with (WA_{tol}) and without (WA) tolerance down-weighting, using unweighted classical and inverse deshrinking, weighted averaging – partial least squares (WA-PLS), partial least squares (PLS), and a modern analog technique (MAT), as implemented in the computer program C2 (Juggins 2003). The minimal adequate model was identified as having a combination of a high coefficient of determination (R^2) between observed and predicted values, a low mean and maximum bias, and a low root mean squared error of prediction (RMSEP), all assessed by (bootstrapping and jack-knifing) cross-validation (9999 permutation cycles; Birks 1995). In PLS and WA-PLS, only components leading to a reduction in RMSEP of 5% or more were retained (Birks 1995). The minimal adequate model was examined for potential outliers, because these can strongly affect transfer function coefficients and may markedly decrease the predictive

Table 2. Environmental variables having significant marginal (i.e. percentage of variance they explain individually) effects ($p < 0.01$) and their unique effects on the cyst assemblages (i.e. percentage of variance they explain after effects of other variables have been removed; only for variables with VIF <20). Asterisks indicate significant marginal effects taking into account multiple testing ($P_{adj} < 0.05$). The gradient length presents the amount of compositional change of the cyst assemblages along the environmental variables measured in SD units. The ratio of the first constrained DCCA eigenvalue to the second unconstrained DCA eigenvalue (λ_1/λ_2) determines the strength of each variable by its ability to maximize the dispersion of the taxon scores. The variables are listed according to their λ_1/λ_2. See Fig. 2 for acronyms and a graphical summary.

	Marginal effects	Gradient length	λ_1/λ_2	Unique effects
Ca^{2+}	9.9*	1.93	1.17	–
Conductivity	9.7*	1.81	1.12	–
Alkalinity	9.8*	1.96	1.06	6.0
pH	9.4*	1.67	1.04	7.4
S_{mix}	7.8*	1.48	0.92	6.5
Mg^{2+}	7.4*	1.41	0.89	5.8
T_{July}	7.2*	1.55	0.83	5.0
T_{June}	7.7*	1.18	0.80	–
Ice cover	7.0*	1.69	0.69	5.1
Cl^-	6.3*	1.30	0.67	7.8
Break-up	7.2*	1.43	0.66	–
T_{Oct}	6.3*	1.43	0.60	4.1
Altitude	5.9*	1.32	0.59	5.8
Rel. depth†	5.7*	1.15	0.57	5.2
P_{dis}	5.3	1.63	0.56	6.3
A_{mix}	5.8*	1.60	0.55	4.8
DOC	5.5	1.22	0.55	5.0

†Ratio of lake-surface area to maximum water depth.

ability of the inference model. Outliers were identified as samples having an absolute residual (observed – predicted) higher than the SD of the environmental variable of interest and a low influence on the model indicated by Cook's D (Cook's D <4/n). For model-building and model-specific descriptive statistics, cyst abundances were square-root transformed stabilizing their variances.

Climate reconstruction. – The minimal adequate regression and calibration model was applied to the sediment-core cyst assemblages from Jezero v Ledvici. Reconstructions were evaluated using (1) analogue statistics based on the χ^2-distance dissimilarity for each individual sediment-core sample in comparison with the calibration data set, (2) the bootstrapped root mean square error of prediction calculated for individual sediment-core samples (sample-specific RMSEP), and (3) goodness-of-fit statistics (Birks 1998).

Reconstructions were validated by comparison with air temperatures which were retrodicted from 20 homogenized instrumental lowland records. The retrodicted air temperatures have a root mean square error of ±1.6°C (Agustí-Panareda & Thompson 2002). We used bi-monthly air-temperature means because these probably had the largest influence on ice break-up dates (Livingstone 1997). We averaged the temperature retrodictions corresponding to the time period each sediment-core sample spanned. Similarly, we averaged the cyst-based reconstructions for sediment-core samples with sub-annual resolution to cover at least one year of cyst production. The averaged bi-monthly mean air temperature retrodictions ($T_{Jan/Feb}$ – $T_{Nov/Dec}$) were correlated with our cyst-based reconstructions taking into account the serial dependency of time series (Mudelsee 2003). Regression analysis followed Fox (2002) using the R/S language.

Results

Relationship of cyst assemblages with environmental variables

A total of 101 cyst types, including two cyst-like morphotypes (*Chrysococcus furcatus* and cf. *Chrysococcus* sp. (Kamenik *et al.* in press b), i.e. a similar type lacking the depressions of *Chrysococcus furcatus*) were found in the 33 recovered sediment traps (max. abundance ≥1%, occurrences in at least two traps). There were no unusual cyst assemblages, as indicated by DCA. DCCA revealed that 15 environmental variables (Table 2) individually explained a significant amount of variation in the cyst assemblages (marginal effects), taking into account multiple testing ($P_{adj} < 0.05$). In addition, DOC and dissolved phosphorus were significant ($p < 0.01$). Weighted correlations and variance inflation factors (VIF) >20 indicated multi-collinearity in a CCA combining the 17 environmental variables. Conductivity, Ca^{2+}, T_{June} and the break-up date were highly correlated with other environmental variables and deleted in a step-wise procedure until all VIF were <20. The remaining 13 variables, summarized in Fig. 2, explained 52% of

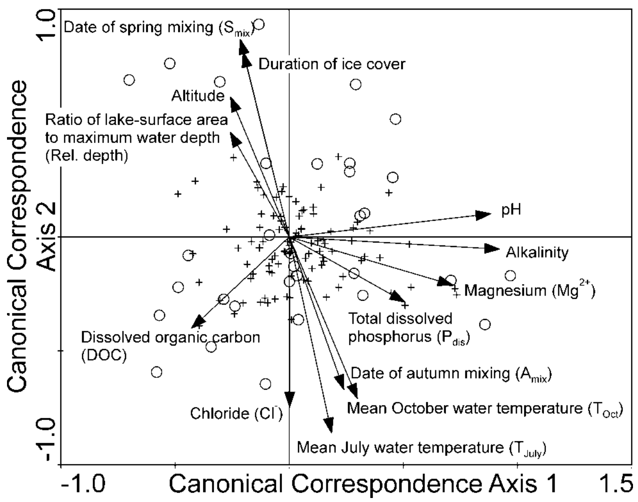

Fig. 2. Canonical correspondence triplot based on 32 sites and 100 cyst types (max. abundance ≥1%, occurrences in at least two lakes) summarizing environmental variables that individually explain a significant amount of variation in the cyst assemblages and that were not co-linear (VIF <20, Table 2). Canonical correspondence axes 1 and 2 indicate changes that are related to catchment characteristics (bedrock mineralogy, vegetation cover) and altitude/air temperature. Circles and crosses indicate sediment-trap samples and cyst types. Their scores are scaled by the factor 0.4.

the variation in the cyst assemblages. Manual forward selection indicated that this set of environmental variables could be further reduced to for example alkalinity, 'date of spring mixing' and relative water depth (i.e. the ratio of lake-surface area to maximum water depth) or pH, 'date of spring mixing' and DOC ($P_{adj} < 0.05$). Both sets explained 21.6% of the variation in the cyst assemblages. CCA axes 1 and 2 were significant ($p < 0.001$). CCA axis 1 was highest correlated with alkalinity or pH. CCA axis 2 was highest correlated with 'date of spring mixing' (S_{mix}). See Table 3 for a summary of both sets. Among the climate-related variables (Table 1), S_{mix} explained the highest amount of variation in the cyst assemblages (Table 2). In a series of partial CCAs, S_{mix} significantly explained 8.6%, 8.5%, 8.1% or 6.5% of the variation in the cyst assemblages after effects of pH, alkalinity, DOC or relative water depth were removed. In two other partial CCAs with either effects of alkalinity and relative water depth (set A in Table 3) or effects of pH and DOC (set B in Table 3) removed, S_{mix} significantly explained 7.4% or 8.8% of the variation in the cyst assemblages.

Transfer functions

The reference date 'date of spring mixing' (S_{mix}) was chosen for developing a cyst-based climate-related regression and calibration model, because (i) it was correlated with altitude (Table 1) and hence with air temperature, (ii) it explained a large amount of variation in the cyst assemblages (marginal and unique effects, Table 2), and (iii) it explained a significant amount of variation in the cyst assemblages when effects of other important variables (pH, alkalinity, DOC or relative water depth) were removed. WA-PLS with 2 components provided the minimal adequate model with the lowest RMSEP, the highest R^2 and the lowest mean and maximum bias (Table 4). The two-components WA-PLS model had a 12.5% lower $RMSEP_{boot}$ and a 9.5% lower $RMSEP_{jack}$ than the one-component model. The sediment-trap samples from Lakes Hüttensee, Kaltenbachsee, Knappenkarsee and Oberer Landwiersee had absolute residuals (observed – predicted S_{mix}) higher than the SD of 19.4 Julian days. Hüttensee had the largest residual; its bootstrapped prediction was 30 days too late. The other three sediment-trap samples had low Cook's D.

Table 3. Summary statistics of canonical correspondence analyses based on 32 sites and 100 cyst types (maximum abundance ≥1%, occurrences in at least two lakes) for two possible minimum sets of environmental variables (A: alkalinity, S_{mix}, relative water depth; B: pH, S_{mix}, DOC) that both have significant conditional effects ($P_{adj} < 0.05$). Inter-set correlations presented in boldface indicate significant canonical coefficients (approximate *t*-values >2.0; ter Braak & Šmilauer 2002). Lake Pfannsee was excluded because it lacks chemical measurements.

	(A)		(B)	
CCA axes ($p < 0.001$)	1	2	1	2
Eigenvalues	0.167	0.128	0.172	0.130
Species environment correlations	0.956	0.932	0.967	0.933
Cumulative percentage variance				
– of species data	10.0	17.6	10.2	18.0
– of species environment relationship	46.2	81.6	47.5	83.4
Inter-set correlations of three forward selected environmental variables with axes				
Alkalinity	**0.94**	**0.10**		
pH			**0.89**	0.18
'Date of spring mixing' (S_{mix})	−0.29	**0.89**	**−0.23**	**0.90**
Relative water depth†	**−0.27**	0.55		
Dissolved organic carbon (DOC)			**−0.39**	**−0.37**

†Ratio of lake-surface area to maximum water depth.

Table 4. Descriptive statistics for the climate-sensitive cyst-based ‘date of spring mixing’ (S_{mix}) inference model after deletion of outliers. Eighteen cyst types were identified in the sediment-core samples (max. abundance >3%, occurrences in at least 2 samples), 7 of which were not included in the training set.

No. of samples	29			No. of taxa	98
N2 values for samples:				N2 values for taxa:	
Minimum	9.57			Minimum	1.20
Median	26.20			Median	9.04
Maximum	47.40			Maximum	23.86
DCCA axis 1:				DCA axis 2:	
λ_1	0.15			λ_2	0.15
Gradient length (SD units)	1.57			Gradient length (SD units)	1.79
% variance explained	8.8			% variance explained	9.1
Monte Carlo *p*-value	0.0001				
Total inertia	1.65				
λ_1/λ_2	0.97				
S_{mix} (Julian days):					
Minimum	134				
Mean	161				
Median	157				
Maximum	199				
Standard deviation (SD)	17				
WA-PLS prediction model (2 components):					
Apparent R^2	0.98	R^2_{boot}	0.85	R^2_{jack}	0.85
RMSE	2.67	$RMSEP_{boot}$	9.62	$RMSEP_{jack}$	7.12
Apparent mean bias	−0.02	Mean $bias_{boot}$	0.49	Mean $bias_{jack}$	0.06
Apparent maximum bias	4.02	Maximum $bias_{boot}$	13.92	Maximum $bias_{jack}$	11.31

All four lakes were skipped in the final model (for a summary, see Table 4). The final model overestimated S_{mix} by less than 7 days in most cases when S_{mix} occurred prior to 170 Julian days. After 170 Julian days the deviations were more pronounced; the model underestimated S_{mix} by more than 10 days. Predicted S_{mix} >160 Julian days were therefore about 10 to 15 days too early. The bootstrapped predictions had higher residuals than the jack-knifed ones (Fig. 3).

Climate reconstruction

Eighteen cyst types were identified in 63 sediment-core samples (max. abundance >3%, occurrences in at least 2 samples), seven of which were not included in the training set. Samples with heavily corroded cysts, frequently found below 10 cm, were skipped from further analysis causing a large gap with no samples between 17.2 and 26.6 cm (see Kamenik (2001) for a detailed cyst stratigraphy). We therefore focused only on the upper core sequence (<17.2 cm). The base of the ^{210}Pb record occurred at 17.4 cm and was dated to AD 1835 ± 25 years standard error (Brancelj *et al.* 2002).

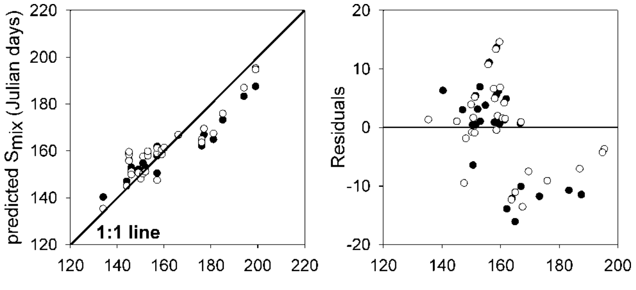

Fig. 3. Observed versus predicted ‘date of spring mixing’ (WA-PLS, 2 components and the residuals (inferred-observed)) of the climate-inference model. Bootstrapped and jack-knifed estimates are indicated by black and white circles. Model parameters are summarized in Table 4.

The reconstructed ‘dates of spring mixing’ (S_{mix}) fluctuated between 107 and 142 Julian days (jack-knifed) or 116 and 145 Julian days (bootstrapped). Jack-knifed and bootstrapped estimates of S_{mix} were highly correlated ($R = 0.995$). The reconstructions indicated late S_{mix} from the beginning of the record (AD 1842 ± 24) to AD 1856 ± 19, intermediate S_{mix} from AD 1863 ± 16 to AD 1878 ± 11, a late S_{mix} at AD 1878 ± 9, and a trend to earlier S_{mix} until AD 1932 ± 4 (Fig. 4). Prior to this date the record was non-continuous, because of samples that could not be used due to heavily corroded cysts. After AD 1932 ± 4 S_{mix} occurred early, except for AD 1955 ± 4, AD 1965 ± 2, AD 1976 ± 2 and the surface sediment (Fig. 4). At *c.* AD 1880 the average time interval covered by sediment-core samples changed from longer than 3 years to shorter than 3 years due to an increase in sedimentation rates. Prior to this date, S_{mix} was therefore more smoothed.

The sediment-core assemblages comprised 2% to 30% (mean 12%) of cyst types absent in the training set. All present cyst types were poorly represented in the training set (<10%). None of the sediment-core samples had good present-day analogues (analogue

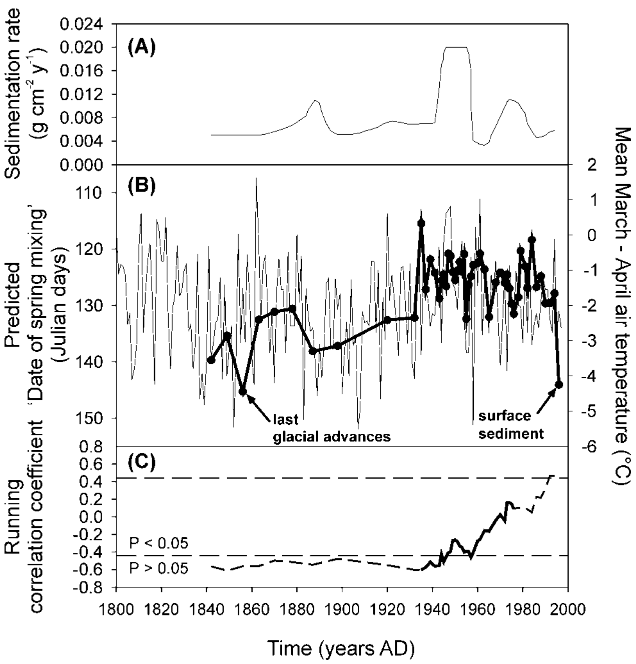

Fig. 4. (A) Sedimentation rates and (B) bootstrapped estimates of the 'date of spring mixing' (S_{mix}) reconstructed from the sedimentary cyst assemblages of Jezero v Ledvici (black circles) using the WA-PLS prediction model (Table 4, Fig. 3). Samples were dated with ^{210}Pb, ^{226}Ra and ^{137}Cs using a piecewise CRS model (Appleby 2000). 'Date of spring mixing' was highest correlated with the mean March–April air temperature ($T_{Mar/Apr}$, thin line in (B)), which drives break-up dates ($R = -0.35$, note the inverted y-axis for S_{mix}). (C) Running correlation coefficients between S_{mix} and $T_{Mar/Apr}$ (solid line: $n = 21$, dashed line: $n < 21$ at the ends of the time series), indicating possible artefacts in the S_{mix} reconstruction at around AD 1950 and after 1960.

statistics: χ^2 distance >5% percentile). Similarly, goodness-of-fit statistics revealed that all sediment-core samples had very poor fits with modern analogues. The sample-specific RMSEP fluctuated between 10 and 12 Julian days from AD 1935 ± 4 to AD 1994 ± 1. The topmost sediment-core sample and samples prior to AD 1935 ± 4 had a sample-specific RMSEP between 12 and 16 Julian days.

The comparison with mean bi-monthly air temperatures retrodicted from meteorological stations revealed that S_{mix} was highest correlated with $T_{March/April}$ ($R = -0.35$, 95% BCa (bias-corrected and accelerated) confidence interval: -0.54 to -0.12, $\alpha = 0.025$). The correlation was significant taking into account the length and the serial dependency of the time series ($n = 51$, $\max(\tau_x, \tau_y) = 2.2$). The surface-sediment sample was excluded from this analysis. Correlations were high prior to 1947 ± 5 and around AD 1957 ± 4 (Fig. 4). According to the linear relationship $T_{March/April} = 6.32 - 0.064\ S_{mix}$, the sample-specific RMSEP corresponded to 0.6°C and 0.8°C from AD 1935 ± 4 to AD 1994 ± 1, and 0.8°C to 1.0°C prior to AD 1935 ± 4. The surface-sediment sample and the sample at 16.2–16.4 cm (AD 1856 ± 19) were excluded from this linear regression analysis because of high leverage (hat values >4× average hat value).

Discussion

Relationship of cyst assemblages with environmental variables

The most important environmental factor affecting the modern cyst assemblages is the acid-base balance (Table 3). Cysts are well known to respond to pH and alkalinity (e.g. Facher & Schmidt 1996; Duff *et al.* 1997; Lotter *et al.* 1997; Pla *et al.* 2003). Bedrock mineralogy is the primary factor driving the acid-base balance of the training-set lakes (Kamenik *et al.* 2001b). Air temperature and ice cover might be other important variables driving pH (Wögrath & Psenner 1995; Koinig *et al.* 1998) and hence cyst assemblages (Table 3). In acid-sensitive lakes pH can thus be used for climate reconstruction (Anderson 2000). Lake ontogeny and anthropogenic disturbances, such as deforestation or acid deposition, can, however, decouple the climate–pH relation (Psenner & Schmidt 1992; Engström *et al.* 2000; Kamenik *et al.* in press b).

The reference date 'date of spring mixing' (S_{mix}) explained the highest amount of variation among the climate-related variables in the training set (see PC axis 1 in Table 1); the ratio of λ_1 to λ_2 indicates that it is almost as important as pH (Table 2). These results might have been influenced by the different sampling strategies (continuous water-temperature measurements versus spot-measurements of water chemistry). However, the seasonal changes of chemical and morphological features of mountain lakes are by far less variable than the seasonal chances of their physical characteristics, except during a very short period around thawing (Catalan *et al.* 2002b), which we therefore did not sample. S_{mix} was highly correlated with altitude, break-up dates and canonical correspondence axis 2 (Tables 1 and 3, Fig. 2). Pla & Catalan (2005) found a similar orthogonal positioning of their altitude gradient with respect to their chemical gradient driven by bedrock mineralogy, suggesting that a portion of the variation in their cyst assemblages was independent from chemical characteristics. Altitude controls air temperature (Wagner 1930; Barry 2001), which drives break-up dates (Livingstone 1997; Magnuson *et al.* 2000). S_{mix} is thus a reliable indicator of late winter or spring temperature.

S_{mix} and break-up dates affect the cyst assemblages probably by changing the timing and magnitude of cyst production. The period of break-up and freezing is often the period of highest cyst production, and the occurrence of specific cyst types has a distinct seasonality: cyst types occurring during break-up differ from cyst types occurring during freezing (Agbeti & Smol 1995; Pla 1999; Kamenik *et al.*

2001a). The former are produced by species living in or under the ice ('cold cysts'), e.g. *Mallomonas akrokomos, M. eoa* and *M. teilingii* (Cronberg 1973, 1980), whereas the latter are produced by open-water species ('warm cysts'), e.g. *Chromulina* sp. and *Mallomonas acaroides* (Siver 1991; Kamenik *et al.* in press a). We hypothesize that late S_{mix} causes a shift towards 'cold cysts' in the cyst assemblage produced during an entire year. In turn, early S_{mix} causes a shift towards 'warm cysts' in the cyst assemblage produced during an entire year.

Nutrition is a potential mechanism driving 'cold cysts' and 'warm cysts'. Chrysophytes are a diverse group of algae with opportunistic feeding strategies (autotrophy, mixotrophy and heterotrophy) that are believed to be species-specific (Holen & Boraas 1995; Raven 1995). Their occurrence is thus governed by the availability of light and nutrients. The response of cyst assemblages to nutrients (Tables 2 and 3), and hence trophic status (Kamenik *et al.* 2001b), corresponds with other studies from mountain lakes (Kamenik *et al.* 2001a; Pla *et al.* 2003). Like pH, the trophic status can be driven by climate (Sommaruga-Wögrath *et al.* 1997) or human disturbances such as deforestation and pasturing (Kamenik *et al.* 2000; Hausmann *et al.* 2002).

S_{mix} explained a significant amount of variation when effects of other important variables such as pH, alkalinity, DOC and relative water depth were removed. The partial CCAs indicate that, besides chemical keys, cyst assemblages are driven by physical factors such as light availability. There are, however, relations between S_{mix} and the latter four variables in our data set, as shown in Table 3. The S_{mix} – DOC – pH/alkalinity relation, for example, is partly caused by the distribution of shrubs and trees along altitude. Large numbers of shrubs and trees in the lower altitudes, indicated by chloride (Fig. 2; Kamenik *et al.* 2001b), cause higher input of nutrients and organic acids from the watershed (Psenner & Catalan 1994; Kamenik *et al.* 2001b; in press b) which potentially influence the cyst assemblages. The positive relationship between S_{mix} and relative water depth in our data set (Fig. 2) might have physical reasons; large lakes heat up slowly (Gorham 1964) and therefore tend to affect cyst assemblages via late dates of spring mixing. Alternatively, we could interpret high values of the ratio of lake surface area to maximum water depth as indicating shallow lakes that potentially include an extensive littoral zone. Cyst assemblages in these lakes might be affected by enhanced nutrient release from sediments during winter (e.g. Wögrath & Psenner 1995). Duff *et al.* (1997) found distinctive cyst types in shallow lakes, probably produced by periphytic chrysophytes. Catalan *et al.* (2002b) and Lotter *et al.* (2002) suggested that climate effects on biota are mediated by ice cover and related factors, such as nutrients and light.

Water temperature *per se* may have little influence on chrysophytes (Koinig *et al.* 2002). Kamenik *et al.* (2001a), Catalan *et al.* (2002a) and Šporka *et al.* (2002) have shown that air temperatures during freezing or break-up have had the largest influence on cyst assemblages in mountain lakes during the past *c.* 150 years, whereas air temperature during the open water season, driving water temperature (Livingstone & Lotter 1998), has been less significant.

Currently, we do not know whether freeze and break-up dates are truly less important than the dates of spring and autumn mixing (Table 2). In the Swedish lake Trummen, *Mallomonas eoa* was observed producing cysts before break-up (Cronberg 1973); however, 2 years later the same species produced cysts only after water temperature rose to about 6°C (Cronberg 1980). Furthermore, there were also technical limitations in our study: the reference dates S_{mix} and A_{mix} were easy to determine using the thermistor readings; in contrast, freeze and break-up dates were more difficult to resolve due to the complexity of the freezing and thawing process (Gabathuler 1999). Obviously, there is need for high resolution field surveys and experiments on for example encystment and its triggers if we are to improve our knowledge of the key factors that govern the cyst assemblage composition.

Transfer functions

Compared with other cyst-based transfer functions (e.g. Rybak *et al.* 1991; Zeeb & Smol 1995; Facher & Schmidt 1996; Brown *et al.* 1997; Duff *et al.* 1997; Lotter *et al.* 1997; Pla *et al.* 2003), we obtained excellent model parameters for our S_{mix} transfer functions (Table 4) despite the short environmental gradient (Birks 1998) and the low number of sediment-trap samples (Wilson *et al.* 1996). This result can probably be attributed to the use of sediment traps that allowed a precise temporal sampling. The R^2_{boot}/R^2_{jack} of the model (Table 4) are similar to or higher than those of other Alpine climate-inference models, such as the diatom-based 'date of autumn mixing' (Schmidt *et al.* 2004a), diatom-based summer water-temperature models (Lotter *et al.* 1997) or chironomid-based air-temperature models (Lotter *et al.* 1997; Heiri *et al.* 2003). Due to a significant relationship between altitude and S_{mix} in our training set ($altitude = 2441 - 3.476\ 10^{10}\ S_{mix}^{-3.6}$; $R^2_{adj} = 0.87$, $p < 0.001$) and the correlation between altitude and air temperature (e.g. Livingstone *et al.* 1999; Agustí-Panareda & Thompson 2002), the cyst-based S_{mix} model allows reconstructing air temperature.

According to cross-validation (Table 4), WA-PLS, which assumes unimodal species responses, works best, although the length of DCCA axis 1 (Table 2) suggests that the use of a linear species-response model may be more appropriate. Ter Braak & Juggins (1993) showed that WA-PLS performs well even with short environmental gradients. This short gradient might, however, have increased the 'edge effect', which is inherent to the regression methods (Birks 1998). It leads to predicted values that are overestimated at the lower end of the environmental gradient and underestimated at the higher end of this gradient. We selected the WA-PLS model regardless of the distinct bias occurring at S_{mix} >160 days (Fig. 3), because our reconstructed S_{mix} values were never that high (Fig. 4).

We retained two components in our final model, thus utilized residual structure in our data set to improve the prediction (ter Braak & Juggins 1993). This residual structure might be related to environmental factors affected by ice cover or mixing, such as the acid-base balance or nutrients (Wögrath & Psenner 1995; Sommaruga-Wögrath *et al.* 1997; Koinig *et al.* 1998). Significant canonical coefficients (Table 3) and the negative shared variation (marginal effects – unique effects) between S_{mix} and pH, alkalinity, DOC and relative water depth point to dependencies between S_{mix} and the latter four variables (Méot *et al.* 1998; ter Braak & Šmilauer 2002; Lepš & Šmilauer 2003). The four model-dependent outliers suggest potential influence of additional factors on the transfer functions. Hüttensee, Kaltenbachsee and Knappenkarsee are lakes that are too warm or too cold for their altitude (Livingstone *et al.* in press). Oberer Landwiersee had a low water transparency which caused the loss of both thermistors after summer 1999. Gabathuler (1999) and Livingstone *et al.* (in press) stressed the importance of meltwater from snowfields and water transparency, which is linked to trophic status, for the heat budget of mountain lakes. Applications of the cyst-based S_{mix} model in lakes that experienced for example long-term changes in pH and nutrients (Schmidt *et al.* 2004a; Kamenik *et al.* in press b) may therefore result in inferred temperatures that are merely artefacts of changing lake-water chemistry (Anderson 2000). In turn, common changes in pH and air temperature in for example poorly buffered lakes (Psenner & Schmidt 1992) could amplify the climate signal obtained from our transfer functions.

Climate reconstruction

The late 'date of spring mixing' (S_{mix}) prior to AD 1860 (Fig. 4) is in agreement with the glacial advances at the end of the Little Ice Age (e.g. Nicolussi & Patzelt 1996). The disagreement between these glacier advances and the retrodicted air temperature (Fig. 4) may be attributed to an increased snow accumulation during winter. Periods of glacier advance are generally cooler and more maritime with reduced sunshine duration and increased precipitation (Schöner *et al.* 2000).

S_{mix} captured a significant amount of variation in the mean March and April air temperatures (Fig. 4). The results correspond with those of Livingstone (1997), who showed that the timing of break-up (and hence of S_{mix}) on lakes at similar altitude in SE Switzerland was strongly related to local and regional surface-air temperatures, centred on the middle of April and integrated over 4–8 weeks. The unusually late reconstructed S_{mix} of the surface-sediment sample of the record might result from a high amount of 'cold cysts' produced prior to sediment-core sampling; the core was taken in summer 1996 and 'warm cysts' from the open water season of this year were probably lacking in the lake sediment.

The validation suggests that cyst assemblages in mountain-lake sediments are a powerful tool for reconstructing late winter and/or spring climate, taking into account the limitations and potential pitfalls of our approach: (i) the reconstructions were based on cyst assemblages having very poor fits with modern analogues; (ii) inferred S_{mix} earlier than 134 Julian days were extrapolated resulting in unreliable estimates, whereas late reconstructed S_{mix} had the largest estimated standard errors of prediction; (iii) retrodicted air temperatures had a root mean square error of ±1.6°C; (iv) dating uncertainties caused potential errors in the comparison of the time series. Additionally, there is evidence that inferred S_{mix} was affected by changes in trophic status or pollution: low correlations between S_{mix} and the mean March–April air temperature around AD 1950 and after AD 1960 (Fig. 4) coincided with (i) earthquakes or a slope collapse and subsequent changes in sedimentation rates at AD 1942 and 1975; (ii) with a sudden increase in diatom-accumulation rates from AD 1942 ± 4 to 1960 ± 3 indicating a period of enhanced trophic status caused by an earthquake; and (iii) with a sudden increase in the flux of spheroidal carbonaceous particles in the early 1950s indicating fuel combustion (Brancelj *et al.* 2000, 2002).

Conclusions

- The 'date of spring mixing' (S_{mix}) of mountain lakes is a climate indicator. It is correlated with altitude and ice break-up dates, and hence with late winter and/or spring air temperature.
- Cyst assemblages store information on S_{mix} and thus on seasonal climate.
- The application of a cyst-based S_{mix} model to a late-Holocene sediment core from a southern Alpine lake indicated agreement of the climate reconstruction with glacier fluctuations and retrodicted spring-air temperature.
- Artefacts in inferred S_{mix} coincided with changes in trophic status or air pollution suggesting that effects of climate on stomatocyst assemblages are not independent from other environmental factors. Hence, we strongly recommend a validation, based

on independent climate proxies, of cyst-based climate reconstructions.

- In sum, chrysophyte stomatocysts are an appropriate tool for reconstructing winter/spring climate from Alpine lake sediments.

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