J Paleolimnol (2017) 57:273–285
DOI 10.1007/s10933-017-9946-2

ORIGINAL PAPER

The ratio between chrysophycean cysts and diatoms in temperate, mountain lakes: some recommendations for its use in paleolimnology

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Received: 13 March 2015 / Accepted: 27 January 2017 / Published online: 7 February 2017

Abstract The ratio between chrysophycean cysts and diatom valves (CD ratio) in lake sediments has been suggested as a useful indicator of changing trophic state conditions in oligotrophic lakes. Other environmental factors, however, may influence the CD ratio because chrysophycean cysts usually reflect conditions in the planktonic environment and diatoms reflect benthic conditions. We investigated the CD ratio in 76 mountain lakes in the Pyrenees to determine the environmental drivers that influence the ratio and assess its value for paleoenvironmental inference. The lakes surveyed included a broad range with respect to bedrock type, altitude and surface area, characteristics that cover much of the variability that can be found in cold, oligotrophic mountain lakes. Lake depth and Ca^{2+} concentration explain most of the variation in the CD ratio. Trophic state factors (e.g. total phosphorus, TP) play a secondary role. As a predictor, CD ratio performs primarily as a lake depth indicator. The predictive models can be improved if trophic state (i.e. TP) and chemical conditions (Ca^{2+}) are known or can be estimated independently. Use of the CD ratio for inferring Ca^{2+} oscillations only makes sense in lakes with Ca^{2+} <200 μeq/L or in those that oscillate below and above this threshold through time. Other interpretations of the CD ratio (e.g. lake trophic state changes, ice-cover duration) make sense if complementary paleolimnological evidence indicates that neither water depth nor Ca^{2+} concentration changed significantly. Indeed, paleolimnological interpretation of the CD ratio requires considering the particular characteristics of the lake and may vary depending on the temporal scale considered. This study provides some guidelines for evaluating critically the use of the CD ratio.

Keywords Environmental reconstruction · Depth · Calcium · Total phosphorus · Pyrenees

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Introduction

Chrysophyceae and Synurophyceae are unicellular or colonial algae that are found across a broad range of environmental conditions (Pla et al. 2003; Zeeb et al. 1994). They produce siliceous resting stages that accumulate in lake sediments, thereby storing information about past environmental conditions (Pla and Catalan 2005; Pla et al. 2009; Zeeb and Smol 2001). Although the species identity of most cysts is unknown, a taxonomic system that uses the morphology of cysts has been developed. The robustness of morphological traits enabled development of an iconographic atlas (Duff et al. 1995; Pla 2001; Wilkinson et al. 2001) that can be used to identify the ecological relations of many cyst morphotypes and use them for inferences about the past (Pla and Anderson 2005; Pla and Catalan 2005; Smol 1995).

Diatoms are microalgae characterized by possessing outer siliceous valves that also preserve extremely well in sediments and thus are used widely in paleolimnological studies (Battarbee et al. 2001). In addition to the indicator value of individual taxa and transfer functions based on the taxonomic composition of assemblages, the simple ratio between the number of chrysophycean cysts and diatom valves (the CD ratio) has been suggested as an environmental indicator for the paleolimnological toolbox (Douglas and Smol 1995; Werner and Smol 2005).

Most chrysophyte species are planktonic and common in oligotrophic lakes, although cysts of benthic chrysophyceae may occasionally be common in Arctic ponds (Douglas and Smol 1995) and in alpine water bodies. Diatoms show habits that are complementary to chrysophytes, in that there are more benthic diatom species than planktonic ones (Lowe 1996). Only occasionally do species that grow in the water column become relevant or dominate the taphonomic assemblages in the sediment record (Ardiles et al. 2012; Ferris and Lehman 2007), depending on a combination nutrient, light and temperature conditions (Saros and Anderson 2015). In general, chrysophytes are highly indicative of planktonic algal growth, whereas diatoms indicate benthic dynamics. If otherwise, planktonic diatom taxa can be excluded from calculations.

Several factors may influence the relative contribution of planktonic and benthic populations to the taphonomic assemblage. Consequently, the interpretation of the CD ratio may vary depending on the lake typology and ecoregion. In lakes where both algal groups can grow without severe physiological constraints, for instance oligotrophic freshwater bodies, the CD ratio has been related to ice-cover duration (Smol 1983), trophic status (Smol 1985) and mixing regimes (Werner and Smol 2005). If there is an alternative factor that constrains the viability of one group, such as elevated salinity for chrysophytes, the CD ratio may be related to this factor (Cumming et al. 1993). On the other hand, in situations of relatively similar physical, chemical and trophic state conditions, the CD ratio might just reflect lake morphology, which determines the relative availability of planktonic and benthic habitats. Therefore, application of the CD ratio in paleolimnological studies requires addressing the fact that interpretations of the ratio may vary depending on the type of lake and ecoregion in which it is applied.

We addressed the application of the CD ratio in temperate mountain lakes, using a data set from the Pyrenees. First, we assessed the explanatory capability of the CD ratio across a broad range of environmental variables. Second, we evaluated the use of the CD ratio in paleolimnological studies. Our results may apply to other mountain lake districts of similar environmental characteristics and provide some general insights into the use of this ratio for paleolimnological inferences.

Materials and methods

This study was based on a survey of 76 lakes in the Pyrenees, conducted between 9 July and 23 August 2000 (Catalan et al. 2009b). The lakes were distributed across broad environmental gradients, determined by bedrock type, altitudinal range (1620–2990 m a.s.l.) and lake morphology (Table 1; Fig. 1). They were selected to ensure a balanced representation of the environmental variability and to cover the geographic extremes. In general, lakes of the Pyrenees are located in basins of plutonic or metamorphic lithology and have poorly developed soils. Bare rock and meadows are the dominant land cover, and less than 20% of the lakes studied have coniferous woodlands in their catchments. The lakes are generally small, but have high relative depth (Table 1) because of their glacial origin in steep valleys (Catalan et al. 2009b). They are ice-covered from 5 to 9 months of the year and are generally dimictic (Catalan et al. 2002).

Variables that describe the physical environment, such as temperature, ice-cover duration, light environment, and littoral substrate were included (de Mendoza and Catalan 2010). Surface-water temperature was measured at the center of each lake. Ice-cover duration was estimated according to Thompson et al. (2005). Maximum depth (z_{max}) was measured with sonar along transects.

Water samples for chemical analyses were collected near the outflow of each lake following protocols of previous pan-European studies on

Table 1 Geographic, morphological, lithological, land cover and chemical characteristics of the lakes studied

Variable	Median (range)
Altitude (m a.s.l.)	2305 (1620–2990)
Lake area (ha)	5.5 (0.2–53.2)
Catchment area (ha)	114.6 (7–5437.9)
Maximum depth—Z_{max} (m)	17 (0.7–123)
Relative depth (%)	6.5 (0.8–18)
Ice-cover duration (days)	185 (115–215)
Lithology (>30% lake catchment area)	
Metamorphic (%)	27.1 (0–100)
Plutonic (%)	48.2 (0–100)
Detrital (%)	17.7 (0–100)
Carbonate (%)	17.5 (0–90)
Land cover	
Glacier presence (%)	0 (0–15)
Bare rock (%)	30 (0–90)
Meadows (%)	15 (0–90)
Shrubs (%)	0 (0–60)
Coniferous (%)	0 (0–40)
Secchi disk (m)	10.0 (0.7–22)
Summer surface temperature (°C)	12.6 (3.8–18.5)
pH	7.0 (4.5–9.0)
Alkalinity (μeq/L)	123 (0–1696)
SO_4^{2-} (μeq/L)	41.3 (10–1240)
Ca^{2+} (μeq/L)	139.5 (20–1195)
Mg^{2+} (μeq/L)	14.6 (4–557)
TP (μg/L)	3.4 (0.94–33.3)
TN (μg/L)	177.1 (43.9–967.9)
NO_3^- (μeq/L)	5.1 (0–19.9)

acidification of sensitive lakes (Wright et al. 2005). The analytical methods were carried out according to the methods agreed upon by the MOLAR Project (The MOLAR Water Chemistry Group 1999), and they are described in Camarero et al. (2009). Measurements included pH, alkalinity, conductivity, calcium, magnesium, sodium, potassium, sulfate, chloride, total phosphorus (TP), nitrate, ammonium, total nitrogen (TN) and total organic carbon (TOC).

Samples for sediment chrysophycean cyst and diatom analyses were collected at each lake at the same time the water chemistry survey was conducted, using a gravity corer deployed in the deepest part of the lake. The upper 0.5 cm of the sediment core was taken for analysis. Samples were cleaned and mounted using standard techniques (Battarbee et al. 2001). Organic matter was oxidized using 1 N HCl and 30% H_2O_2 in a water bath at 70 °C. Once cleaned, samples were mounted on permanent slides using Naphrax (Brunel Microscopes LTD, refractive index = 1.74). Chrysophycean cysts and diatoms were counted until 500 diatom valves were enumerated, using a Zeiss Axio Imager A.1 differential interference contrast microscope with a plan-apochromatic 100 × objective.

The CD ratio was calculated as the $\log_e$-transformed absolute number of chrysophycean cysts per 500 diatom valves. The use of this value and a constant number of diatom valves cancel variability in the denominator of the ratio. Consequently, only variability in cyst numbers is reflected in the ratio and oscillations in diatom abundance do not influence the ratio or its value as an indicator.

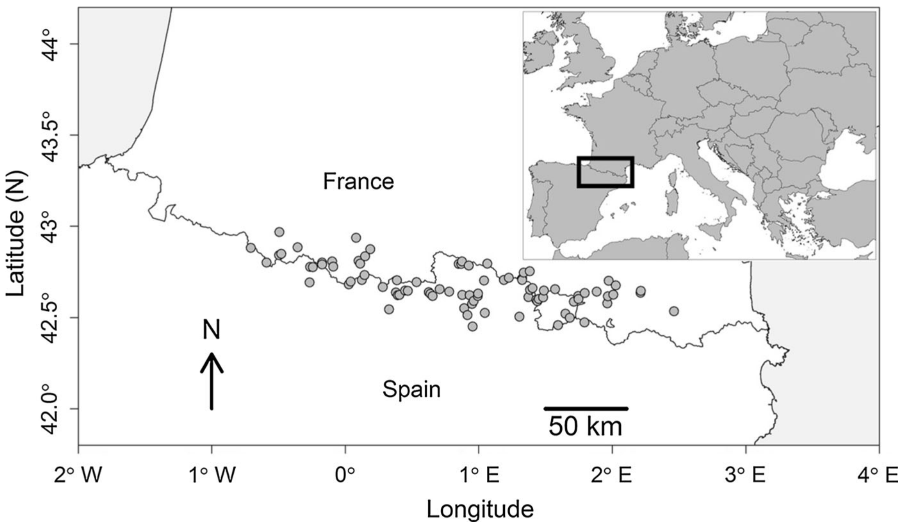


Fig. 1 Geographic distribution of the lakes surveyed in the Pyrenees

Relationships between variables were explored by Principal Component Analysis (PCA), in which the CD ratio was included as a passive variable. Except for altitude, ice-cover duration, temperature, Na^+, and lake area, variables were transformed using $\log_e(x + 1)$. Then, the relation of the CD ratio with explanatory variables was further characterized using linear regression. The variables were $\log_e$-transformed and standardized to compare models. The most appropriate models were identified using the coefficient of determination (R^2), the standard error of the models (SE) and the Akaike information criterion (AIC). A regression tree was performed using some selected explanatory variables of the CD ratio to identify possible subsets with different relationships (Legendre and Legendre 2012). Variables were $\log_e$-transformed and the tree was pruned using the complexity factor with the minimum cross-validated error. Structural equation models were developed to investigate the configuration of the relationships between variables associated with the CD ratio (Legendre and Legendre 2012). Finally, the predictive potential of the CD ratio was explored using linear regression models, which were validated using jackknife resampling. All analyses and models were performed using R language and the packages stats 3.2.2, rpart 4.1-10 and lavaan 0.5-20 (R Core Team 2015; Rosseel 2012).

Results

The ratio of chrysophycean cysts to diatom valves (cyst/diato) ranged from zero to 1.77, with a mean of 0.16 and a median of 0.06 (Fig. 2a). In most samples, cysts showed lower abundance than diatoms; in only 9% of the lakes did the cysts exceed the diatom cells (cyst/diato > 1.0). These lakes showed an average depth of 51 m (16 – 100 m). The only two lakes without recorded cysts were shallow (2.1 and 9.5 m maximum depth), had high pH (7.95 and 8.60) and TP concentrations close to the median of the lake set (2.6 and 3.9 µg/l).

The cyst/diato distribution was markedly skewed, with 84% of lakes <0.25 (Fig. 2a). The logarithmic transformation of the cyst/diato ratio did not fully correct the observed asymmetry. The number of cysts counted by the time 500 diatom valves were enumerated, did however show a central distribution when transformed logarithmically (Fig. 2b). As mentioned, fixing the number of diatoms counted pegs variation of the ratio to the number of cysts enumerated, and does not produce spurious effects related to higher or lower diatom abundance (i.e. counts). From here on, the CD ratio refers to the $\log_e$-transformed cyst abundance per 500 valves.

The PCA showed that the first axis was mainly related to TP (r = 0.80, $p < 0.001$), ice-cover duration (r = −0.66, $p < 0.001$), DOC (r = 0.67, $p < 0.001$), temperature (r = 0.65, $p < 0.001$), alkalinity (r = 0.63, $p < 0.001$) and altitude (r = −0.63, $p < 0.001$). The second axis correlated with SO_4^{2-} (0.82, $p < 0.001$), Mg^{2+} (r = 0.76, $p < 0.001$), and Ca^{2+} (r = 0.65, $p < 0.001$). The two axes explained a similar amount of variability, about 20% (Fig. 3).

The CD ratio, introduced passively in the PCA, fell between the two principal axes (Fig. 3). Univariate regression models (Table 2) showed that Ca^{2+} and z_{max} were the best explanatory variables of the CD ratio ($R^2 > 0.30$), followed by a second group of variables including alkalinity, SO_4^{2-}, Mg^{2+} and pH

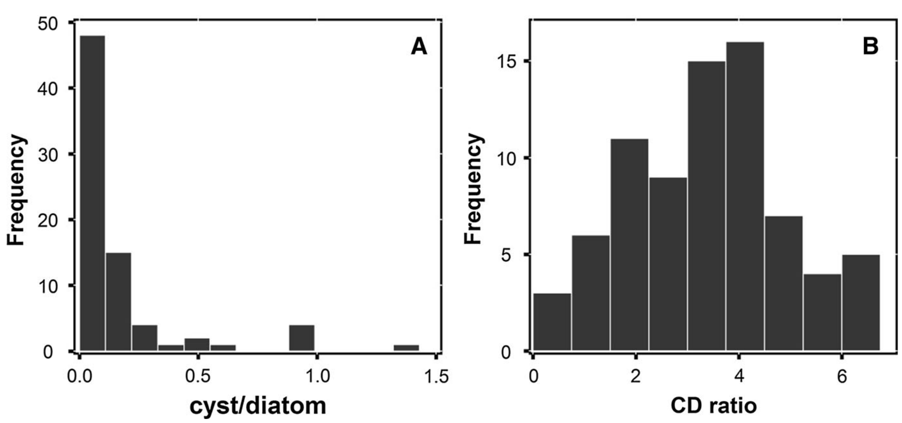


Fig. 2 Histograms of **a** cyst/diatom ratio and **b** the $\log_e$-transformed cyst abundance (CD ratio)

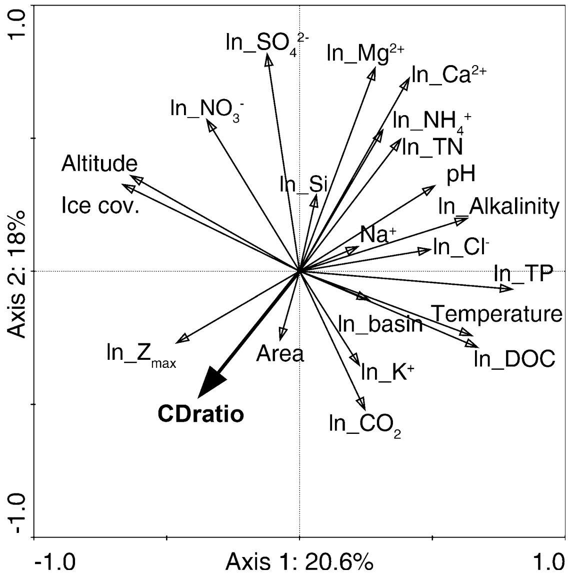


Fig. 3 Biplot of the principal component analyses of the environmental variables considered. The CD ratio is plotted as a passive variable. z_{max} is the maximum depth. The variance explained for each axis is indicated. Variables $\log_e$ transformed (ln) are indicated

($R^2 > 0.10$). Other variables with significant relationships (TP, and NH_4^+) had little explanatory power for the CD ratio ($R^2 < 0.05$). Other variables that are highly relevant in defining environmental variation (Fig. 1), such as altitude, ice-cover duration and lake area, were not significantly related to the ratio.

Regression models using two and three variables showed that z_{max} and Ca^{2+} played a complementary role in explaining the variation in CD ratio (Table 2). The models in which they were included achieved the highest explanation (R^2), and the lowest standard error and AIC. The rest of the variables, which were significant in univariate models (SO_4^{2-}, Mg^{2+}, pH, TP, and NH_4^+), were usually non-significant or had a low influence in models with two or three variables.

Further exploration of the relationship of z_{max} and Ca^{2+} with the CD ratio was not linear throughout the range of values. The regression tree showed that the influence of z_{max} and Ca^{2+} on the CD ratio changes at about 200 μeq Ca^{2+}/L (Fig. 4). The model that includes only lakes with Ca^{2+} ≥216.6 μeq/L (Group II) showed the highest z_{max} explanation of the CD

Table 2 Linear regressions explaining the CD ratio using the standardized explanatory variables

Variable(s)			R^2	Standard error	AIC
Univariate models					
−0.62 Ca^{2+}***			0.38	0.79	183.1
0.59 z_{max}***			0.34	0.81	187.8
−0.42 Alkalinity***			0.17	0.91	205.8
−0.39 SO_4^{2-}***			0.14	0.92	207.8
−0.39 Mg^{2+}***			0.14	0.92	208.0
−0.38 pH***			0.13	0.93	208.7
−0.24 TP*			0.04	0.98	216.1
−0.23 NH_4^+*			0.04	0.98	216.4
Models with two variables					
−0.52 Ca^{2+}***	+0.47 Z_{max}***		0.59	0.64	152.6
+0.53 z_{max}***	−0.33 Alkalinity***		0.43	0.75	176.8
+0.54 z_{max}***	−0.29 pH**		0.42	0.76	179.7
Models with three variables					
+0.53 z_{max}***	−0.52 Ca^{2+}***	+0.11 TP	0.60	0.64	152.7
−0.59 Ca^{2+}***	+0.50 z_{max}***	+0.13 Mg^{2+}	0.60	0.64	152.7
−0.54 Ca^{2+}***	+0.48 z_{max}***	+0.07 NH_4^+	0.59	0.64	153.9
−0.56 Ca^{2+}***	+0.47 z_{max}***	+0.07 pH	0.59	0.64	154.0
−0.50 Ca^{2+}***	+0.47 z_{max}***	−0.04 SO_4^{2-}	0.59	0.64	154.3
−0.54 Ca^{2+}***	+0.47 z_{max}***	+0.03 Alkalinity	0.59	0.64	154.5

The best models were identified using R^2, the standard error of the models and Akaike's information criterion (AIC). z_{max} is the maximum depth. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Note that models with three variables do not improve the best models with two variables

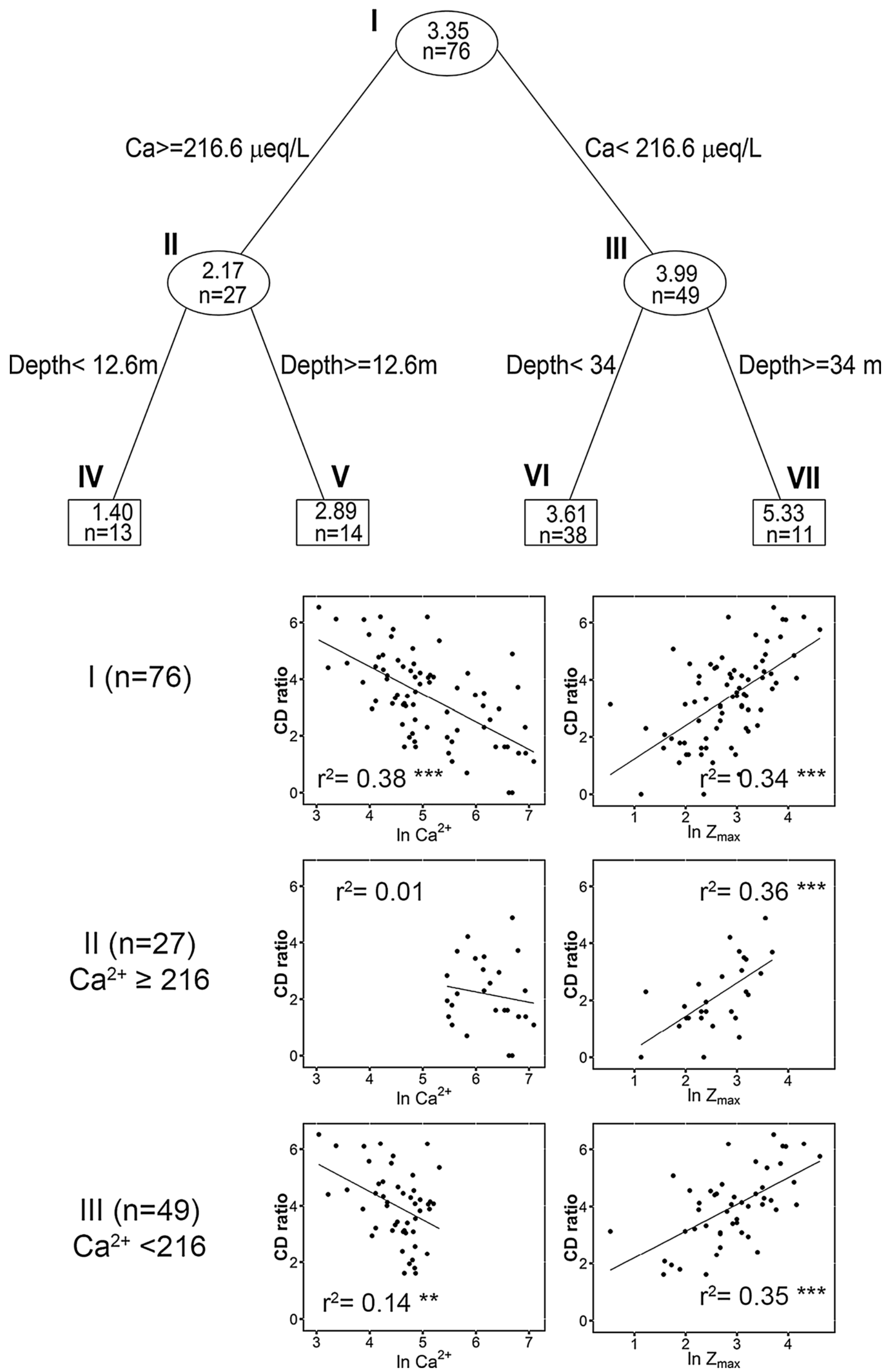


Fig. 4 Regression tree for the function CD ratio $\sim$ln_Ca^{2+} + ln_Z_{max}. The tree branches show the decision value for significant variables and the nodes show the number of lakes and the average of the CD ratio for each group of data. Regressions between variables are shown. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

ratio, whereas Ca^{2+} was not significant. For lakes with Ca^{2+} <216.6 μeq/L (Group III), models for Ca^{2+} and z_{max} were both significant, although z_{max} showed higher explanatory capacity than Ca^{2+} (Fig. 4). The z_{max} maintained a similar effect on the CD ratio in groups II and III as it had in the whole set of lakes (Group I, Fig. 4). The z_{max} values of 12.6 and 34 m produced other significant branches in the regression tree for the low and high Ca^{2+} groups, respectively. This result further indicates the relevance of z_{max}, but the low number of lakes in the groups prevents further interpretation.

To this point, it was clear that z_{max} and Ca^{2+} have relatively independent influences on the CD ratio in mountain lakes, with saturation of the Ca^{2+} effect occurring at about 200 μeq/L. The role of TP, a variable often associated with the CD ratio in paleolimnological studies, was weak. We might surmise, however, that the low explanatory capacity of TP in space would be greater in time, if the other two variables were maintained nearly constant. We could not directly explore this question with the available data, but we investigated further the statistical links using structural equation models (Fig. 5) of groups I, II and III of the regression tree. The structural equation model for group I ($p = 0.48$, $n = 76$, $df = 1$) suggests a similar influence of z_{max} and Ca^{2+} on the CD ratio (Fig. 5). z_{max} was also related to TP, but there was not a significant influence of TP on the CD ratio, indicating that the z_{max} effect on the CD ratio was not mediated by TP at all. The model for group II ($p = 0.29$, $n = 27$, $df = 1$) only showed a significant effect of z_{max} on CD ratio. The model for group III was not significant ($p = 0.025$, $n = 49$, $df = 1$), suggesting a different relationship between the variables than the assumed pathways. The limitations in degrees of freedom did not allow us to consider the causal effect of Ca^{2+} on TP, which may exist, as suggested by the correlation between the two variables.

As a final step, we investigated the performance of the CD ratio in predicting the explanatory variables (Table 3), considering the three groups of lakes from the regression tree (Fig. 6). As could be expected from the previous analyses, the models indicate relatively high prediction for z_{max} and for Ca^{2+} (Table 3). The relationship between z_{max} and TP shown by the structural equations appears in the residuals of the predictive models (Fig. 6). Therefore, the prediction of z_{max} increases when an estimation of TP is included in the model (Table 3). For Ca^{2+}, the prediction capacity of the CD ratio does not improve if TP is involved, and only slightly if depth is considered.

Discussion

The CD ratio in the sediments of the Pyrenean lakes is mainly explained by lake depth. Chrysophytes are the dominant planktonic algal group in many oligotrophic lakes, including mountain lakes (Nicholls 1995; Olrik 1998; Tolotti et al. 2003; Zeeb et al. 1994). Although

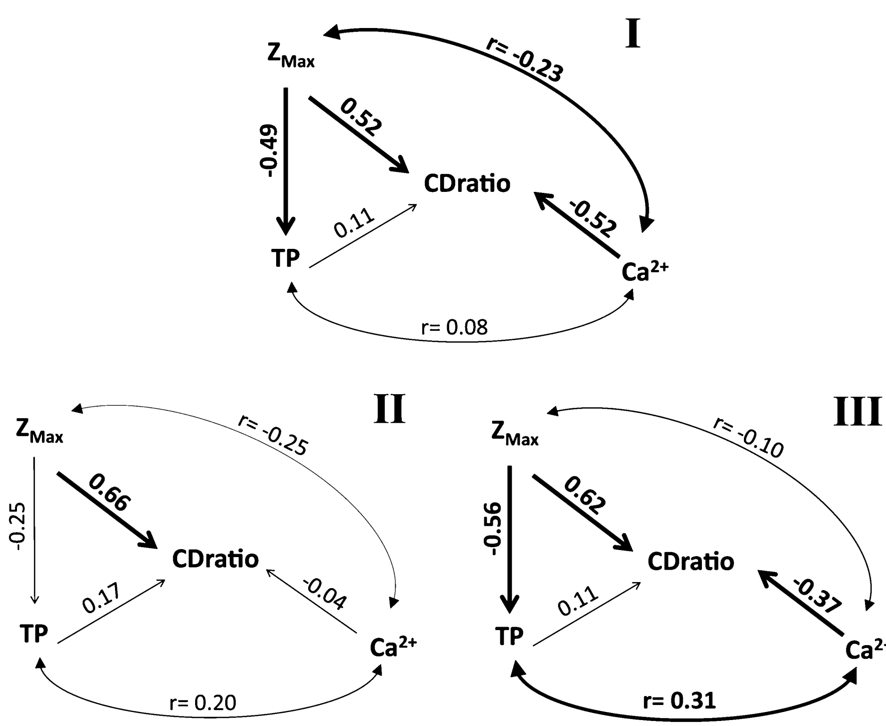


Fig. 5 Structural equation models for groups of lakes. *I* all data ($n = 76$), *II* lakes with $Ca^{2+} \geq 216$ μeq/L ($n = 27$), *III* lakes with Ca^{2+} <216 μeq/L ($n = 49$). Significant relations are plotted using *thick solid lines*. *Single straight arrows* are the path coefficient of the model and *curved double arrows* indicate a correlation between variables. All coefficient are standardized

diatoms may occupy a large variety of benthic substrates (DeNicola et al. 2004; McCabe and Cyr 2006), the chrysophyte abundance relative to benthic diatoms increases with depth. The photic zone in the Pyrenean lakes occupies a large proportion of the water column, as is indicated by the large Secchi disk values (Table 1). This is a feature common to temperate mountain lakes, which are poor in DOC and display low productivity (Catalan et al. 2002, 2009a).

With increasing depth, the available benthic area for diatoms increases at a lower rate than the volume for chrysophytes in the water column. Therefore, despite the fact that several diatom species grow in the plankton and there are some rare benthonic chrysophytes (Douglas and Smol 1995), the relative abundance of chrysophycean cysts relative to diatom valves should serve as an indicator of the importance of the planktonic environment in a lake. In mountain lakes, the sedimentary diatom record is largely dominated by benthic species (Cameron et al. 1999). Even in the exceptional cases of a large abundance of planktonic diatom species, they do not exceed the proportion of benthic ones (Koinig et al. 2002). In our data, excluding the planktonic diatom species does not make any real difference in the CD ratio value.

Our results indicate the CD ratio may be used to study past changes in lake depth, i.e. water level oscillations. Lake level fluctuations may respond to climate changes, geomorphological and tectonic processes, ontogenic lake dynamics and human disturbance. In any case, changes in lake level have substantial consequences for the organization of aquatic ecosystems (Leira and Cantonati 2008) and, eventually, are recorded in the lake sediments. When oscillations of several meters occur throughout the time period considered, the relative change in habitat

Fig. 6 Regression models using the CD ratio to predict ▶ ln_Ca^{2+}, ln_z_{max} and ln_TP, and the correlation between the residuals of regression and these variables. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

availability for diatoms and chrysophytes can be captured by the CD ratio in the sediments. The reconstruction of lake-level fluctuations has been undertaken using different proxy variables: lithology (Magny 2001), sediment accumulation rate (Shuman et al. 2001), biomarkers (He et al. 2014), macroinvertebrates (Luoto 2009) and diatom assemblages (Gomes et al. 2014; Shinneman et al. 2010; Yang and Duthie 1995). Chrysophyte assemblages and the CD ratio, however, have not been applied to the reconstruction of lake-level fluctuations. The complexity of the response of the system to changes in lake depth usually requires a multi-proxy approach, tailored to the characteristics of the lake and catchment system. The simplicity of the CD ratio provides a quick assessment, in contrast to other proxies. The use of diatom assemblages (Moos et al. 2005) requires more effort and a detailed knowledge of the species identification and ecology.

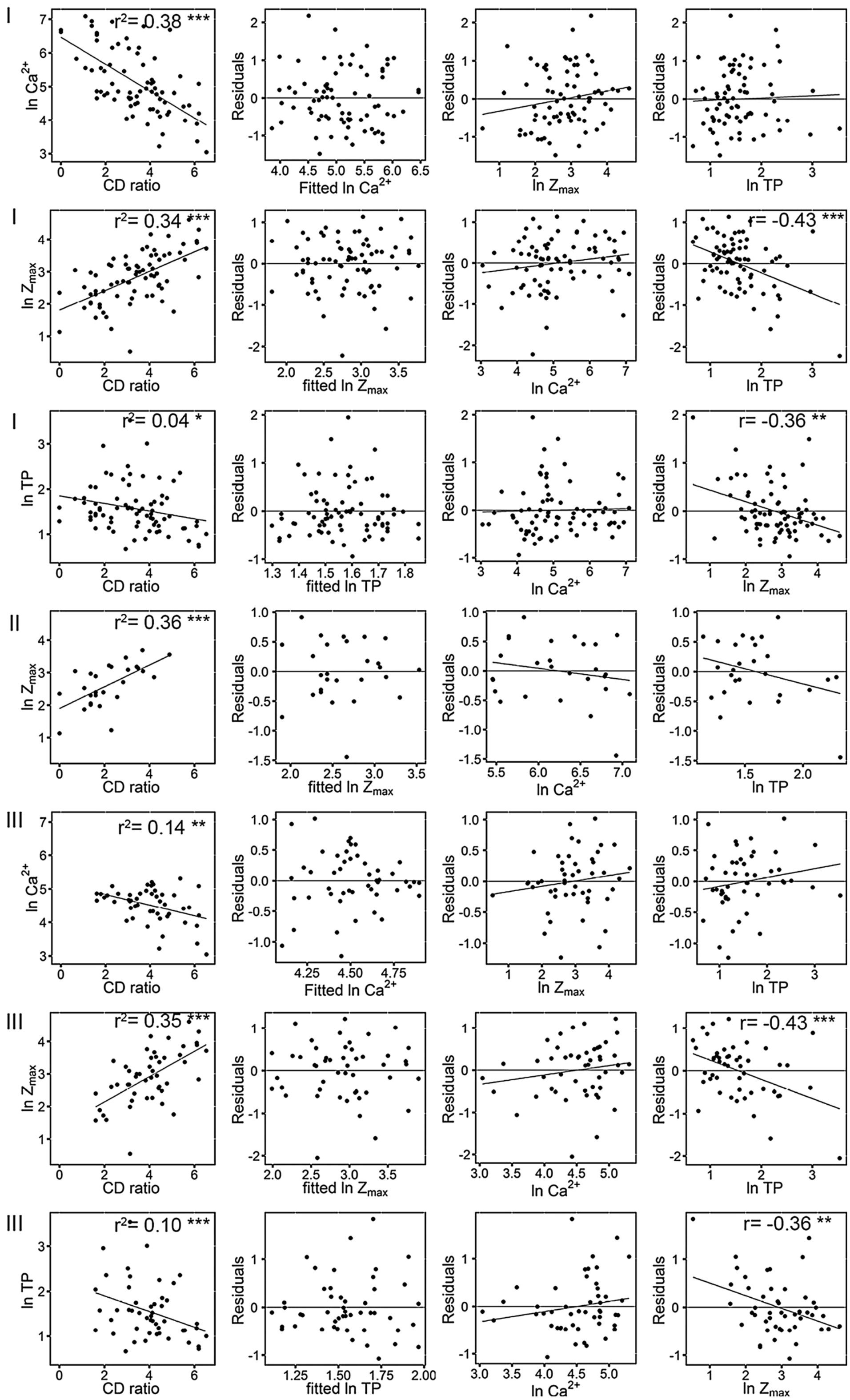
The CD ratio in lakes of the Pyrenees is more sensitive to lake depth than to other factors such as lake productivity (i.e. TP) and chemical composition (Ca^{2+}, Mg^{2+}, pH). In other studies, the CD ratio has been inversely related to phosphorus concentration in both deep and shallow lakes (Werner and Smol 2005), and to lake trophic state (Hobæk et al. 2012; Smol 1985). The weak relation found here might be related to the generally low trophic state of the lakes studied, which range from ultra-oligotrophic to barely mesotrophic (Table 1). In that respect, the average phosphorus concentration and maximum range in the Pyrenean lakes are lower than in lakes studied

Table 3 Summary of the regression models for selected variables using all data (n = 76)

	Model	R^2 Jackk.	RMSE Jackk.	AIC
(a)	$ln\_z_{max} \sim +0.33$ CD ratio*** $+ 0.19\ ln\_Ca^{2+}* - 0.54$ ln_TP*** $+ 1.54*$	0.50	0.58	131
(b)	$ln\_z_{max} \sim +0.25$ CD ratio*** $- 0.53$ ln_TP*** $+ 2.79***$	0.46	0.59	134
(c)	$ln\_z_{max} \sim +0.30$ CD ratio*** $+ 1.80***$	0.34	0.64	149
(d)	$ln\_Ca^{2+} \sim -0.48$ CD ratio*** $+ 0.27\ ln\_z_{max} + 6.46***$	0.40	2.58	178

Models were validated using jack-knife resampling (Jackk.). The use of these models for environmental reconstruction requires a thorough analysis of issues indicated in the discussion text

$*p < 0.05$, $***p < 0.001$


I
r²= 0.38 ***
ln Ca2+
CD ratio
Residuals
Fitted ln Ca2+
ln Zmax
ln TP
I
r²= 0.34 ***
ln Zmax
CD ratio
fitted ln Zmax
ln Ca2+
r= -0.43 ***
ln TP
I
r²= 0.04 *
ln TP
CD ratio
fitted ln TP
ln Ca2+
r= -0.36 **
ln Zmax
II
r²= 0.36 ***
ln Zmax
CD ratio
fitted ln Zmax
ln Ca2+
ln TP
III
r²= 0.14 **
ln Ca2+
CD ratio
Fitted ln Ca2+
ln Zmax
ln TP
III
r²= 0.35 ***
ln Zmax
CD ratio
fitted ln Zmax
ln Ca2+
r= -0.43 ***
ln TP
III
r²= 0.10 ***
ln TP
CD ratio
fitted ln TP
ln Ca2+
r= -0.36 **
ln Zmax

elsewhere, in which significant relationships between CD and TP were found. Nonetheless, the relationship between the residuals of the CD ratio – z_{max} predictions and TP (Fig. 6) indicate that estimating lake fluctuations with the CD ratio can be improved if TP is determined independently, e.g. by using diatoms (Hall et al. 1997).

Ca^{2+} is also an important explanatory variable of the CD ratio. The statistical analyses suggest that Ca^{2+} affects the CD ratio, independent of the correlation between Ca^{2+} and the pH-alkalinity gradient. The species composition of chrysophyte and diatom assemblages is known to respond to alkalinity and pH (Hadley et al. 2013; Pla et al. 2003) and, consequently, Ca^{2+} significance in the assemblage composition is usually considered an effect of the contribution of Ca^{2+} to alkalinity. Our results, however, indicate that Ca^{2+} concentrations, rather than total alkalinity, favor diatom productivity over chrysophyte productivity. The regression tree shows that the Ca^{2+} influence is only significant when the concentration is <216.6 μeq/L. How Ca^{2+} levels favor diatoms over chrysophytes remains a subject for speculation. It could be a consequence of physiological constraints or to some biogeochemical pathway that specifically affects the benthic community. Our results support the need for studies that address the role of Ca^{2+} in these lakes, beyond its influence in the acid–base balance. In a regional analysis of water chemistry in European mountain lakes, 200 μeq Ca^{2+}/L was suggested as a threshold that reflects a change in the nature of rock weathering processes in the watersheds of the Pyrenean lakes (Camarero et al. 2009); this coincidence also merits future consideration.

It may be argued that preservation of biogenic silica structures also affects the relative abundance of chrysophyte cysts versus diatom valves in sediment samples. Because chrysophycean cysts are more heavily silicified, dissolution of diatoms occurs first (Smol 1985). Thus, the CD ratio may be influenced by differential preservation in sediments. High alkalinity and certain ionic ratios in particular are the primary cause of valve dissolution (Barker et al. 1994). The diatom valves were well preserved in the sediments of all the lakes studied. In fact, the CD ratio showed an inverse relationship with pH and alkalinity, indicating a negligible effect on the CD ratio of valve dissolution in these lakes. In any case, a diatom preservation index can always be applied to take into account potential artifacts (Ryves et al. 2001).

Other factors such as lake thermal stratification and ice-cover duration could affect the CD ratio. Climate variability causes changes in the heat budgets of the lakes and modifies their thermal stability (Hadley et al. 2014). These changes, in turn, provoke shifts between the dominance of benthic and planktonic diatoms (Michelutti et al. 2015a, b). *Cyclotella*-like forms (*Cyclotella* and *Discostella*) are considered planktonic, and their abundances are related to changes in temperature and physical structure of the water column (Winder et al. 2009). In our samples, the cyst/diatom ratio calculated excluding *Cyclotella*-like forms showed a high correlation with the ratio calculated using the whole diatom assemblage ($r = 0.97$, $p < 0.001$, $n = 76$) and the abundance of *Cyclotella*-like forms was not related to the CD ratio ($r = 0.07$, $p = 0.56$, $n = 76$). Chrysophyte and diatom assemblages both respond to variations in ice cover duration (Keatley et al. 2008; Pla and Catalan 2005). Likewise, extended periods of ice cover could change the relative importance of benthic versus planktonic diatoms (Lotter and Bigler 2000). Therefore, the ice-cover duration could also affect the CD ratio, as observed in other areas (Smol 1985). Lake ice-cover duration, however, did not show any significant influence on the CD ratio in our samples, perhaps related to the dominance of benthic diatom species.

In conclusion, we suggest that the CD ratio may be used as a proxy for water level fluctuations in mountain lakes and oligotrophic lakes in which diatoms and chrysophytes are the principal components of the benthic and planktonic communities. The ratio may complement information provided by other environmental proxies (Magny et al. 2007, 2012) in the toolbox of paleolimnologists. The CD ratio can be particularly useful in lakes that have experienced periods of contrasting lake level in the past (e.g. Late Glacial dynamics). Seepage lakes with marked water level fluctuations are also candidates for use of the ratio.

In lakes that did not experience large lake level fluctuations, the CD ratio may reflect chemical changes (Ca^{2+}). There are many indicators that can provide insight into these alternative causes, including the diatom composition per se, which is a good indicator for chemical and trophic state changes (Chen et al. 2008; Cremer et al. 2009).

To sum up, we provide the following guidelines for use of the CD ratio in paleolimnological studies in mountain lakes and similar aquatic ecosystems:

1. The CD ratio should be relatively constant in a lake without marked past changes in z_{max} or Ca^{2+}. Equations (c) and (d) in Table 3 can help evaluate potential fluctuations of these variables according to the observed CD ratio. If small, the CD ratio is not particularly helpful for use in a paleolimnological study.
2. If any of the variables (depth or Ca^{2+}) has fluctuated in a range of paleolimnological interest, check for complementary evidence for changes in water level (e.g. geomorphology, laminated sediments, changes in the spatial distribution of the aquatic vegetation, changes in littoral lithology, etc.) and water chemistry (e.g. sediment elemental chemistry, diatom assemblages, changes in indicator aquatic vegetation, etc.). If there is no evidence for changes in any of these variables, investigate further what other factors may affect the CD ratio at the site (e.g. TP, ice-cover duration). The "space-for-time" substitution is commonly used in paleolimnological inference (Cremer et al. 2009; DeNicola et al. 2004; Jong et al. 2013; Millet et al. 2012; Pla and Catalan 2005), but has some weaknesses in that the range of temporal variation in one site and the range of variation across sites may differ substantially among variables (Walker et al. 2010).
3. If there is evidence of z_{max} changes, you can apply equation (c) if you do not have any estimate of the current Ca^{2+} and TP concentrations. If you do know them, you can use constant values in equations (a) or (b) for a better estimate of z_{max} fluctuations. In case you have independent reconstructions of Ca^{2+} or TP, you can use the same equations, but change the Ca^{2+} and TP values through time.
4. If there is evidence of chemical changes, but it is thought that Ca^{2+} concentrations were always above 200 μeq/L, it makes no sense to estimate Ca^{2+} fluctuations using the CD ratio. It does, however, make sense to determine Ca^{2+} with the CD ratio (equation d in Table 3) if the lake could have fluctuated above and below that threshold, or has always been below. It is worth including z_{max} as a constant if depth has not changed markedly during the period studied. Alternatively, include independent estimates for z_{max} over time.
5. Lakes with high alkalinity could experience strong dissolution of diatom valves. The influence of dissolution on the CD ratio can be checked through the coherence with a diatom dissolution index (Ryves et al. 2001).
6. Finally, there is no standardized way to calculate the CD ratio. The crude approach is to divide the number of cysts by the number of diatom valves. If the number of diatom valves counted is not constant, then part of the variation in the CD ratio may result from variability in the diatom counts. It is advisable to base the CD ratio on a constant number of diatom valves. Even in this case, as we have shown, the distribution of the ratio is rather biased (Fig. 2), though log transformation of the absolute number of cysts may provide a normal distribution of the ratio. Use of the equations in Table 3 requires following the CD ratio estimate used in our study.

Acknowledgements This study was funded by the EU EMERGE Programme (EVK1-CT-1999-00032), the Spanish Government LACUS (CGL2013-45348-P) and CUL-PA (Parques Nacionales 998/2013). CRR was supported by a Ph.D. scholarship from the Departamento Administrativo de Ciencia, Tecnología e Innovación de Colombia (COLCIENCIAS-ICETEX-LASPAU, 070-2007) and the Pontificia Universidad Javeriana (DJE-025-2007).

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