INTRODUCTION

Palaeolimnological tools can be applied to reveal information retained in aquatic sediments (Smol 1992, European Union 2000). Cladocerans (Branchiopoda: Phyllopoda), which have been demonstrated to be excellent indicators of environmental change (Eggermont and Martens, 2011), have been used in palaeolimnological studies to reconstruct past environmental conditions of lake ecosystems since the 1950s (Frey, 1986). Despite their obvious value, methodological limitations and sources of error still exist. Cladoceran remains, for example, preserve selectively in lake sediments, as some species and some components preserve better than others. In the case of Daphniids O.F. Müller, only postabdominal claws and resting spores (ephippia) are typically found in sediments (Schmidt et al., 1998; Korhola, 1999; Sarmaja-Korjonen, 2002), while the small size of their claws inflicts an elevated risk of losing claws during sieving. This may lead to an underestimation of Daphnia O. F. Müller abundance when it is based on the number of claws (Nykänen et al., 2009). The poor preservation of Daphnia is particularly problematic, because Daphnia is by far the most studied cladoceran taxon (Ebert, 2005). For example, Peters and de Bernardi (1987) and Lampert (2011) published comprehensive reviews of Daphnia ecology, and the number of scientific articles on Daphnia indexed in the Thomson Reuters Web of Science exceeds 10 000 (Seda and Petrusek, 2011). Nevertheless, Daphnia remains do preserve well in some lakes, such as in Lake Kivijärvi (Finland), although the reasons behind this variation in preservation are not yet fully understood. The preservation issues in sedimentary cladocerans have been studied before (Deevey, 1964; Kerfoot, 1981), but we are unaware of previous research attempting to assess the variations in Daphnia degradation in a sediment core.

In this study we combined data on Daphnia preservation to sedimentary geochemistry, diatom-inferred lake water pH, predation indices and known land use and fishing history of Lake Kivijärvi in order to shed new light on the preservation issues of Daphnia remains.

METHODS

Study area

Lake Kivijärvi is a small (1.8 km²), relatively deep (max depth 12 m), brown-coloured lake, which is surrounded by mires and coniferous forests. It is located at 165 m asl in central northern Finland (N 63° 55.998’ E 27° 54.281’). During the summer stratification in August 2006, which corresponds to the age of our topmost sedi-
ment sample, the lake surface water (1 m) was slightly acidic (pH 6.2), with an alkalinity of 59 µmol L⁻¹ and conductivity of 19 µS cm⁻¹. The total phosphorus and total nitrogen concentrations measured during this period were 33 µg L⁻¹ and 540 µg L⁻¹, respectively, and the chlorophyll-a concentration 22 µg L⁻¹. The lake is fed by River Lumijoki and the brook Myllypuro. Human activities, such as land use and fishing have affected the lake since the beginning of the 20th century. The catchment area (53 km²) has been heavily ditched during forestry practices, which were most intense during the 1960s and 1970s. In addition, one peat extraction area (33 ha) located in the western part of the Kivijärvi catchment operated between 1986 and 2012 (N. Huotari, personal communication).

Today the proportion of the ditched area in the lake catchment is over 40%. More recent disturbances (since 2008) include sulphate and metal pollution from the Talvivaara Ni-Cu-Zn-Co mine, which is connected to Lake Kivijärvi via Lumijoki River and is located approx. 6 km NE from Lake Kivijärvi (Kauppi et al., 2013). The average (1971-2000) yearly air temperature and precipitation are +2°C and ~600 mm, respectively (Mikkonen et al., 2009). Annual precipitation has increased by an estimated 0.92 mm±0.50 mm year⁻¹ (Irannezhad et al., 2014) during the last century and mean air temperature has increased by 2°C during the past 166 years (Mikkonen et al., 2014).

Bathymetric data was obtained from the Finnish Environment Institute electronic GIS library (http://www3.ymparisto.fi/d3/wmsrajapinta.htm), the catchment area was determined using the online tool VALUE provided by the Finnish Environmental Institute (http://paikkatieto.ymparisto.fi/value/) and was analysed using the geographical information system program ESRI ArcMap 10.2.1. Water chemistry data was obtained from the OIVA database (Finnish Environment Institute, https://wwwp2.ymparisto.fi/scripts/oiva.asp). Data regarding forestry activity between 1966 and 1994 was obtained from historical map archives of the National Land Survey of Finland (http://vanhatpainetutkartat.maanmittauslaitos.fi/).

Coring

Two short sediment cores were retrieved from the northern section of Lake Kivijärvi (N 63° 55.998’E 27° 54.281’ WGS84, at the depth of 8 m) using a HTH-Kajak sediment corer with a diameter of 9 cm (Renberg and Hansson, 2008). Core A (26 cm length) was used for sediment dating, and for analyses of sub-fossil cladocerans and diatoms, core B (24 cm) was used for elemental analysis. The cores were subsampled at 0.25 cm intervals for the first 5 cm and at 1 cm intervals between 5 and 26 cm. The initial reason for different subsampling for the first 5 cm was to provide possibility for high resolution assessment of known mine pollution, which is affecting lake water quality since 2008. Mining effluent has resulted in elevated lake metal (Fe, Mn, Ni, Zn) and salt (NaSO) concentrations, and has turned the lake meromictic (Kauppi et al., 2013). Subsampling was conducted in the field and the samples were stored in plastic zip lock bags and stored in a dark cold room at 4°C. As the two cores were retrieved from the flat and large center of the lake and less than 1.5 m apart from each other, the temporal resolution of these two cores can be assumed as comparable. Nevertheless, the cores were correlated based on their visual features. Both cores exhibited analogous and clear changes in sediment composition in the top first cm, where the sulfur rich layer originating from the mine pollution disappeared strictly at 0.75-1 cm level. Moreover, both cores showed a clear transition layer between 4 and 6 cm, where the sediment color changed to darker brown. Below this, both cores showed unchanged sediment color and texture. The top 0-1 cm core samples were omitted from the study, as they were deposited after the onset of mine pollution and therefore strongly affected by metals and sulphur, which caused the almost complete disappearance of Daphnia remains.

Dating and sediment chemistry

Freeze dried sediment samples from core A were radiometrically dated at the Liverpool University Environmental Laboratory, using Ortec HPGe GWL well-type coaxial low background intrinsic germanium detectors (Appleby et al., 1986). The samples were analysed for ²¹⁰Pb, ²²⁶Ra, ¹³⁷Cs and ²⁴¹Am by direct gamma assay. Elemental concentrations of erosion indicators magnesium (Mg), potassium (K) and sodium (Na) were analysed in the acid soluble (NHO₃) sediment fractions in the Metropolilab Environmental laboratory at Helsinki, which is an accredited testing laboratory (FINAS T058).

Daphnia analysis

Each sediment subsample (~1 cm³ of wet material) was treated using 10 % KOH solution, heated (80°C) on a hotplate for 30 min, and sieved through 48 µm mesh size with tap water. Samples were mounted with safranine stained glycerol. For more information regarding the protocol, see Szeroczyńska and Sarmaja-Korjonen (2007). Daphnia remains were identified and counted using both 200 and 400 x magnifications. During the preliminary microscopic screening, a broad variation in the preservation of postabdominal claws was observed, e.g. notable variations in type and magnitude of damage in claws, denticles and setae. In addition, also the size of the claws showed large variability, which, in turn, has a direct impact on their resistance to damage. Classification of claws was thus found to be extremely difficult, whereas the differences in degree of preservation were clearer for caudal spines. Caudal spines were therefore identified as the most
suitable component to study the variance in preservation of subfossil *Daphnia*. To evaluate these preservation differences, 100 spines from each one of the 20 sediment subsamples were identified and assessed for a taphonomic grade. Taphonomic grading is used in palaeoecology to assess the deposition speed and reworking of fossil assemblages (Brandt, 1989). The studies regarding microbial decomposition of *Daphnia* remains are rare, but it has been reported that *Daphnia* remains are rapidly and heavily colonized by chitin degrading microbes (Tang *et al.*, 2009) and fungi (Czezuga *et al.*, 2002). The colonization by degrading organisms would imply a rather even and comprehensive degradation within a certain sediment layer than mechanical impacts. In the present work each spine was graded in relation to its stiffness, folding and breakage (Tab. 1; Fig. 1). We assumed that folded or broken spines have been damaged due to mechanical impact, and spines that have completely lost their form (slack spines) have been affected by microbial or chemical degradation.

**Predation assessment**

To assess changes in predation on cladocerans in Lake Kivijärvi, we conducted morphological measurements of *Eubosmina longispina* Leydig exoskeletal remains (36-46 measurements of carapace and mucro length per sample), and counted the *Chaoborus* spp. Lichtenstein, mandibles detected in the sediment subsamples to calculate the ratio (number of *Chaoborus* spp. mandibles: 100 *Daphnia* spines). The value as an ecological indicator of morphological changes within the genus *Bosmina* has been recently reviewed by Korosi *et al.* (2013), but the applicability of subgenera *Eubosmina* morphology is not so straightforward due to contrasting results in literature (Sprules *et al.*, 1984; Johnsen and Raddum, 1987). In contrast, the abundance of *Chaoborus* larvae has been demonstrated to reflect the abundance of fish and to affect the cladoceran community structure (Sweetman and Smol, 2006).

**Numerical analysis**

The non-parametric Mann-Kendall trend test (Gilbert, 1987) was used to detect significant monotonic trends in the taphonomic grade data of sedimentary *Daphnia* remains. Constrained optimal sum of squares partitioning with untransformed species percentage data (Birks and Gordon, 1985) was used to detect significant zones in taphonomic stratigraphy. The number of statistically significant zones was calculated using the broken-stick model described in Bennett (1996). Optimal partitioning was conducted using the program ZONE 1.2 (Lotter and Juggins, 1991). The Mann-Kendall test was conducted with PAST statistics 3.10 software (Hammer *et al.*, 2001).

**Tab. 1. Taphonomic grades for *Daphnia* caudal spines.**

| Excellent preservation | Fair preservation | Poor preservation |
|------------------------|-------------------|------------------|
| Stiff and straight spines with no visible damage | Stiff and straight spines with breakage and/or folding | Slack spines. Initial shape is no longer sustained |

**Fig. 1.** A) An example of a well-preserved caudal spine graded as excellent. B) A relatively well preserved, but visibly damaged caudal spine graded as fair. C) A poorly preserved caudal spine graded as poor.
Diatom-inferred lake water pH (DI-pH)

The pH history of Kivijärvi was quantitatively reconstructed using an independent modern diatom-water pH calibration data set consisting of 98 surface-sediment diatom assemblages from northern Finland and corresponding pH measurements (for more details see Seppä and Weckström, 1999; Väliranta et al., 2011). After testing different models (WA-inverse deshrinking, WA-classical deshrinking, Partial-Least-Squares) the 1-component weighted average partial least squares (WA-PLS) model provided the best performance with a coefficient of determination ($r^2$) of 0.68 and a root mean square error of prediction (RMSEP) of 0.31 pH units. This diatom-based quantitative pH model was then applied to the fossil diatom data analyzed from Lake Kivijärvi. Methods and taxonomic literature used for diatom analyses are described in Weckström et al. (1997). The quantitative DI-pH is inferred only for the depths of 1-16 cm, as the original reason for constructing the DI-pH model was to study the recent impact of the Talvivaara mine on the Lake Kivijärvi water chemistry (unpublished data).

RESULTS

Dating and sediment geochemistry

According to the radiometric dating, the sediment depth of 2.5 cm records the fallout from Chernobyl reactor accident (AD 1986) and the depth of 4.5 cm records the fallout maximum from atmospheric nuclear weapons testing (AD 1963, Appleby et al., 1991). The dating provided a sedimentation rate of approximately 0.1 cm year$^{-1}$. Unsupported lead concentrations reach zero values at the depth of 10 cm, which corresponds to the beginning of the 20th century (1910±8). Concentrations of K and Mg increased after 1925 and reached maximum values in the late 1990s (Fig. 2).

Fig. 2. Results of taphonomical stratigraphy, geological characteristics, predation indices and diatom-inferred lake pH. *Eubosmina* measurements are in µm (bar, average carapace length or mucro length; vertical line inside bar, standard deviation). Note that *Eubosmina* were not measured between depths 1-5 cm. *Chaoborus* spp., number of *Chaoborus* spp. claws counted in each sample per 100 *Daphnia* spines. Horizontal line indicates the statistically significant shift in the preservation pattern.
**Daphnia remains**

Many *Daphnia* remains were detected throughout the core, except for the top 0-1 cm section, where they were extremely rare, likely in relation to the impact of the mine pollution. In the other sediment layers, postabdominal claws, carapace, ephippia, caudal spines and head shields were present in large numbers. Only *Daphnia longispina* O.F. Müller -type postabdominal claws were detected. Head shields showed various degrees of preservation including many very well preserved helmet-type head shields (Fig. 3A), with one head shield still attached to the carapace (Fig. 3B). There was a significant increasing trend towards present in the proportion of caudal spines graded as excellent (Mann-Kendall: Z=3.88, S=103, P<0.001) and fair (Mann-Kendall: Z=3.53, S=94, P<0.001), and a significant decreasing trend in the proportion of caudal spines graded as poor (Mann-Kendall: Z=3.94, S=-105, P<0.001). Zoning revealed two significant zones at depths 1-9 and 9-26 cm (Fig. 2).

**Predation assessment**

The length of the mucro and carapace of *E. longispina* exhibited no clear shifts within the core (Fig. 2). Measurements were terminated at the depth of 5 cm because of the appearance of *Bosmina longirostris* O. F. Müller in the sediment, as carapaces of *E. longispina* and *B. longirostris* are extremely difficult to be discriminated from each other. *Chaoborus* mandibles were detected in very small numbers (0-2 mandibles per sample) throughout the core.

**DI-pH**

The DI-pH of the deepest core layers (which were likely deposited during the 18th century), was comparable to DI-pH values around year 2000 (Fig. 2). However, DI-pH started to decrease since the late 19th century reaching the lowest values during the 1960s (Fig. 2). DI-pH remained at lower values between ca. 1920 and 1995, but later increased towards modern levels.

**DISCUSSION**

**Daphnia remains in Lake Kivijärvi**

Usually only *Daphnia* postabdomal claws and ephippia are found in lake sediments. Sometimes, as reported by Frey (1991), Mancini *et al.* (1999), and Sarmaja-Korjonen (2007), different components of *Daphnia* subfossils can stay relatively intact in the sediment for extended periods of time. Also *Daphnia* head shields have been detected in sediment samples (Frey, 1991; Mancini *et al.*, 1999), but to our knowledge, helmet-type head shields of *Daphnia longispina* -group species have not been reported from lake sediment samples before. In the sediments of Lake Kivijärvi sediments, the *Daphnia* head shields appeared folded with fornices clearly visible, and

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*Fig. 3. A) Daphnia* helmet type headshields. Scale bar: 100 µm. B) *Daphnia* headshield attached to carapace.
Changes in Daphnia preservation in Lake Kivijärvi

According to the statistically significant core zonation, the greatest shift in Daphnia preservation occurred at the depth of 9 cm (~1925) and was clearly reflected by changes in proportions of different grades. Though not statistically significant, also the depth of 12 cm (~ pre 1900) clearly emerged as the level where early changes in Daphnia preservation onset. The 9 cm shift is most pronounced for the excellent and poor grades, whereas the change regarding spines graded as fairly preserved is not as distinct. Earlier studies suggest that Daphnia preservation is controlled by temperature (Szeroczyńska and Zacisza, 2005) or water chemistry (Sarmaja-Korjonen, 2007). However, changes in hypolimnetic temperatures of Lake Kivijärvi during the 1920s are not likely, because a most notable warming occurred in N-Finland only after the 1960s (Mikkonen et al., 2014). Moreover, hypolimnetic temperatures during stratification are not easily altered by relatively small scale increase in summer air temperatures (Arvola et al., 2010).

DI-pH showed a slight decrease since ~1910, which may have been related to natural acidification i.e. continuous leaching of buffering mineral elements like calcium (Ca), potassium (Na), magnesium (Mg), and sodium (K) (Pennington, 1984). In fact, the proportion of naturally acidified lakes is higher in central and northern Finland compared to S Finland, where anthropogenic acidification is more common (Meriläinen and Huttunen, 1990). The decrease in DI-pH since ca. 1910 corresponds relatively well to the change in Daphnia preservation, but effects of pH on preservation changes is not straight forward. In fact, preservation was not affected when pH increased since ca. 1995, thus contradicting the response during slight acidification at the beginning of the 20th century. The lowest DI-pH values around 1960 could be due to the increased impact of acid rain on small freshwaters as sulphur emission in Finland increased substantially during the period of 1950-1970 (Kauppi et al., 1990). However, this period of lower pH seemed not to affect the preservation of Daphnia remains (Fig. 2).

The level of preservation of all subfossil material is usually related to accumulation rates, with good preservation (high taphonomic grade) reflecting fast accumulation and burial (Brandt, 1989). Since the core was only 210Pb-dated, we lack dates previous to 1900 (i.e., below the depth of 10 cm). As a consequence, the possibility of low sedimentation rates and subsequent poor preservation of Daphnia before ca. 1925 cannot be ruled out. Possible reasons behind major changes in the sedimentation rate during the pre-industrial era should be principally related to hydrological perturbations, such as water level manipulation and channel building. However, according to the map by Gylden (1848), shape and size of Kivijärvi did not change since the mid-19th century, which excludes any large-scale water works during the early 20th century.

Cladoceran remains are directly affected by microbial degradation of chitin, which occurs both in the water and in the sediment (Swiontek Brzezinska et al., 2008). Bacteria are the most important responsible of chitin decomposition in aquatic environments (Aumen, 1980; Goody, 1990), but there are indications that particularly Daphnia remains are attacked also by aquatic fungi (Czezuga et al., 2002). In addition, chemical changes of the sediments (diagenesis) may alter the conditions within the sediment and the degree of ongoing degradation of chitinous subfossil remains (Kidwell and Flessa, 1995). Forestry activities may affect lake water characteristics such as chemical oxygen demand (Ahtiainen, 1992; Rask et al., 1998), which in their turn may affect the fungal community (Wurzbacher et al., 2010) and slow down the bacterial decomposition of chitin (Köllner et al., 2012) at the sediment-water interface. However, documented forestry activities occurred well after the 1920s, while the recent increasing concentrations of erosion indicators (Virkkainen and Tikkanen, 1998) in the studied sediment core started only during the last decades of the 19th century. In addition to forestry actions, also changes in lake productivity could affect microbial community (Naether et al., 2012). However, according to the available maps and per-
sonal communication by S. Peronius, no farmland existed within the catchment during the early 20th century. Moreover, the first residents occupying the lake’s shoreline in the early 20th century were not farmers, but employed, thus the agricultural land use was minimal. This is also supported by the diatom data (not shown here), which did not show any indication of eutrophication as no taxa preferring elevated nutrient concentrations occurred during the last centuries.

The sole known potentially relevant event that occurred in Lake Kivijärvi during the 1920s was the beginning of intense fishing activity, which resulted in large vendace (Coregonus albula Linnaeus) catches since the 1920s (S. Peronius, personal communication). Planktivorous fish are known to target large-size zooplankton (Zaret, 1980), and vendace has been noted to prefer large cladocerans, such as Eubosmina and Daphnia, in Swedish (Hamrin, 1983) and Finnish (Viljanen, 1983) forest lakes. Cladoceran remains are poorly digested during their passage through fish guts (Sutela and Huusko, 1993; Riccardi, 2000) and Daphnia remains have been proved to survive the passage through vendace and whitefish digestive apparatus (Sutela and Huusko, 2000). The poor preservation level of Daphnia spines before the 1920s may therefore reflect a higher proportion of partly digested Daphnia in the correspondent sediment samples. The intensive removal of fish may have also increased the abundance of invertebrate predators (Milardi et al., 2016). Elevated invertebrate predation has been noted to induce thickening and hardening of Daphnia carapaces (Rábus et al., 2013). This might be the reason for the appearance of hard and thick remains, which are more resistant to post-mortem degradation. However, the lack of variations of Eubosmina size suggests that no high magnitude changes in invertebrate predation have occurred during the shift in Daphnia preservation. Moreover, the very low number of Chaoborus mandibles in our samples (<2 per sample) allow no assumptions regarding changes of predation regime in Lake Kivijärvi, because according to Quinlan and Smol (2010) a minimum of 5 to 10 mandibles is needed to reliably assess Chaoborus assemblages. High head helmet and a long spine are also considered as general defensive structures, which are grown by Daphnia when they are subjected to invertebrate predation (Laforsch and Tollerian, 2004). The fact that only Daphnia remains with long spine and high helmet were identified throughout the core studied, further supports the hypothesis that only little change in the intensity of invertebrate predation occurred at Lake Kivijärvi during the last ~250 years.

The results presented here suggest the potential of Daphnia caudal spine to be used as indicator of cladoceran degradation in sediments. However, more work is still necessary to further evaluate this potential. For example, type and degree of degradation of caudal spines caused by fish ingestion, should be experimentally assessed. Similarly, the microbial degradation of Daphnia remains should be tested in a controlled environment, in order to clarify the degradation process and the actual microbial community involved in Daphnia degradation.

CONCLUSIONS

This work provides first evidence for the potential use of caudal spine as indicator of Daphnia degradation in sediments. Historical information and sediment geochemistry suggest that no large-scale environmental or hydrological changes have affected the remote Lake Kivijärvi during the early decades of the 20th century, when major changes in Daphnia preservation occurred. On the other hand, the historical data and the diatom-inferred pH profile indicate the increase in fishing activity after 1920 and the steady decrease in water pH from the beginning of 20th century till the 1960s as possible drivers of spine preservation of Daphnia. Though more work is clearly needed to experimentally verify the role of these factors in affecting Daphnia preservation, these preliminary results shed new light on the issue of large cladoceran preservation in lake sediments. This aspect is still poorly explored, but might have the potential to improve the reliability of palaeolimnological reconstructions.

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