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Spectral absorbance of benthic cladoceran carapaces as a new method for inferring past UV exposure of aquatic biota

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ABSTRACT

We developed a method for measuring fossil cladoceran (Branchiopoda) carapace absorbance to infer past ultraviolet radiation (UV) exposure in lakes. This was done under the presumptions that cladocerans synthesize photoprotective compounds, of which melanin is the main UV-absorbing pigment, to their exoskeletons and melanin is preserved in sedimentary cladoceran remains. We extracted large-sized cladoceran (benthic *Alona* spp.) carapaces from subsections of sediment cores from two environmentally divergent lakes; a humic boreal forest lake in eastern Finland (past 1500 years) and a clear-water mountain lake in the Austrian Alps (past 300 years). We measured the absorbance of extracted carapaces with a spectrophotometer under visible light and UV wavelengths using an adapter, which was designed to hold the microfossils. When compared to the spectrum of synthetic melanin, the shapes of absorbance spectra at the 700–280 nm range suggested that the fossil carapaces contained melanin. The carapace absorbance under UV throughout the sediment cores was significantly higher in the clear-water alpine lake than in the humic boreal lake reflecting differences in the general underwater UV and optical environments between the sites. In addition, carapace absorbance was significantly higher during the Little Ice Age (LIA) than during pre- or post-LIA periods in both lakes. In the alpine lake, this was most likely a response to increased underwater UV induced by reduced primary production and more transparent water column during the cold summers of LIA, whereas reduced input of carbon compounds from the catchment through elongated permafrost and ice-cover periods likely induced higher water transparency in the boreal lake during this cold climate phase. We conclude that fossil melanin provides a good estimation of past underwater UV exposure in lakes with large cladoceran carapaces preserved in sediments and that the method introduced here is easy and cost- and time-efficient technique to be widely used in paleoaquatic UV inferences.

1. Introduction

Among the numerous environmental threats to climatically sensitive aquatic ecosystems, highly energetic ultraviolet radiation (UV) has many biologically deleterious effects and its increase results in changes in productivity and species composition, ultimately altering ecosystem structure, functioning and biogeochemical cycles (Perin and Lean, 2004; Häder et al., 2011). Exposure of aquatic organisms to UV is controlled by the inherent and extrinsic properties of a given water body. The most important inherent property is the concentration of dissolved organic carbon (DOC) (Schindler et al., 1996) although algal biomass also plays a role (Laurion et al., 2000). Both absorb UV before it penetrates deep in the water column. Extrinsic properties include the geographical location of the water body (latitude, altitude), seasons, and changes in the UV-protective ozone layer. While DOC and algal biomass vary naturally, e.g. due to lake succession in time (Williamson et al., 2001a), they are also highly influenced by anthropogenic effects such as catchment disturbance and the resultant changes in carbon export to lakes. Severe ozone depletion and increased UV since the 1970s are caused by manmade chlorofluorocarbons and halons, which interact with and destroy stratospheric ozone. Despite some recent global advances in reducing ozone-depleting chemicals, ozone depletion is expected to persist and worsen, resulting in significantly increased UV doses (Björn et al., 1998; ACIA, 2005; Manney et al., 2011). On the other hand, there are also signs that ozone layer has begun to recover regionally and will continue to

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recover in the coming decades (McKenzie et al., 2011; UNEP, 2012). Apart from changes in the ozone layer, climate warming that results in reduced ice-cover period and catchment-driven limnological changes will change underwater UV intensities in the future via direct increase in underwater exposure as well as through changes in water optical properties and UV attenuation (Vincent et al., 2008).

Aquatic microcrustaceans, such as cladocerans (Branchiopoda), are negatively affected by high intensities of UV (Williamson et al., 2001b; Rautio and Korhola, 2002a). However, these animals can also be well adapted to high UV, because they can synthesize and/or accumulate photoprotective compounds; melanin, carotenoid, scytonemin and mycosporine-like amino acids, repeatedly during their life (Hessen, 1996; Rautio et al., 2009). These compounds increase survival under intensive UV (Hairston, 1976; Hebert and Emery, 1990; Hessen et al., 1999), being adaptive responses to the underwater UV environment (Rhode et al., 2001). Pigment synthesis is considered to be energetically costly and there occurs a tradeoff between damage from UV exposure and the costs of pigmentation as well as increased predation from fish hunting the most visible prey, i.e. pigmented individuals (Hansson, 2000). Of the UV protective compounds, melanin pigment results in brown/black color of cladoceran carapace and if pigmentation is strong, it can be visually detected. Strongly melanic forms of cladoceran plankton and benthos have been observed from high latitude and altitude sites (Manca et al., 1998, 2006; Rautio and Korhola, 2002b; Sommaruga, 2010; Van Damme and Eggermont, 2011).

Despite the detrimental impacts of UV in nature, little is known about its natural variability or long-term effects on ecosystems and individual organisms since meteorological and biological monitoring does not extend longer than 1970's to record past changes (Rozema et al., 2002). Natural variability in UV attenuation in lakes is strongly related to long-term climatic variation in temperature and precipitation patterns via catchment characters, water depth, productivity, and ice-cover period. For example, permafrost and surface runoff resilience impacts in releasing or holding DOC (Schindler et al., 1996; Rosén et al., 2009b), developed soils or vegetation and catchment characters act as sources of DOC (Pienitz and Vincent, 2000; Saulnier-Talbot et al., 2003; Schmidt et al., 2008), water depth affects the size of UV-free refugia in the water column (Leavitt et al., 2003), and persistent snow and ice-cover effectively attenuates UV (Vincent et al., 2007; Lami et al., 2010).

Previously, UV absorbing pigments in benthic and planktonic algae and in cyanobacteria, extracted from lake sediments, have been used as aquatic biological proxies in inferring historical patterns in UV (Leavitt et al., 1997, 1999, 2003; Verleyen et al., 2005; Lami et al., 2010). Furthermore, fossil diatom assemblages combined with bio-optical models have been used in assessing past variation in underwater light regime, and accordingly, magnitude of UV (Pienitz and Vincent, 2000; Saulnier-Talbot et al., 2003). UV absorbing compounds in plants (phenolic acids in pollen, spores, cuticles, seed coats, and wood) have proven to retain potential as a proxy for UV inferences in terrestrial and lacustrine cores (Rozema et al., 2001, 2009). However, the existing UV reconstructions are scarce and far from straightforward; it is extremely difficult to completely isolate the effects of UV, especially in aquatic ecosystems where DOC and water depth play a significant role in the range and magnitude of UV penetration (Leavitt et al., 2003, 2009; Verleyen et al., 2005). Accordingly, new supplementary approaches for long-term UV inferences are needed.

The degree of melanin pigmentation in cladocerans is related to the UV exposure of a given water body (Rautio and Korhola, 2002b; Tollrian and Heibl, 2004). Melanin is also chemically inert in chitinous sedimentary cladoceran remains after death or molting of the organism (Rautio, 2007) and it has been suggested that fossil melanin in cladoceran remains can be a valuable indicator of past underwater light regimes (Jeppesen et al., 2001; Rautio, 2007). However, no down-core studies have tested this yet. Although there exists standardized protocols for measuring extracted melanin content from modern zooplankton (e.g. Hebert and Emery, 1990), they are not optimum for sedimentary cladoceran remains, since it would be too time consuming to extract enough remains from lake sediments for the analysis due to the small size and weight of the remains. To overcome this problem, we report here a new method for evaluating past fluctuations of UV protective phenotypic features (melanin pigment) in sedimentary cladoceran remains. Our aim is to develop an easy and cost-efficient method for paleoaquatic inferences of past underwater UV and to apply and test this method for disentangling fluctuations in pigmentation, and hence in the UV exposure, in limnologically and geographically divergent (alpine versus boreal) lakes. We hypothesize that melanin is preserved in fossil cladoceran remains in centuries old lake sediment deposits and can be inferred via spectrophotometric carapace absorbance measurements. Furthermore, we presume that the UV absorbance of cladoceran carapaces would be more pronounced in the clear-water alpine site than in the boreal humic lake.

2. Regional setting

The cladoceran carapaces, which are used for developing and testing the new method for paleoaquatic UV inferences, originate from sediment cores from lakes Pieni-Kauro and Oberer Landschitzsee. Pieni-Kauro is a north boreal forest lake in eastern Finland with slightly acidic and mesohumic lake-water (Table 1). The Pieni-Kauro basin is open, connected to other lakes via streams, and its catchment consists mainly of boreal coniferous forests and mires. The Pieni-Kauro core (35 cm) was investigated for the current study at 2-cm intervals, resulting in 18 samples. The time span of the core is from *ca* 1500 yr BP to present. The details of core collection and dating of the Pieni-Kauro core are available in Luoto and Helama (2010) and environmental settings in Luoto (2010). The general cladoceran community development of the Pieni-Kauro core is previously presented and discussed in Nevalainen et al. (2013). Oberer Landschitzsee is located in the Austrian Alps above the present-day tree line and it is a clear-water and slightly alkaline mountain lake (Table 1). Oberer Landschitzsee is an enclosed basin and its catchment consists of alpine meadows with grasses and scattered dwarf pines. The core from Oberer Lanschitzsee (17 cm) was investigated with 1-cm intervals (17 samples) and the temporal range of the core is from *ca* 300 yr BP to present. Detailed environmental characteristics and coring and dating details for Oberer Landschitzsee are given by Nevalainen and Luoto (2012), who also depict and discuss the general cladoceran community succession.

Table 1
General geographical and limnological features of the study sites in Finland (Pieni-Kauro) and Austria (Oberer Landschitzsee).

	Pieni-Kauro	Oberer Landschitzsee
Location	64°17′ N, 30°07′ E	47°15′ N, 13°52′ E
Altitude	188 m a.s.l.	2076 m a.s.l.
Biome	Boreal forest	Alpine tundra
Water color	Darkbrown	Clear
Trophic status	Oligo-dystrophic	Ultra-oligotrophic
pH	Acidic-circumneutral	Alkaline-circumneutral
Maximum depth	7.9 m	13.6 m
Hydrology	Open basin	Enclosed basin

3. Material and methods

Large-sized carapaces (~500 µm) of abundant benthic cladocerans (Chydoridae) *Alona affinis* and *Alona quadrangularis* (*Alona* spp.) were extracted from the subsections of lake sediment cores (lakes Oberer Landschitzsee and Pieni-Kauro) by rinsing the sediment samples with a 100-µm mesh under running tap water and hand-picking the remains with fine forceps under a binocular microscope. The absorbance of the *Alona* spp. carapaces was measured with a Shimadzu UV/VIS-2401PC dual-beam spectrophotometer connected to Shimadzu UV Probe program (Shimadzu Corporation, Kyoto, Japan). To prevent scattering and to reduce the spectrophotometer beam diameter appropriate for the size of cladoceran remains, an adapter was developed to be placed in the spectrophotometer cuvette holder (Fig. 1). The adapter (0.1 × 1.5 × 4.5 cm) was prepared from aluminum and a suitable shutter with a diameter of 300-µm was made with a micro drill. The carapaces (double valves) were attached into the adapter individually with a UV transparent cellophane tape using fine forceps under binocular microscope and placing their anterior-dorsal part on the shutter (Fig. 1).

The adapter and cellophane tape on the shutter were used in determining the baseline and served as a reference sample. Absorbance spectra were measured from randomly selected carapaces from the sediment cores in order to assess whether the remains contained photoprotective features (melanin pigment). Spectral analysis was performed with the following settings; 5 nm slit width, 700–280 nm range, 1 nm sampling interval, and medium scan speed. A previously available absorbance spectrum of synthetic melanin (Sigma–Aldrich M8631 with a concentration 5 mg L^{-1}) was used as a standard for the melanin absorbance spectrum. A photometric approach was applied to investigate long-term trends in fossil melanin in the sediment stratigraphies of the case study sites. Ten *Alona* spp. carapaces were extracted from each section and subjected to absorbance measurements at visible light (700 and 400 nm) and UVR, which were selected to cover UV-A (380 and 320 nm) and UV-B (280 nm) wavelengths. The following settings were adapted for these photometric measurements: 5 nm slit width, 1 sample repetition, and point wavelength type.

Medians, 25–75% quartiles, and minimum and maximum values of the average measurements through the sediment cores were used to depict general trends in the absorbance values between the five selected wavelengths and two study sites. To illustrate long-term trends in absorbance of the remains through the two sediment stratigraphies, average values and 95% confidence intervals, together with lowess (locally weighted scatterplot smoothing) smoothing (span 0.3), were used to depict general trends. Lowess curves were generated with the C2 program (Juggins, 2007). Pearson's product moment correlation coefficient (r) and statistical significance ($p < 0.05$) were used to determine the relationships between the absorbance spectra of synthetic melanin and fossil carapaces. Shapiro–Wilk test was used to examine the normality of data and a t-test was used to determine whether the differences in stratigraphic absorbance values are statistically significant ($p < 0.05$) between the study lakes and within the time series at different wavelengths. The tests were performed with Paleontological Statistics (PAST) software (Hammer et al., 2001).

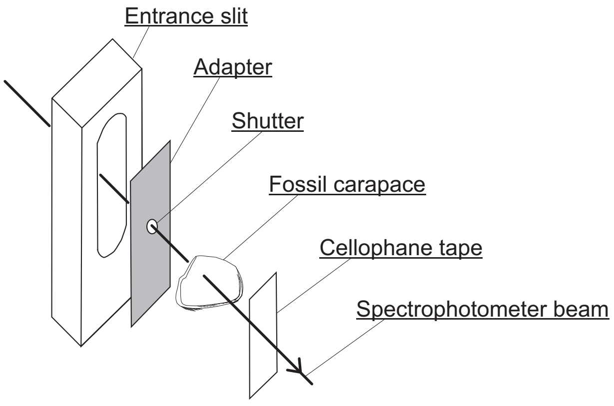


Fig. 1. Developed spectrophotometer adapter. Schematic illustration of the developed adapter for measuring UV absorbance of sedimentary cladoceran remains. Direction of the light beam is illustrated with an arrow.

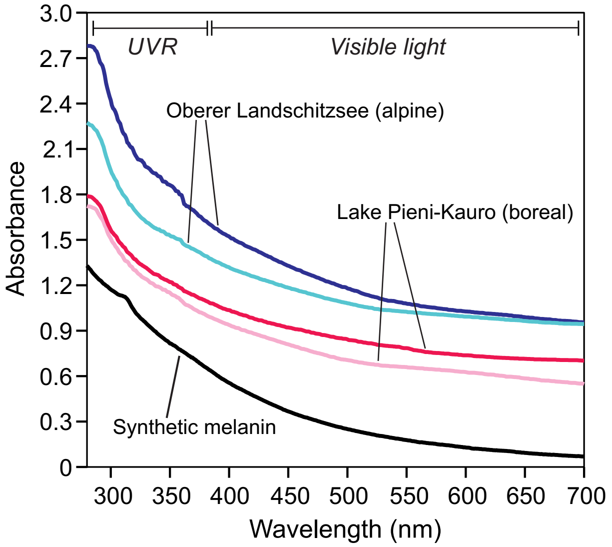


Fig. 2. Absorbance spectra of fossil carapaces. Absorbance spectra of fossil *Alona* spp. carapaces from the case study lakes Pieni-Kauro from sediment depths 32 [dark red] and 18 [pink] cm) and Oberer Landschitzsee (11 [navy-blue] and 6 [light blue] cm) and of synthetic melanin (Sigma–Aldrich M8631 with a concentration 5 mg L^{-1}) in the 280–700 nm wavelength range. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Results and discussion

4.1. Melanin pigmentation in sedimentary cladoceran remains

Spectral analysis indicated that the absorbance of *Alona* spp. carapaces from lakes Pieni-Kauro (from 0.75 to 1.75) and Oberer Landschitzsee (from 1 to 2.5) was progressively higher with shorter UV than that under visible light wavelengths. The absorbance reached the maximum at 280 nm (UV-B) within the measured wavelength range (Fig. 2). The high similarity ($r = 0.98$–0.99, $p < 0.001$) between the absorption of synthetic melanin and the carapaces suggests that the absorbance of fossil carapaces were due to melanin pigment (Fig. 2). This supports the hypothesis that melanin remains inert in fossil cladoceran remains and thus can be measured. As such, a summary of the stratigraphical approach (Fig. 3) showed that absorbance was systematically higher at UV wavelengths (>1.5) and highest at the most hazardous UV-B wavelength (>2.25). The outcomes presented here (Figs. 2 and 3) are in accordance with the first, and at present the only available absorbance results for fossil cladoceran remains. Rautio (2007) had compared the absorbance values of contemporary and fossil *Daphnia middendorffiana* (Daphniidae) carapaces and showed similar absorbance spectra when compared with each other; high absorbance under UV and low absorbance under visible light.

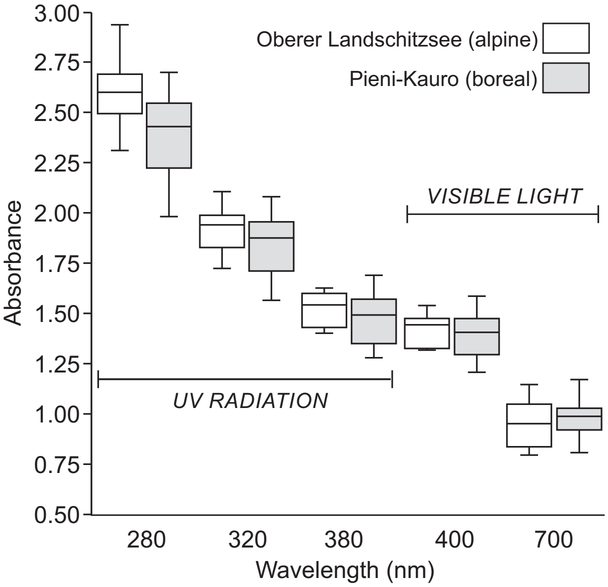


Fig. 3. Summary of carapace absorbance measurements. Boxplots summarizing the variance of fossil *Alona* spp. carapace absorbance measurements with the 25–75% quartiles (boxes), medians (horizontal lines), and minimum and maximum values (vertical error bars) in the sediment cores from lakes Pieni-Kauro and Oberer Landschitzsee at wavelengths 280, 320, 380, 400, and 700 nm.

Our results indicated that the UV absorbance was consistently higher in the clear-water Oberer Landschitzsee (medians for 380, 320 and 280 nm: 1.55, 1.95, and 2.61, respectively) than in the humic Pieni-Kauro (1.48, 1.87, and 2.41) (Fig. 3). The *t*-test also supported the differences in the carapace UV absorbance between Oberer Landshcitzsee and Pieni-Kauro showing significantly higher values for the clear-water alpine site under UV-B (280 nm: $t = 3.65$, $p = 0.0009$) and UV-A (320 nm: $t = 2.05$, $p = 0.048$), whilst such differences were not observed under 380, 400 and 700 nm. The absorbance values in both lakes increased clearly between UV-A and UV-B approximately from 1.5 to 2.5 (Fig. 3). Accordingly, the carapace absorbance and, hence, melanin pigmentation of the *Alona* spp. carapaces was higher in the clear-water alpine site than in the humic forest lake, as was expected according to our hypothesis. The results likely reflect differences in the general underwater UV and optical environments between the sites (Table 1, Figs. 2 and 3). UV is more intensive in mountainous regions due to the altitudinal increase of UV under a thinner atmosphere (Sommaruga, 2001; Rose et al., 2009; Rautio and Tartarotti, 2010) and in clear-water alpine lakes due to lack of UV attenuating carbon compounds (e.g. DOC), which are generally more abundant in lakes located at the boreal forest biome (Laurion et al., 2000; Molot et al., 2004). In fact, DOC concentration of Oberer Landschitzsee is 0.6 mg L^{-1} and that of Pieni-Kauro 6.1 mg L^{-1} creating significant differences in water optical properties and UV environment between the sites. Therefore, it is likely that the intensity of underwater UV is higher in Oberer Landschitzsee than in Pieni-Kauro, where allochtonous carbon compounds enter the lake from the surrounding forested and paludified catchment and where UVR is not so intensive due to lower altitudinal and higher latitudinal position (Table 1).

Many previous studies on pigmentation in aquatic organisms have focused on zooplankton revealing the negative effects of UV on aquatic biota, reviewed by Hansson and Hylander (2009) and Rautio and Tartarotti (2010). In addition to the above mentioned paleoaquatic implication, for the first time, the present study sheds light on UV responses of benthic cladocerans (Chydoridae). Our results indicate that these benthic microcrustaceans, despite their shaded sediment- and vegetation-associated habitat preferences, are exposed to harmful UV and synthesize UV protective melanin for survival and adaptation (Figs. 2 and 3). Other studies have also shown that shallow-water invertebrate communities in the littoral zone of lakes are adversely affected by UV (Vinebrooke and Leavitt, 1999). Previous faunistic (visual) observations have confirmed the existence of strongly melanic forms of Chydoridae under extreme UV exposure in high altitude lakes (Manca et al., 1998; Van Damme and Eggermont, 2011), but the presence of melanin has not been confirmed by absorbance measurements.

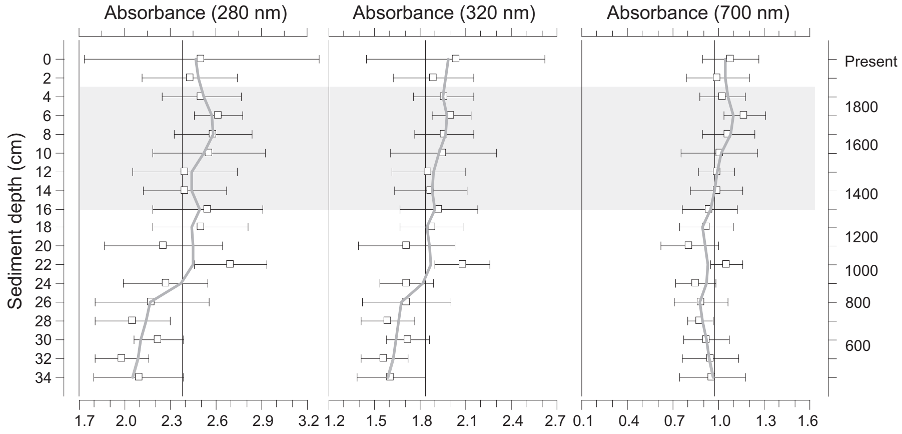
4.2. Inferring past underwater UV exposure

The stratigraphic approach revealed that there was relatively high variation in the *Alona* spp. carapace UV-B absorbance throughout the Pieni-Kauro (2–2.6) and Oberer Landschitzsee (2.3–2.9) cores. The variation was more subtle in the visible light and UV-A range than under UV-B (Figs. 3 and 4), indicating differential content of melanin throughout the cores. These are the first results to illustrate cladoceran population pigmentation in a long-term temporal context (Fig. 4) and they imply that pigmentation of the two geographically divergent *Alona* populations experienced centennial changes. The centennial succession of fossil melanin in the two *Alona* populations showed similar features during the culmination of the cold Little Ice Age (LIA) around 1700–1800 AD, when the UV-B carapace absorbance reached highest values in both cores (Fig. 4). UV-B absorbance values in the Pieni-Kauro core during the LIA (samples at 16–4 cm) were not significantly higher when compared to those during the warm Medieval Climate Anomaly (MCA *ca* 800–1300 AD, 26–18 cm; $t = 1.33$, $p = 0.161$), but considerably higher when compared to the pre-MCA period (34–28 cm; $t = 7.41$, $p = <0.001$). In addition, UV-B absorbance increased significantly from the pre-MCA period to the MCA ($t = 2.49$, $p = 0.042$). Similarly, significantly higher absorbance values occurred in Oberer Landschitzsee (Fig. 4b) in the LIA samples (16–9 cm) than during the post-LIA period (8–0 cm), as verified by the *t*-test ($t = 2.15$, $p = 0.048$). The results suggest that the *Alona* populations responded to altered underwater UV regimes during the LIA by increasing their pigmentation.

While productivity is likely to control UV transparency in clear-water alpine lakes, changes in DOC are expected to be more important in underwater UV control of humic boreal lakes (Sommaruga and Psenner, 1997; Sommaruga et al., 1999; Laurion et al., 2000; Molot et al., 2004). Accordingly, the higher underwater UV exposure of Oberer Landschitzsee (Fig. 4b) during the LIA may have been related to a more transparent water column through reduced primary production during the relatively cooler summers of the 18th and 19th centuries (Nevalainen and Luoto, 2012) that would have favored stronger pigmentation of benthic cladocerans. In agreement, previous sedimentary records indicate that productivity increased in Oberer Landschitzsee over the post-LIA period (Nevalainen and Luoto, 2012, 2013) along with slightly elevated DOC concentrations (Schmidt et al., 2008). Accordingly, the reduced post-LIA carapace absorbance values may have been induced by increased UV attenuation due to phytoplankton development (cf. Sommaruga and Psenner, 1997; Sommaruga et al., 1999).

The *Alona* carapace UV-B absorbance in Pieni-Kauro showed clear and significant shifts from the distinctively lower values in the pre-MCA period to higher values during the MCA and LIA (Fig. 4a). The underwater UV environment of boreal humic lakes is largely controlled by changes in the forested and paludified catchments that either promote or reduce the amount of UV-absorbing humic compounds that enter the lakes. However, other environmental

a) Pieni-Kauro (boreal)



b) Oberer Landschitzsee (alpine)

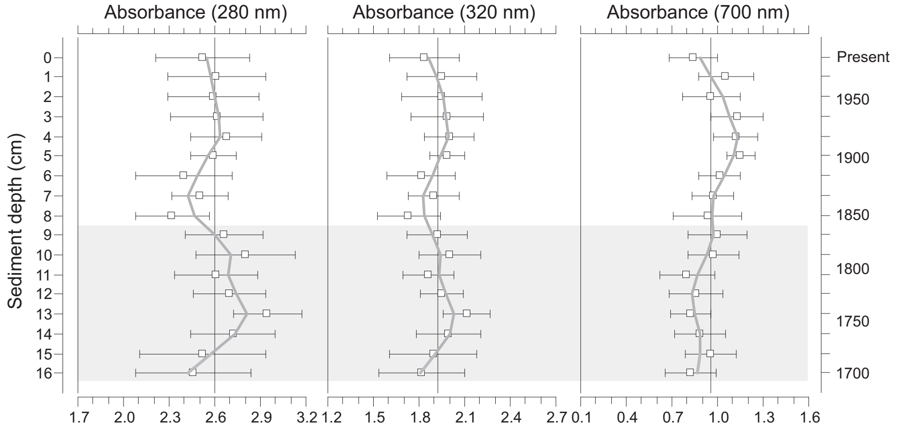


Fig. 4. Stratigraphic variation of carapace absorbance measurements. Absorbance of fossil *Alona* spp. carapaces ($n = 10$) in lakes a) Pieni-Kauro and b) Oberer Landschitzsee at selected UV (280 and 320 nm) and visible light (700 nm) wavelengths. The average absorbance for the topmost sample (0 cm) in Pieni-Kauro was based on lower number of carapaces ($n = 4$), because of a shortage in the sediment material. The vertical black lines indicate stratigraphic average values, error bars indicate 95% confidence intervals, and thick gray lines indicate general trends with lowess smoothing (span 0.3). The gray bands indicate the Little Ice Age, which lasted until late 19th century in eastern Finland (Luoto and Helama, 2010) and mid-19th century in the Austrian Alps (Schmidt et al. 2008).

changes, such as depth and shelter from vegetation may also have an important role in determining the water column UV exposure. Previous paleoenvironmental investigations from the Pieni-Kauro basin suggest that the lake level was 1.5–2 m lower than presently, with maximum depth of about 5 m and 80% of lake area <1 m during the pre-MCA until ~800 AD (Nevalainen et al., 2013). At the same time stream flow through the basin was reduced (Luoto and Helama, 2010; Luoto et al., 2013), being coincident with a regional drought period (Helama et al., 2009). Such conditions favor the growth of extensive littoral macrophyte zones that may have covered majority of the lake providing UV shelter to benthic cladocerans. The low carapace absorbance in the pre-MCA period therefore suggests that underwater UV exposure was low, possibly due to a combination of abundant humic compounds, shallow lake depth, and dominance of macrophytes during this period.

The absorbance of carapaces was generally highest during the cold LIA in the Pieni-Kauro core (Fig. 4a) suggesting more transparent water column and higher UV exposure when compared to previous centuries. During the culmination of the LIA, lake level in the basin was at its 1500-year maximum, being consistent with high summer precipitation (Luoto and Helama, 2010; Nevalainen et al., 2013). The lake basin therefore returned to conditions where there was a real pelagic area and likely less dominant macrophyte coverage, which could have reduced UV shading. In addition, the lower LIA temperatures have likely reduced the input of humic carbon compounds from the catchment into the lake, inducing more transparent water column. Low temperatures are known to control organic carbon entrance to lakes through longer permafrost and ice-cover period and reduced runoff season that constrain DOC (Weyhenmayer and Karlsson, 2009; Rosén et al., 2009b). The lower temperatures during the LIA could also have further caused changes in catchment vegetation and soil type through losses in photosynthetic tissue, root disfunction, and reduced organic matter accumulation (Kullman, 1987) that would have reduced the DOC export from the catchment. Present day DOC export from tundra is on average 0.7 mg m^{-2} yr^{-1} in comparison to 6.3 mg DOC m^{-2} yr^{-1} from boreal catchments (Aitkenhead and McDowell, 2000). Therefore, it is likely that the Pieni-Kauro record of high water transparency and UV exposure during cold climate conditions of the LIA were controlled by temperature-mediated factors, i.e. duration of permafrost and ice-cover period, and catchment vegetation type, holding DOC.

Since heavy fish predation can reduce cladoceran pigmentation as an escape mechanism from visually feeding fish predators (cf. Hansson, 2000), the observed stratigraphic changes in cladoceran carapace absorbance may have additionally been impacted by altered predation regimes. Although both of the current study lakes are, and likely have been during the time span of the cores, inhabited by fish, benthic cladocerans do not usually constitute a significant part of fish diet, planktonic species being more vulnerable to fish predation (e.g. Brooks and Dodson, 1965). Therefore, it is unlikely that the long-term changes in *Alona* spp. pigmentation would have been related to top down control in lakes Pieni-Kauro and Oberer Landschitzsee. However, if the carapace absorbance method will be used in the future for examining pigmentation in sedimentary remains of planktonic taxa, such as *Daphnia* or *Bosmina*, food web interactions should be taken carefully into account.

4.3. Concluding remarks

We conclude that the developed method of spectrophotometric cladoceran carapace absorbance measurement is an easy and cost- and time-efficient technique to infer past (and modern) variability of UV induced melanin pigmentation from sedimentary cladoceran remains preserved in lake sediments. The preliminary results indicate that the carapace absorbance values are indicative for the melanin pigment content of fossil carapaces and that melanin is preserved in ancient cladoceran remains. Melanin in sedimentary cladoceran remains may provide a good estimation of past underwater UV regimes and it may also help to reveal the specific past environmental controls to which organisms in a given lake were subjected. We emphasize that the method needs to be further calibrated with wider spatial data sets to quantify more precisely how cladoceran carapace absorbance values relate with extracted melanin pigment content and UV absorbance of lake-water in terms of e.g. DOC concentration. Further temporal calibration of the method with existing paleolimnological UV proxies will also be required to fully promote the usefulness of the cladoceran carapace absorbance values in UV inferences. We also note that biotic predator–prey interactions may hamper the UV-indicator value of fossil melanin in lakes with complex food webs and therefore, we recommend that this aspect is always taken into account when discussing of carapace absorbance results in fish-populated lakes. In addition to inferring past UVR exposure on centennial-millennial scales, the method introduced here can have wide ecological applications, because it can be also used in detecting recent aquatic responses to anthropogenically induced ozone depletion and increased UV irradiance. As microbenthic communities play a crucial role in biogeochemical cycles of lakes by recycling nutrients and carbon as grazers and detritivores, their modern and past UV responses should be assessed in more detail in the future for macro- and paleoecological implications of organism–environment relationships.

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