

Role of terrestrial carbon in aquatic UV exposure and photoprotective pigmentation of meiofauna in subarctic lakes

LIISA NEVALAINEN*, TOMI P. LUOTO*[†], MARTTIINA V. RANTALA[†], ANNUKKA GALKIN[†] AND MILLA RAUTIO[‡]

*Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland

[†]Department of Geosciences and Geography, University of Helsinki, Helsinki, Finland

[‡]Département des Sciences Fondamentales, Centre for Northern Studies (CEN) & Group for Interuniversity Research in Limnology and aquatic environment (GRIL), Université du Québec à Chicoutimi, Chicoutimi, QC Canada

SUMMARY

1. Aquatic organisms are adversely influenced by ultraviolet radiation (UV) and utilise photoprotective strategies, including pigmentation. We examined UV-protective melanin pigmentation of aquatic meiofauna (Cladocera) in relation to the UV exposure across 25 tree line lakes in Finland to address the potential effects of increased UV and altered input of UV-screening terrestrial dissolved organic carbon (DOC) on aquatic organisms.
2. Bio-optical parameters, including concentration of DOC, the coloured dissolved organic matter (CDOM) fraction, a suite of carbon quality indices and chlorophyll *a*, were analysed from lake water, and their role in controlling underwater UV environment (measured as diffuse UV attenuation coefficient K_d at 305 and 340 nm) was examined. Cladoceran (*Alona affinis*) carapaces were extracted from the surface sediments, and their melanisation was assessed with spectroscopic UV-visible light absorbance measurements.
3. DOC, CDOM and specific UV absorbance (SUVA) had strong positive relationships with the attenuation of UV in the lakes, suggesting that terrestrial organic carbon controls underwater UV exposure in the examined lakes. The absorbance measurements indicated the presence of melanin in the cladoceran carapaces, the degree of melanisation varying strongly among the lakes. Melanisation had significant relationships with SUVA and fluorescence index (FI). It was higher in lakes with low SUVA and high FI, indicating that cladocerans exhibit strong melanisation in lakes with low contribution of UV-attenuating allochthonous DOC (i.e. high UV exposure).
4. The results suggest that cladoceran meiofauna respond to UV by utilising photoprotective pigmentation and that the degree of pigmentation is affected by site-specific underwater UV exposure, which is ultimately controlled by UV-attenuating DOC of terrestrial origin.
5. Although cladoceran meiobenthos are able to adapt to varying underwater UV doses, climate change with its multiple consequences on hydrology, limnology and catchment vegetation in the tree line zone may cause major changes in underwater UV environment for the organisms to adapt.

Keywords: Cladocera, dissolved organic carbon, melanin, palaeolimnology, UV radiation

Introduction

Small and shallow high-latitude lakes are sensitive to environmental changes due to their close connection to the catchments (Rautio *et al.*, 2011). Their location in the

extremely harsh climates with long winters and short open-water seasons makes them susceptible to changes in climate conditions. The late 20th-century climate warming is known to cause major changes in physical and chemical lake-water properties (Adrian *et al.*, 2009;

Correspondence: Liisa Nevalainen, Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, 40014 University of Jyväskylä, Finland. E-mail: liisa.e.nevalainen@ju.fi

Luoto & Nevalainen, 2013), resulting, for example, in longer ice-free season and altered thermal properties with consequent impacts on primary production and food-web structure (Smol *et al.*, 2005; Wrona *et al.*, 2006). Increased solar ultraviolet radiation (UV) is an environmental hazard causing negative ecological effects in aquatic ecosystems (ACIA, 2005; Häder *et al.*, 2015). UV impacts are especially pronounced at high latitudes due to the arctic ozone losses and intensive solar irradiance during the polar summer. The shallowness and high water transparency of many high-latitude lakes make them particularly susceptible to UV irradiance (Rautio, Bonilla & Vincent, 2009). Arctic ozone loss (Manney *et al.*, 2011) that is superimposed on climate warming during the late 20th and early 21st centuries may cause cumulative environmental stress to organisms and ecosystems through altered underwater UV regimes (Vincent, Rautio & Pienitz, 2007; Williamson *et al.*, 2014; Häder *et al.*, 2015).

A central component in the underwater UV environment and UV attenuation of lake water is organic carbon of terrestrial origin (allochthonous carbon) that arrives to the lakes from surrounding soils and vegetation (Tranvik *et al.*, 2009; Jansen, Kalbitz & McDowell, 2014). It impacts optical properties of lake waters and accordingly also underwater UV exposure (Vincent & Pienitz, 1996; Vincent *et al.*, 2007; Forsström *et al.*, 2015). Climate warming has increased the risk of this carbon becoming mobile (Davidson & Janssens, 2006; Friedlingstein *et al.*, 2006), which can cause significant changes in aquatic systems and their underwater UV regimes opposing stress to organisms and changing ecosystem functions (Schindler *et al.*, 1996; Vincent *et al.*, 2007).

Microinvertebrates, such as zooplankton and benthic meiofauna, are a key component of high-latitude freshwater food webs. Zooplankton possesses multiple coping mechanisms against biologically harmful UV radiation (Rhode, Pawlowski & Tollrian, 2001; Rautio & Tartarotti, 2010). Among these, photoprotective pigmentation is of high significance in the shallow and transparent arctic waterbodies because the systems usually lack deep-water or vegetative UV refugia and are exposed to high diurnal doses of solar radiation during the polar summer. Most common UV-protective pigments of arctic zooplankton include melanins, carotenoids and scytonemin that induce brown–black or red coloration of the exoskeletons (Hessen, 1996; Rautio *et al.*, 2009). Of the zooplankton, cladocerans (Crustacea: Cladocera) are able to synthesise melanin into their exoskeletons (Hebert & Emery, 1990; Rautio & Korhola, 2002a), whereas other photoprotective compounds are

derived from phytoplankton or benthic food sources (Rautio *et al.*, 2009). Apparently also littoral benthic cladocerans utilise photoprotection under high UV exposure and produce melanin pigment (Manca *et al.*, 1998; Van Damme & Eggermont, 2011; Nevalainen & Rautio, 2014).

It has been shown that UV-screening melanin pigmentation of the cladoceran genus *Daphnia* is clearly highest in subarctic lakes with low dissolved organic carbon (DOC) concentration when compared to lakes with higher DOC (Rautio & Korhola, 2002a). Thus, cladoceran melanisation seems to be dependent on site-specific underwater UV exposure that is controlled by UV-screening DOC concentration of the lake water and ultimately by catchment characteristics and climate. This has also been recorded using long-term palaeolimnological approach, as climate dynamics and catchment succession through the past centuries and millennia are known to have influenced cladoceran carapace melanisation in fossil specimens preserved in lake sediments (Nevalainen & Rautio, 2014; Nevalainen *et al.*, 2015). Understanding of spatial and temporal patterns in UV-protective pigmentation and its connections to the lake-catchment coupling and climate conditions is important, since increasing UV doses and accelerating climate warming in future may create unique and unexpected aquatic environmental settings in underwater UV regimes for the aquatic organisms to adapt.

To address the potential effects of increased UV radiation and altered input of terrestrial UV-screening DOC on aquatic organisms, we examined the degree of meiofaunal melanisation in relation to aquatic carbon pool and underwater UV exposure in a set of tree line lakes in subarctic Finland. We hypothesised that high underwater UV exposure, related to low DOC concentrations and associated high water transparency, induces UV-protective melanisation in meiofauna and that this can be detected by spectroscopic UV absorbance measurements. We presumed that highest UV exposure is evident in tundra lakes located above the tree line with low DOC content and lowest UV stress is observed in humic lakes with forested and paludified catchments. First, we examined the relative contribution of terrestrial carbon, which is the coloured and most UV-attenuating fraction of DOC, in the lakes. Second, we determined the UV absorbance of a meiobenthic cladoceran *Alona affinis* (Chydoridae) carapaces extracted from surface sediments of 25 small and shallow lakes in subarctic Finnish Lapland. Third, we compared the carapace UV absorbance measurement with a set of lake-water optical properties that indicate carbon pool of the lakes and are

closely connected with amount of terrigenous organic carbon in the aquatic systems. This study aimed to provide new empirical evidence on the adaptation potential of meiobenthos to varying UV doses with significant implications on aquatic UV responses under changing environmental conditions.

Methods

A set of 25 lakes in northern Finnish Lapland were sampled for UV profiles, bio-optical water properties and surface sediments (Fig. 1). The lakes were chosen to form an ecological gradient across the northern and altitudinal tree line from north boreal to subarctic ecoregions, exhibiting a succession from mixed pine and birch forest through mountain birch woodland to barren tundra (Table 1). All the lakes are small (mean size 3.8 ha) and shallow (mean depth 3.0 m) with catchments covered by natural forest and tundra vegetation (Table 1). The lakes are considered as pristine because they are not subjected to direct anthropogenic influence due to their remote location (Table 1, Fig. 1).

Underwater UV was determined *in situ* with a submersible profiler radiometer PUV-2500 (Biospherical Instruments Inc., San Diego, CA, U.S.A.) from a small boat at central basins. Diffuse attenuation coefficients (K_d) for UV wavelengths 305 and 340 nm (K_{d305} and K_{d340}) were obtained from the slope of the linear regression of the natural logarithm of down-welling irradiance (E_d) versus depth (Z), $\ln(E_{d(Z)}) = -K_d Z + c$, where the constant $c = \ln(E_{d(0^-)})$, with $E_{d(0^-)}$ being the irradiance

just below the water surface. Per cent UV left in the water column at 0.5 m depth was calculated with the equation $EZ = E0 \exp - K_d Z$.

The bio-optical measurements included in the study were DOC, coloured dissolved organic matter (CDOM), specific UV absorbance at 254 nm (SUVA), fluorescence index (FI, an index to analyse the origin of fulvic acids from microbial versus terrestrial sources) and chlorophyll *a* (chl-*a*). DOC concentrations were analysed from epilimnetic water samples with a Shimadzu Total Organic Carbon analyser at the Lammi Biological Station of University of Helsinki. A set of indicators for the quality of carbon was measured using spectrophotometric and spectrofluorometric methods. The measurements were carried out from epilimnetic water samples filtered through 0.7 μm pre-rinsed and combusted GF/F filters and stored in the dark at +4 °C. Scanning of absorption coefficient at 320 nm (ABS_{320} , indicator for CDOM concentration) and SUVA (DOC normalised absorbance at the wavelength 254 nm) were performed with a dual-beam mode with Cary 100 UV-Vis spectrophotometer (Agilent, Santa Clara, CA, U.S.A.) using a 10-cm quartz cuvette. Samples were corrected against MilliQ water. Fluorescence emission scans (400–700 nm) with single excitation at 370 nm were measured with Cary Eclipse fluorescence spectrophotometer (Agilent, Santa Clara, CA, U.S.A.) for the FI, which was calculated as relative fluorescence emission intensities at 450/500 nm (McKnight *et al.*, 2001). Chl-*a* concentrations were determined from epilimnetic water samples that were filtered (250 mL) through pre-combusted GF/F filters and freeze-dried. Spectrophotometric and spectrofluorometric analysis of chl-*a* was performed following Nusch (1980). Bias caused by phaeopigments were removed by measuring the concentration from acidified chl-*a* samples (Yentsch & Menzel, 1963), and final chl-*a* concentration calculations were made following Jeffrey & Walschmeyer (1997). Chl-*a* and optical quality measures of DOM were performed at the Aquatic Science Laboratory of Université du Québec à Chicoutimi.

Surface sediments (topmost 0–2 cm) were sampled with a Limnos gravity corer from a small boat at the central basins from water depth range of 0.5–7.5 m (Table 1). The samples were stored in plastic bags in a cold room at +4 °C. Carapace absorbance method was used to determine the degree of melanisation in meiofauna (Nevalainen & Rautio, 2014). Sedimentary carapaces (shells) of a large and common cladoceran taxon *Alona affinis* Leydig (Chydoridae, Aloninae), originating from moulted or dead animals, were extracted from the sieved (100- μm mesh) sediment-water solution under a

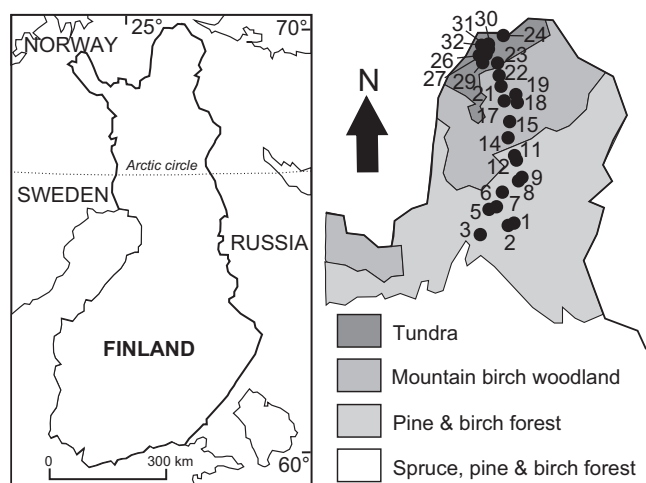


Fig. 1 Location of the 25 study lakes in northern Finland across the general vegetation zones. The lake numbers indicate original sampling identification codes (see geographical and limnological details in Table 1).

Table 1 Catchment and basin characteristics, bio-optical water properties and *Alona affinis* carapace absorbance values (melanisation, last column) of the 25 study lakes in northern Finland

ID	Veget.	Latitude (N)	Longitude (E)	Altitude (m a.s.l.)	Catchment (km ²)	Area (ha)	Mires (%)	Depth (m)	DOC (mg L ⁻¹)	SUVA			Kd340 (m ⁻¹)	UV ₃₀₅ (%)	UV ₃₄₀ (%)	Melanisation (ABS _{UV})		
										FI (ratio)	Chl- <i>a</i> (µg L ⁻¹)	CDOM (m ⁻¹)						
1	PB	68°52.211	27°01.333	174	1.39	3.90	2.4	4.1	2.2	2.5	1.23	0.50	4.6	7.3	4.1	13.1	0.88	
2	PB	68°52.077	26°59.885	191	0.64	3.05	3.9	3.1	3.8	3.2	1.21	1.40	11.2	15.1	10.5	0.1	0.5	0.95
3	PB	68°47.494	26°31.365	239	0.35	4.77	6.6	5	3.8	2.4	1.17	0.99	7.5	11.2	6.1	0.4	4.6	1.20
5	PB	68°57.123	26°44.250	149	0.21	9.29	5.5	0.5	5.2	0.8	1.47	4.79	3.3	na	na	na	1.27	1.20
6	PB	68°57.244	26°44.581	148	0.13	3.21	0.0	7.5	2.6	0.8	1.59	1.20	1.6	2.2	1.2	33.2	54.4	1.26
7	P	69°00.906	27°01.537	172	0.55	3.98	16.6	5	5.7	2.8	1.24	1.49	13.7	19.4	11.8	<0.1	0.3	0.96
8	P	69°03.898	27°14.799	167	1.03	9.21	6.4	6.3	3.2	2.1	1.19	1.17	5.2	7.8	4.1	2.1	12.8	1.10
9	PB	69°04.456	27°16.285	165	1.07	0.91	6.3	1.4	5.9	3.1	1.28	0.77	17.6	22.3	15.2	<0.1	<0.1	1.39
11	P	69°10.106	27°15.630	158	0.12	2.42	1.4	4	4.3	0.9	1.49	1.60	2.9	4.1	3.0	13.0	22.3	1.35
12	P	69°09.625	27°15.345	157	0.24	3.91	10.4	2	9.6	1.7	1.23	1.63	13.7	20.2	11.3	<0.1	0.4	1.22
14	MB	69°18.725	27°12.792	211	0.67	5.43	49.3	0.7	12.0	3.0	1.23	3.56	34.9	40.5	30.2	<0.1	<0.1	1.07
15	MB	69°24.074	27°15.572	222	1.80	6.35	25.9	0.7	10.0	2.4	1.28	2.70	21.4	28.4	15.2	<0.1	<0.1	1.22
17	MB	69°33.752	27°14.021	202	0.34	6.01	5.7	2.5	4.9	0.7	1.48	0.67	2.5	3.1	1.6	21.3	45.4	1.32
18	ST	69°34.472	27°19.395	225	0.40	8.02	22.5	0.8	10.7	3.2	1.23	3.81	33.7	50.3	33.0	<0.1	<0.1	1.15
19	ST	69°34.525	27°19.594	225	0.16	3.51	22.8	0.7	16.6	3.0	1.39	6.08	79.7	50.8	46.5	<0.1	<0.1	1.07
21	MBP	69°38.519	27°08.262	109	0.66	0.70	0.1	6.3	3.9	1.9	1.20	2.39	5.6	na	na	na	na	1.11
22	MBP	69°40.786	27°04.688	96	0.22	2.51	0.1	5.7	2.5	1.6	1.41	1.04	3.3	na	na	na	na	1.21
23	MBP	69°46.951	27°00.908	85	0.36	2.41	8.9	4.2	3.6	1.0	1.38	0.88	1.8	na	na	na	na	1.13
24	MB	69°54.743	27°04.290	71	0.55	2.46	1.7	1.6	7.4	2.9	1.30	2.28	18.6	27.6	15.2	<0.1	<0.1	1.28
26	ST	69°50.663	26°57.166	219	0.38	3.54	0.0	3.5	1.8	0.8	1.53	0.64	1.1	2.0	0.8	37.2	65.8	1.54
27	ST	69°50.724	26°57.075	214	0.37	1.47	0.0	1.9	2.5	1.5	1.25	0.34	3.4	4.7	2.4	9.7	29.9	1.33
29	MB	68°50.401	26°56.469	169	9.01	3.19	0.0	1.7	1.8	2.4	1.21	0.56	4.0	5.9	4.5	5.3	10.3	1.14
30	MB	69°53.897	26°58.940	223	0.30	1.48	7.8	3.1	4.0	2.0	1.29	3.00	6.7	11.4	7.7	0.3	2.2	0.98
31	BT	69°53.215	26°57.112	241	0.20	0.91	3.8	1.7	6.0	2.3	1.22	1.77	12.8	16.6	10.9	<0.1	0.4	1.10
32	BT	69°53.049	26°55.893	255	1.99	2.97	5.5	1.2	3.4	2.3	1.23	1.46	7.6	11.1	7.3	0.4	2.6	1.09

PB = pine and birch forest; P = pine forest; MB = mountain birch woodland; MBP = mountain birch woodland with pine; ST = shrub tundra and BT = barren tundra, and for limnology: DOC = dissolved organic carbon; SUVA = specific UV absorbance; FI = fluorescence index; chl-*a* = chlorophyll *a*; CDOM = coloured dissolved organic matter as absorbance at 320 nm; K_d = UV attenuation coefficient for 305 and 340 nm; UV_{305/340} = percentage UV left at 0.5 m water depth.

binocular microscope. The carapaces were attached individually with fine forceps on a spectrophotometer adapter having a shutter of 300 μm and sealed with a UV transparent cellophane tape. Spectral (UV to visible light) absorbance of randomly selected carapaces from each site was measured under 280–600 nm to verify the presence of melanin with the shape of the absorbance spectra. LOESS smoothing (span 0.5) was applied to the absorbance spectra to reduce variance induced by the current method (i.e. adapter with the attached microfossil) since cuvette and liquid solution (normally used in absorbance measurements) were not applicable. Estimation of melanisation in carapaces at each site was performed by UV absorbance measurements, where a minimum of 12 carapaces per site was measured under 305 and 340 nm. Highest and lowest absorbance values from each site were omitted, and an average of absorbance values ($n = 10$) at 305 and 340 nm was used as a final lake-specific carapace UV absorbance value (ABS_{UV} , indicating the degree of melanisation).

To standardise the scale of optical measurements and carapace absorbance values in the surface sediments, data were log-transformed (base 10 logarithm). The relationships between carapace UV absorbance and lake-water optics were estimated first with multiple linear regressions and then with Pearson's correlation coefficients (linear regressions, r). Pearson's correlation coefficients were also used to assess the relationships between DOC and its fractions and UV exposure of the water column. Statistical analyses were performed with PAST v. 3.06 software (Hammer, Harper & Ryan, 2001).

Results

UV attenuation and bio-optical water properties varied largely among the 25 sites (Table 1). Mean $K_{\text{d}305}$ and $K_{\text{d}340}$ were 17.2 and 11.6 m^{-1} , and calculated mean percentage of surface UV at 0.5 m depth for 305 and 340 nm was 6.0 and 12.6%, respectively. Mean value for DOC of the lakes was 5.5 mg L^{-1} , for SUVA 2.1 $\text{mg C L}^{-1} \text{m}^{-1}$, for chl-*a* 1.9 $\mu\text{g L}^{-1}$, for CDOM 12.7 m^{-1} and for FI 1.31. Among the bio-optical parameters, DOC exhibited strong positive ($r \geq 0.80$) and statistically significant ($P \leq 0.001$) correlations with CDOM and UV attenuation coefficients (Table 2). Correlation was lower with SUVA ($r = 0.41$, $P = 0.043$) and insignificant with FI (Table 2).

In the spectral analysis at 600–280 nm, *Alona affinis* carapace absorbance was lowest under visible light wavelengths and increased under UV wavelengths (<400 nm) towards maximum absorbance values at 280 nm (Fig. 2). At the selected sites and specimens,

Table 2 Pearson's correlation coefficients (r) between DOC and UV attenuation coefficients and other bio-optical parameters in the 25 lakes in northern Finland (abbreviations explained in Table 1). All correlations, except between DOC and FI, are statistically significant ($P < 0.05$)

	DOC	$K_{\text{d}305}$	$K_{\text{d}340}$
DOC		0.84	0.83
SUVA	0.41	0.85	0.85
FI	-0.15	-0.55	-0.53
CDOM	0.85	0.99	0.99

DOC = dissolved organic carbon; SUVA = specific UV absorbance; FI = fluorescence index; CDOM = coloured dissolved organic matter; K_{d} = UV attenuation coefficient for 305 and 340 nm.

carapace absorbance was highest in the barren tundra lake and lowest in the boreal lakes (Fig. 2). Among the 25 lakes, *A. affinis* carapace UV absorbance (degree of melanisation) varied between 0.88 (#1, boreal forest) and 1.54 (#26, shrub tundra) absorbance units (Table 1). In addition, high absorbance values (>1.3) were recorded in lakes #9, 17 and 27 and low (<1.0) in lakes #2, 7 and 30 (Table 1). The mean UV absorbance in the lakes was 1.17 absorbance units.

Multiple linear regressions did not identify any significant relationships between *Alona affinis* carapace absorbance and bio-optical water parameters (Table 3). Linear regressions indicated significant ($P < 0.05$) monotonic relationships between carapace absorbance and SUVA and FI (Table 2). Carapace absorbance had a negative correlation ($r = -0.55$, $P = 0.004$) with SUVA and a positive correlation ($r = 0.54$, $P = 0.005$) with FI (Fig. 2, Table 3), whereas other correlations remained above

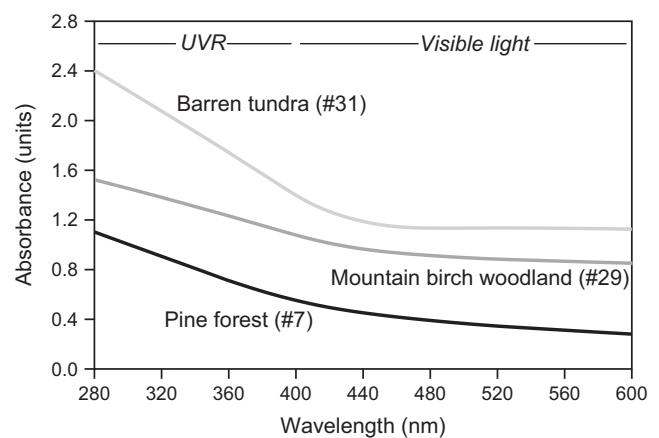


Fig. 2 *Alona affinis* carapace absorbance spectra under UV and visible light wavebands from three lakes in different ecoregions. The absorbance spectra are single measurements from individual carapaces smoothed with LOESS (span 0.5).

Table 3 Multiple linear regressions with coefficient of determination (r^2) and linear regressions with correlation coefficient (r) between *Alona affinis* carapace absorbance and limnological variables in the 25 study lakes

	r^2	P
Multiple linear		
DOC	0.01	0.554
SUVA	0.31	0.268
FI	0.30	0.375
Chl- <i>a</i>	0.04	0.181
CDOM	0.12	0.317
K_{d305}	0.14	0.195
K_{d340}	0.17	0.177
	0.51	0.057
Linear		
DOC	-0.08	0.687
SUVA	-0.55*	0.004
FI	0.54*	0.005
Chl- <i>a</i>	-0.21	0.309
CDOM	-0.35	0.086
K_{d305}	-0.38	0.086
K_{d340}	-0.42	0.061

DOC = dissolved organic carbon; SUVA = specific UV absorbance; FI = fluorescence index; chl-*a* = chlorophyll *a*; CDOM = coloured dissolved organic matter; K_d = UV attenuation coefficient for 305 and 340 nm.

Statistically significant relationships ($P < 0.05$) are indicated with an asterisk.

the level of statistical significance. Close to significant negative relationships occurred between carapace absorbance and CDOM ($r = -0.35$, $P = 0.086$) and UV attenuation coefficients (K_d) at 305 ($r = -0.38$, $P = 0.086$) and 340 nm ($r = -0.42$, $P = 0.061$) as shown in Table 2.

Discussion

DOC controls on UV attenuation

The selected tree line lakes exhibited a large diversity in bio-optical properties as their waters ranged from nearly transparent to highly humic (Table 1). The most significant factor affecting water transparency, and hence underwater UV irradiance, is generally deemed to be DOC and especially the coloured fraction of dissolved organic matter of terrestrial origin, namely CDOM (Morris *et al.*, 1995; Williamson *et al.*, 1996; Laurion, Vincent & Lean, 1997). In alpine lakes, chl-*a* has also been shown to significantly contribute to UV attenuation (Sommaruga & Psenner, 1997; Sommaruga & Augustin, 2006), but in the current data set chl-*a* concentrations remained extremely low and therefore unlikely caused any UV screening (Table 1). The lake set contained a DOC gradient of 1.8–16.6 mg L⁻¹ (Table 1)

that is quite typical for subarctic lakes in northern Finland (Rautio *et al.*, 2011), where lakes located above the tree line are significantly lower in DOC than the lakes below the tree line (Roiha *et al.*, 2012). The highest DOC in the data set was measured from lake #19 that is located above the tree line with shrub tundra vegetation and where the high DOC is likely due to the location of the lake next to palusa mires providing humic substances to the basin (Table 1). DOC was in strong positive correlation ($r \geq 0.80$) with UV attenuation coefficients ($K_{d305/340}$) in the data set, suggesting that it is a major controller of underwater UV exposure in subarctic lakes (Table 2).

The significant role of the terrestrial carbon fraction in lakes' UV attenuation was also evident, as indicated by the strong correlations of SUVA and CDOM with $K_{d305/340}$ (Table 2). These parameters are indicators of autochthonous and allochthonous carbon sources in natural waters. Increasing SUVA is an indicator for increasing terrestrial contribution for the aquatic DOC pool (Hood, Williams & McKnight, 2005). It does not necessarily depend on DOC concentrations (Jaffe *et al.*, 2008; Forström *et al.*, 2015), although in the current lake set there was a significant correlation between SUVA and DOC (Table 2). Highest SUVA values in the current study occurred in lakes with widely paludified catchments (#2, 9, 14, 18–19), suggesting that humic substances from the surrounding mires are major components of the allochthonous carbon transported into the lakes. Catchment vegetation did not seem to show any specific trends since the lakes with high SUVA occurred across the vegetation gradient (Table 1). Additional support for this is provided by the significant negative relationships between FI and UV attenuation coefficients (Table 2). These data suggest that lakes with low terrestrial carbon contribution (high FI) are more exposed to underwater UV (low K_d).

The coloured (chromophoric) fraction of dissolved organic matter (CDOM) is known to largely control underwater UV environment in northern lakes (Laurion *et al.*, 1997). This was the case also in the studied lakes where CDOM showed a very strong relationship with UV attenuation coefficients ($r = 0.99$, Table 2). Lowest CDOM values were measured from lakes located in the tundra (#26) and sand-gravel formations (#6, 23) with low proportions of mires in their catchments (Table 1), which has likely caused a low amount of terrestrial organic carbon entering the lakes. Generally, UV attenuation of low-DOC lakes (<4 mg L⁻¹) is highly sensitive to even small changes in CDOM (Laurion *et al.*, 1997, 2000) and in DOC (Williamson *et al.*, 1996). The

results suggest that, in addition to mere DOC concentrations, underwater UV environment of the lakes was largely related to the amount of terrestrially originated coloured carbon compounds (Tables 1 & 2). Considering the DOC-related controlling factors on aquatic UV exposure in the high-latitude lakes across the tree line (Table 2, Laurion *et al.*, 1997; Pienitz & Vincent, 2000; Saulnier-Talbot, Pienitz & Vincent, 2003; Forsström *et al.*, 2015), climate change and its impacts on catchment vegetation and hydrology are likely to strongly control underwater UV doses in future and set up challenging environments for the aquatic biota to adapt.

Impacts of UV exposure on pigmentation

Of the cladocerans, planktonic and also some benthic taxa, including *Alona affinis*, are suggested to use melanisation as a UV-protective strategy (Hessen, 1996; Rautio *et al.*, 2009; Nevalainen & Rautio, 2014). In the current study, increasing carapace absorbance of the absorbance spectra under shortening wavelengths and especially under UV waveband indicated the presence of UV-protective compounds in the carapaces (Fig. 2). The absorbance spectra of *A. affinis* carapaces in the current study (Fig. 2) were highly similar than those measured for synthetic melanin (Rautio & Nevalainen, 2013), suggesting that the carapaces contained melanin pigments. The absorbance spectra also closely follow those published previously from cladoceran carapaces extracted from

lake sediments (Rautio & Nevalainen, 2013; Nevalainen & Rautio, 2014; Nevalainen *et al.*, 2015).

It has been suggested that DOC concentrations impact UV-protective pigmentation in cladocerans through UV-attenuating properties (Rautio & Korhola, 2002a,b). In contrast to this suggestion and the presumption of the current study, concentrations of DOC did not exhibit any clear relationship with carapace melanisation (Table 3). Instead, the parameters indicating contribution of coloured terrestrial carbon in lake waters, SUVA and FI, were the most significant in explaining cladoceran carapace melanisation (Table 3). Lake-water SUVA had a strong negative ($r = -0.55$, $r^2 = 0.31$) and FI a positive correlation ($r = 0.54$, $r^2 = 0.30$) with *A. affinis* carapace melanin (Fig. 3), suggesting that meiobenthos in lakes with high terrestrial carbon contribution are less exposed to UV than populations in lakes with high autochthonous carbon contribution. Additionally, CDOM, indicating the amount of coloured carbon compounds, and $K_{d305/340}$, indicating the actual underwater UV environment, had negative, although statistically insignificant, relationships with melanisation (Table 3). Although UV controls on photoprotective pigmentation of zooplankton are previously well known (Hessen *et al.*, 1999; Tartarotti, Laurion & Sommaruga, 2001; Rautio & Korhola, 2002b) and investigated within wide geographical gradients (Rautio *et al.*, 2009), these results are the first to show the presence of UV-protective pigments in meiobenthos along a natural gradient of UV exposure

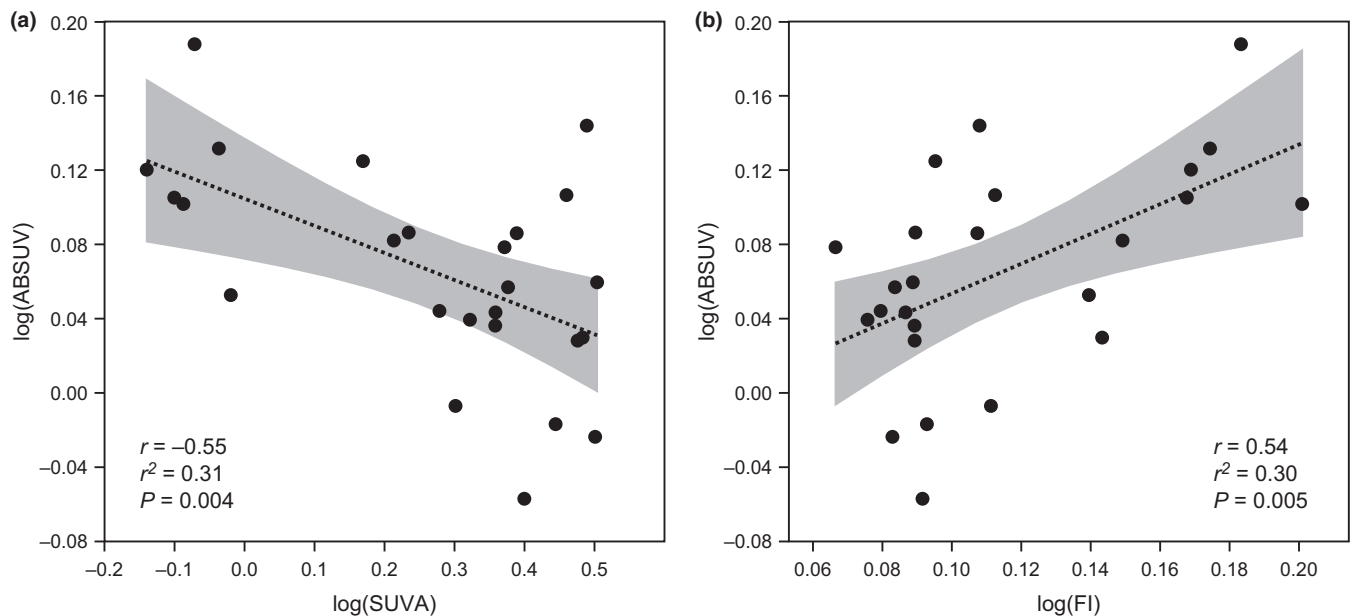


Fig. 3 Relationship between *Alona affinis* carapace UV absorbance and (a) specific UV absorbance (SUVA) and (b) fluorescence index (FI) of lake waters in the study sites. The grey bands indicate 95% confidence intervals.

and in relation to the UV absorbance properties of DOC. These results provide evidence that also lake benthos is subjected to hazardous UV and need adaptation strategies.

Water column depth is also relevant for the underwater UV regimes since deep-waters may provide UV refugia for mobile aquatic organisms (Rhode *et al.*, 2001; Rautio & Korhola, 2002a; Rautio, Korhola & Zellmer, 2003) and UV may not reach the bottom in deeper lakes. UV reaching the bottom of the lakes is especially important for benthic cladocerans that live on the sediment (cf. Vinebrooke & Leavitt, 1999). The observed relationship between lakes' UV exposure and the degree of melanisation was more scattered at the sites with lower UV exposure according to high SUVA and low FI (Fig. 3). This may be caused by variation in water depth causing higher UV exposure of certain shallow sites despite high UV-attenuating properties of the water column. For example, outlier lakes #9 and 24, which both contained high degree of melanisation in cladocerans and low UV exposure according to bio-optical properties, were very shallow (Table 1). Accordingly, the shallowness and abundant near-surface habitats of *Alona affinis* (e.g. mossy shorelines) may have caused higher melanisation despite UV-shaded waters. The variation observed in the degree of melanisation may have additionally been caused by differences in fish predation pressure. There occurs a trade-off between benefits and costs of pigmentation under high UV exposure since fish tend to prey on the most pigmented (high visibility) individuals (Hansson, 2000; Tollrian & Heibl, 2004). It is therefore possible that lakes with dark UV-attenuating waters and high cladoceran melanisation (Fig. 3) were fish-free, allowing the animals to utilise stronger pigmentation. This may be the case in the outlier lakes where both shallowness and lack of fish could have induced higher melanisation.

Aquatic UV responses under global change

Increased UV irradiance due to anthropogenically induced ozone depletion is still an environmental threat to the humans and as well as an ecological threat to ecosystems and organisms (ACIA, 2005; Williamson *et al.*, 2014). Increased UV *per se* (McKenzie *et al.*, 2011) and its cumulative impacts on photodegradation of DOM in aquatic ecosystems (Cory *et al.*, 2013) cause stress on the organisms and likely favour those taxa with efficient UV-protective strategies (Rautio & Korhola, 2002a; Rautio & Tartarotti, 2010). The ongoing climate change adds to the challenge in climate-sensitive

lakes (Williamson *et al.*, 2014; Häder *et al.*, 2015), causing significant reorganisation of aquatic communities and ecosystem structure (Smol *et al.*, 2005; Thienpont *et al.*, 2015). It also significantly alters bio-optical lake-water properties and underwater UV doses by impacting, for example, ice break-up time, catchment vegetation and surface run-off (Pienitz & Vincent, 2000; Saulnier-Talbot *et al.*, 2003; Clark *et al.*, 2013; Nevalainen *et al.*, 2014, 2015). It has been shown in palaeolimnological studies that cladoceran meiobenthos responds to centennial–millennial scale changes in underwater UV exposure through changing the degree of melanin pigmentation (Nevalainen & Rautio, 2014; Nevalainen *et al.*, 2015). The current results also suggest that cladoceran meiobenthos are able to adapt to large differences in aquatic UV exposure by melanin pigmentation along a geographical gradient through the subarctic ecoregion (Fig. 3). Hence, it is well established that melanin is preserved in fossil cladoceran carapaces, which are archived in lake sediments allowing its use in tracking spatiotemporal aquatic UV regimes and organisms' UV responses. In addition to assessing aquatic UV responses under existing ecological gradients and ongoing climate change scenarios, the current results are also applicable to long-term palaeolimnological studies providing unique reference material for estimating past aquatic UV responses under abrupt catchment disturbances and climate changes with respect to currently occurring changes in high-latitude lakes. A combination of concurrent environmental stressors related to the global change such as earlier ice break-up, higher absolute UV doses, increased CDOM photodegradation, and higher terrestrial DOC input may ultimately result in major ecological changes in aquatic ecosystems also impacting the need and ability of the organisms for photoprotection.

Acknowledgments

This study was funded by the Academy of Finland VIOLET project (#287547), the Doctoral Program in Geosciences of the University of Helsinki, Natural Sciences and Engineering Research Council of Canada and the Canadian Foundation for Innovation. We thank the staff of the Kevo Subarctic Research Station for their support during the fieldwork, Tobias Schneider for assistance with profiling radiometer measurements, and Toni Roiha and Jonna Kuha for helpful discussions on spectroscopic methodologies. John Smol and an anonymous reviewer provided constructive comment that improved the manuscript.

References

- ACIA (2005) *Arctic Climate Impact Assessment*. Cambridge University Press, Cambridge.
- Adrian R., O'Reilly C.M., Zagarese H., Baines S.B., Hessen D.O., Keller W. *et al.* (2009) Lakes as sentinels of climate change. *Limnology and Oceanography*, **54**, 2283–2297.
- Clark G.F., Stark J.S., Johnston E.L., Runcie J.W., Goldsworthy P.M., Raymond B. *et al.* (2013) Light-driven tipping points in polar ecosystems. *Global Change Biology*, **19**, 3749–3761.
- Cory R.M., Crump B.C., Dobkowski J.A. & Kling G.W. (2013) Surface exposure to sunlight stimulates CO₂ release from permafrost soil carbon in the Arctic. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 3429–3434.
- Davidson E.A. & Janssens I.A. (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.
- Forsström L., Rautio M., Cusson M., Sorvari S., Albert R.-L., Kumagai M. *et al.* (2015) DOM concentration, optical parameters and attenuation of solar radiation in high-latitude lakes across three vegetation zones. *Écoscience*, in press.
- Friedlingstein P., Cox P., Betts R., Bopp L., von Bloh W., Brovkin V. *et al.* (2006) Climate-carbon cycle feedback analysis: results from the (CMIP)-M-4 model intercomparison. *Journal of Climate*, **19**, 3337–3353.
- Häder D., Williamson C., Wängberg S.Å., Rautio M., Rose K.C., Gao K. *et al.* (2015) Effects of UV radiation on aquatic ecosystems and interactions with other environmental factors. *Photochemical and Photobiological Sciences*, **14**, 108–126.
- Hammer Ø., Harper D.A.T. & Ryan P.D. (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontologica Electronica*, **4**, online. URL: http://palaeo-electronica.org/2001_1/past/issue1_01.htm.
- Hansson L.A. (2000) Induced pigmentation in zooplankton: a trade-off between threats from predation and ultraviolet radiation. *Proceedings of the Royal Society of London B*, **267**, 2327–2331.
- Hebert P.D.N. & Emery C.J. (1990) The adaptive significance of cuticular pigmentation in *Daphnia*. *Functional Ecology*, **4**, 703–710.
- Hessen D.O. (1996) Competitive trade-off strategies in Arctic *Daphnia* linked to melanism and UV-B stress. *Polar Biology*, **16**, 573–576.
- Hessen D.O., Borgeraas J., Kessler K. & Refseth U.H. (1999) UV-B susceptibility and photoprotection of Arctic *Daphnia* morphotypes. *Polar Research*, **18**, 345–352.
- Hood E., Williams M.W. & McKnight D.M. (2005) Sources of dissolved organic matter (DOM) in a Rocky Mountain stream using chemical fractionation and stable isotopes. *Biogeochemistry*, **74**, 231–255.
- Jaffe R., McKnight D.M., Maie N., Cory R., McDowell W.H. & Campbell J.L. (2008) Spatial and temporal variations in DOM composition in ecosystems: the importance of long-term monitoring of optical properties. *Journal of Geophysical Research*, **113**, G04032.
- Jansen B., Kalbitz K. & McDowell W.H. (2014) Dissolved organic matter: linking soil and aquatic systems. *Vandose Zone Journal*, **13**, doi:10.2136/vzj2014.05.0051.
- Jeffrey S.W. & Walschmeyer N.A. (1997) Spectrophotometric and fluorometric equations in common use in oceanography. In: *Phytoplankton Pigments in Oceanography* (Eds S.W. Jeffrey, R.F.C. Mantoura & S.W. Wright), pp. 361–381. UNESCO Publishing, Paris.
- Laurion I., Ventura M., Catalan J., Psenner R. & Sommaruga R. (2000) Attenuation of ultraviolet radiation in mountain lakes: factors controlling the among- and within-lake variability. *Limnology and Oceanography*, **45**, 1274–1288.
- Laurion I., Vincent W.F. & Lean D.R.S. (1997) Underwater ultraviolet radiation: development of spectral models for northern high latitude lakes. *Photochemistry and Photobiology*, **65**, 107–114.
- Luoto T.P. & Nevalainen L. (2013) Long-term water temperature reconstructions from mountain lakes with different catchment and morphometric features. *Scientific Reports*, **3**, 2488. doi:10.1038/srep02488.
- Manca M., Ruggiu D., Panzani P., Asioli A., Mura G. & Nocentini A.M. (1998) Report on a collection of aquatic organisms from high mountain lakes in Khumbu Valley (Nepalese Himalayas). *Memorie Dell'Istituto Italiano Di Idrobiologia*, **57**, 77–98.
- Manney G.L., Santee M.L., Rex M., Livesey N.J., Pitts M.C., Veefkind P. *et al.* (2011) Unprecedented Arctic ozone loss in 2011. *Nature*, **478**, 469–475.
- McKenzie R.L., Aucamp P.J., Bais A.F., Björn L.O., Ilyas M. & Madronich S. (2011) Ozone depletion and climate change: impacts on UV radiation. *Photochemical and Photobiological Sciences*, **10**, 182–198.
- McKnight D.M., Boyer E.W., Westerhoff P.K., Doran P.T., Kulbe T. & Andersen D.T. (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography*, **46**, 38–48.
- Morris D.P., Zagarese H., Williamson C.E., Balseiro E.G., Hargreaves B.R., Modenutti B. *et al.* (1995) The attenuation of UV radiation in lakes and the role of dissolved organic carbon. *Limnology and Oceanography*, **40**, 1381–1391.
- Nevalainen L., Lami A., Luoto T.P. & Manca M. (2014) Fossil cladoceran record from Lake Piramide Inferiore (5067 m a.s.l.) in the Nepalese Himalayas – biogeographical and paleoecological implications. *Journal of Limnology*, **73**, 156–166.
- Nevalainen L., Rantala M.V., Luoto T.P., Rautio M. & Ojala A.E.K. (2015) Ultraviolet radiation exposure of a high

- arctic lake in Svalbard during the Holocene. *Boreas*, **44**, 401–412.
- Nevalainen L. & Rautio M. (2014) Spectral absorbance of benthic cladoceran carapaces as a new method for inferring past UV exposure of aquatic biota. *Quaternary Science Reviews*, **84**, 109–115.
- Nusch E.A. (1980) Comparison of different methods for chlorophyll and phaeopigment determination. *Archiv für Hydrobiologie-Beihefte Ergebnisse der Limnologie*, **14**, 14–36.
- Pienitz R. & Vincent W.F. (2000) Effects of climate change relative to ozone depletion on UV exposure in subarctic lakes. *Nature*, **404**, 484–487.
- Rautio M., Bonilla S. & Vincent W.F. (2009) UV photoprotectants in arctic zooplankton. *Aquatic Biology*, **7**, 93–105.
- Rautio M., Dufresne F., Laurion I., Bonilla S., Vincent W.F. & Christoffersen K.S. (2011) Shallow freshwater ecosystems of the circumpolar Arctic. *Écoscience*, **18**, 204–222.
- Rautio M. & Korhola A. (2002a) UV-induced pigmentation in subarctic *Daphnia*. *Limnology and Oceanography*, **47**, 295–299.
- Rautio M. & Korhola A. (2002b) Effects of ultraviolet radiation and dissolved organic carbon on the survival of subarctic zooplankton. *Polar Biology*, **25**, 460–468.
- Rautio M., Korhola A. & Zellmer I.D. (2003) Vertical distribution of *Daphnia longispina* in a shallow subarctic pond: does the interaction of ultraviolet radiation and *Chaoborus* predation explain the pattern? *Polar Biology*, **26**, 659–665.
- Rautio M. & Nevalainen L. (2013) Cladocera. In: *Encyclopedia of Quaternary Science* (Ed. S.A. Elias), pp. 271–280. Elsevier, Amsterdam.
- Rautio M. & Tartarotti B. (2010) UV radiation and freshwater zooplankton: damage, protection and recovery. *Freshwater Reviews*, **3**, 105–131.
- Rhode S.C., Pawlowski M. & Tollrian R. (2001) The impact of ultraviolet radiation on the vertical distribution of zooplankton of the genus *Daphnia*. *Nature*, **412**, 69–72.
- Roiha T., Tirola M., Gazzanelli M. & Rautio M. (2012) Carbon quantity defines productivity while its quality defines community composition of bacterioplankton in subarctic ponds. *Aquatic Sciences*, **74**, 519–525.
- Saulnier-Talbot E., Pienitz R. & Vincent W.F. (2003) Holocene lake succession and palaeo-optics of a Subarctic lake, northern Québec, Canada. *Holocene*, **13**, 517–526.
- Schindler P., Curtis J., Parker B.R. & Stainton M.P. (1996) Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes. *Nature*, **379**, 705–707.
- Smol J.P., Wolfe A.P., Birks H.J.B., Douglas M.S.V., Jones V.J., Korhola A. et al. (2005) Climate-driven regime shifts in the biological communities of arctic lakes. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 4397–4402.
- Sommaruga R. & Augustin G. (2006) Seasonality in UV transparency of an alpine lake is associated to changes in phytoplankton biomass. *Aquatic Sciences*, **68**, 129–141.
- Sommaruga R. & Psenner R. (1997) Ultraviolet radiation in a high mountain lake of the Austrian Alps: air and underwater measurement. *Photochemistry and Photobiology*, **65**, 957–963.
- Tartarotti B., Laurion I. & Sommaruga R. (2001) Large variability in the concentration of mycosporine-like amino acids among zooplankton from lakes located across an altitude gradient. *Limnology and Oceanography*, **46**, 1546–1552.
- Thienpont J.R., Korosi J.B., Cheng E.S., Deasley K., Pisarcic M.F.J. & Smol J.P. (2015) Recent climate warming favours more specialized cladoceran taxa in western Canadian Arctic lakes. *Journal of Biogeography*, **42**, 1553–1565.
- Tollrian R. & Heibl C. (2004) Phenotypic plasticity in pigmentation in *Daphnia* induced by UV radiation and fish kairomones. *Functional Ecology*, **18**, 497–502.
- Tranvik L.J., Downing J.A., Cotner J.B., Loiselle S.A., Striegl R.G., Ballatore T.J. et al. (2009) Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography*, **54**, 2298–2314.
- Van Damme K. & Eggermont H. (2011) The Afromontane Cladocera (Crustacea: Branchiopoda) of the Rwenzori (Uganda-DR Congo): taxonomy, ecology and biogeography. *Hydrobiologia*, **676**, 57–100.
- Vincent W.F. & Pienitz R. (1996) Sensitivity of high latitude freshwater ecosystems to global change: temperature and solar ultraviolet radiation. *Geoscience Canada*, **4**, 231–236.
- Vincent W.F., Rautio M. & Pienitz R. (2007) Climate control of underwater UV exposure in polar and alpine aquatic ecosystems. In: *Arctic Alpine Ecosystems and People in a Changing Environment* (Eds J.B. Orbaek, R. Kallenborn, I. Tombre, Hegseth E., A. Falk-Petersen & A.H. Hoel), pp. 227–492. Springer-Verlag, Berlin.
- Vinebrooke R.D. & Leavitt P.R. (1999) Differential responses of littoral communities to ultraviolet radiation in an alpine lake. *Ecology*, **80**, 223–237.
- Williamson C.E., Stemberger R.S., Morris D.P., Frost T.M. & Paulsen S.G. (1996) Ultraviolet radiation in North American lakes: attenuation estimates from DOC measurements and implications for plankton communities. *Limnology and Oceanography*, **41**, 1024–1034.
- Williamson C.E., Zepp R.G., Lucas R.M., Madronich S., Austin A.T., Ballaré C.L. et al. (2014) Solar ultraviolet radiation in a changing climate. *Nature Climate Change*, **4**, 434–441.
- Wrona F.J., Prowse T.D., Reist J.D., Hobbie J.E., Lévesque L.M.J. & Vincent W.F. (2006) Climate change effects on aquatic biota, ecosystem structure and function. *Ambio*, **35**, 359–369.
- Yentsch C.S. & Menzel D.W. (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea Research*, **10**, 221–231.

(Manuscript accepted 6 August 2015)