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# UV photoprotectants in arctic zooplankton

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ABSTRACT: High latitude zooplankton must contend with continuous ultraviolet (UV) exposure in summer, increased UVB fluxes as a result of stratospheric ozone depletion and little UV protection from their transparent waters. In the present study, we evaluated the presence and concentration of 4 types of UV-protectants in arctic zooplankton: carotenoids, melanins, scytonemin and mycosporine-like amino acids (MAAs). We analysed 12 commonly occurring crustacean species from 27 freshwater bodies in northern Canada and Alaska. Pigments were detected in all species, and most populations had multiple pigments, suggesting a combination of photoprotection strategies, including broadband screening of UV radiation and carotenoid quenching of reactive oxygen species. Scytonemin, a UVA-screening pigment of cyanobacterial origin that has not been previously detected in zooplankton, was found in 2 crustacean species: the cladoceran *Daphnia middendorffiana* and the fairy shrimp *Branchinecta paludosa*. MAAs were detected in all populations, providing the first records of high concentrations of these compounds in the genus *Daphnia* (1 µg mg$^{-1}$) and in the fairy shrimp *Artemiopsis stefanssoni* (up to 37 µg mg$^{-1}$). Concurrent analyses of food sources showed that scytonemin, carotenoids and MAAs in zooplankton originated in phytoplankton or benthic algal mats. Thus, in addition to providing a measure of UV protection, the pigments also indicate zooplankton food sources and potential benthic–pelagic coupling.

KEY WORDS: UV radiation · Carotenoids · MAAs · Scytonemin · Melanins · Arctic · Zooplankton · Ponds



## INTRODUCTION

High-latitude water bodies often have low concentrations of coloured dissolved organic matter (CDOM) and hence a deep penetration of ultraviolet (UV) radiation into their water columns (Schindler et al. 1996, Laurion et al. 1997, Molot et al. 2004). UV radiation has a broad range of deleterious effects on aquatic biota (Hessen & Færøvig 2001), including zooplankton (Rautio et al. 2003). Many arctic and subarctic waters are also shallow, exposing a large part of the water column to UV effects. In recent years, ozone loss rates in the arctic region have reached values comparable to those recorded over the Antarctic, and further increases of 20 to 90% have been predicted for the period from 2010 to 2020 (Taalas et al. 2000). Increased UV radiation over the poles is of special ecological concern, because the biota have adapted to substantially lower UV irradiance than that imposed by ozone depletion (Caldwell 1972). Additionally, cold temperatures may inhibit the enzymatic processes involved in repairing UV damage to proteins, nucleic acids and other biomolecules, and thereby exacerbate the impacts of UV radiation exposure (Roos & Vincent 1998). However, pigments and other photoprotective compounds could mitigate these effects and thereby confer an adaptive advantage to organisms living in these waters.

Red, brown, or black colouration is typical of high-latitude zooplankton and has long been suggested as

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a protection strategy against UV radiation (Brehm 1938). More recent studies have also concluded that pigmented individuals survive better than pale individuals when exposed to UV radiation (Hairston 1976, Hessen 1996), and that there is a strong seasonal variation in pigment content. Subarctic *Daphnia umbra* synthesize melanin only during summer months, beginning immediately after ice-out (Rautio & Korhola 2002), and alpine copepods peak in their mycosporine-like amino acid (MAAs) concentrations during the open-water period (Tartarotti & Sommaruga 2006, Persaud et al. 2007). Pigments and the colourless MAAs in zooplankton are mostly considered to be UV photoprotectants (Perin & Lean 2004), acting together with other photoprotectants such as antioxidants and with behavioural responses such as vertical migration.

Some zooplankton are able to synthesize melanin; however, other UV photoprotectants must be derived from feeding. In most aquatic systems phytoplankton is the dominant food for zooplankton and, hence, their source of photoprotective compounds. For shallow-water ecosystems other sources must also be considered. The substantial reserves of organic matter stored in the microbial mats on the bottom of most high-latitude ponds can be grazed directly or are even available for obligate planktonic feeders by the resuspension of mats (Hansson & Tranvik 2003, Rautio & Vincent 2006, 2007).

In the present study, we aimed to evaluate the diversity of UV protectants in high-latitude zooplankton. We analysed populations of 12 crustacean zooplankton species to determine the concentration of scytonemin (peak absorbance in the UVA waveband), carotenoids (quenching agents of UV-produced reactive oxygen species), melanins (highest absorbance in the UVB range) and MAAs (peak absorbance in UVB and short wavelength UVA). Additionally, we investigated the origin of these photoprotective compounds. We hypothesised that different species possess different strategies against UV radiation, and that these are determined by species-specific characteristics and by trophic transfer of these compounds (or their precursors) contained in different algal food sources. To address these objectives, we sampled phytoplankton, phytobenthos and zooplankton from subarctic and arctic ponds in northern Canada and Alaska. We evaluated zooplankton pigmentation patterns in more detail at one site in Canadian High Arctic, with analyses of zooplankton, phytoplankton and benthic algal pigments. To our knowledge, this is the first attempt to measure all major types of pigments in multispecies communities of zooplankton and to detect pigment transfer from phytoplankton and benthic microbial food sources.

## MATERIALS AND METHODS

**Study sites.** We sampled 27 tundra lakes and ponds during July and August of 2002, 2003 and 2004. Five of the water bodies were located in coastal subarctic northern Quebec (55 to 56° N, 77 to 78° W), 9 in subarctic Alaska (68° N, 149° W), 4 in the subarctic Mackenzie Delta (69° N, 133 to 134° W), and 5 on Cornwallis Island (74 to 75° N, 94 to 95° W) and 5 on Ellesmere Island in the Canadian High Arctic (81 to 83° N, 68 to 75° W) (Fig. 1). The autotrophic biomass in all of these systems was dominated by the nutrient-rich phytobenthos (Table 1), and the water column contained several species of zooplankton in high abundance. All sites were fishless, which allowed zooplankton to express their visual pigmentation without increasing predation risk (Hylander et al. 2009a).

One sampling site, Pond A1 (Cornwallis Island), was chosen for a closer examination of pigments because of its representativeness of high arctic sites, with shallow transparent water, a large standing stock of benthic microbial biomass and dense populations of pigmented zooplankton. Of the 4 crustacean species at this site, 3 were analysed for pigments, and measurements of pigments and MAAs were also made of its seston and phytobenthos. This pond is located on Cornwallis Island in high arctic Nunavut, Canada (74° 42.48′ N, 95° 00.85′ W). It is a small (0.4 ha), shallow (1.5 m maximum depth) and fishless clear-water pond surrounded by frost-shattered limestone rubble. The pond is covered with ice for approximately 10 mo of the year, and for much of this period it is completely frozen. As a result of low concentrations of UV-absorbing dissolved organic carbon (DOC) (2.8 mg $l^{-1}$) and shallow depth, >60% of the ambient $UV_{320}$ (ultra-

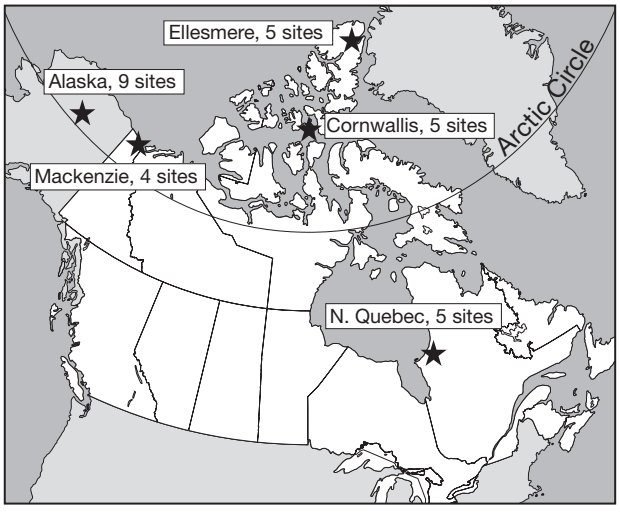


Fig. 1. Map of the study area indicating the number of sites visited in each region

Table 1. Selected environmental variables from the subarctic and arctic sampling sites. TP: total phosphorus; DOC: dissolved organic carbon; $UV_{320}$: ultraviolet radiation at 320 nm; PP: primary productivity; n: number of ponds sampled; ND: no data. Analyses for TP, DOC chl *a* and PP as in Rautio & Vincent (2006), which also gives site-specific information for N. Quebec and Cornwallis ponds

| | Depth (m) | Temperature (°C) | TP (µg $l^{-1}$) | DOC (mg $l^{-1}$) | Bottom $UV_{320}$ (%) | Chl *a* (mg $m^{-2}$) Water | Chl *a* (mg $m^{-2}$) Mat | PP (mg C $m^{-2}$ $h^{-1}$) Water | PP (mg C $m^{-2}$ $h^{-1}$) Mat |
|---|---|---|---|---|---|---|---|---|---|
| **N. Quebec, n = 5** | | | | | | | | | |
| Median | 0.3 | 16.6 | 12.4 | 10.3 | 0.03 | 1.2 | 260.5 | 2.0 | 32.4 |
| Minimum | 0.3 | 14.9 | 4.0 | 4.5 | 0.0 | 0.3 | 1.2 | 1.2 | 1.8 |
| Maximum | 1.0 | 23.5 | 33.4 | 12.2 | 3.9 | 3.8 | 378.7 | 3.4 | 35.4 |
| **Alaska, n = 9** | | | | | | | | | |
| Median | 0.3 | 12.9 | 8.8 | 9.1 | 0.52 | 0.3 | 60.2 | 0.8 | 64.6 |
| Minimum | 0.1 | 11.8 | 0.5 | 4.5 | 0.0 | 0.1 | 39.7 | 0.3 | 28.9 |
| Maximum | 0.4 | 20.9 | 15.8 | 12.2 | 20.4 | 2.0 | 259.8 | 1.3 | 84.8 |
| **Mackenzie, n = 4** | | | | | | | | | |
| Median | 0.3 | 12.1 | 12.5[a] | 30.0 | 1.07 | 0.2[a] | ND | 0.7[a] | 119.7[a] |
| Minimum | 0.1 | 8.0 | | 23.0 | 0.0 | | | | |
| Maximum | 0.4 | 13.2 | | 37.3 | 6.4 | | | | |
| **Cornwallis, n = 5** | | | | | | | | | |
| Median | 1.5 | 4.5 | 5.3 | 2.0 | 73.63 | 1.0 | 84.9 | 0.9 | 5.5 |
| Minimum | 0.2 | 3.6 | 4.4 | 1.7 | 0.4 | 0.2 | 19.5 | 0.2 | 2.4 |
| Maximum | 9.0 | 6.3 | 20.9 | 3.0 | 91.2 | 7.3 | 122.4 | 3.8 | 21.5 |
| **Ellesmere, n = 5** | | | | | | | | | |
| Median | 1.0 | 10.0 | 25.4 | 5.7 | 6.36 | 0.5 | 71.7 | ND | ND |
| Minimum | 0.5 | 3.1 | <0.5 | 4.0 | 0.2 | 0.04 | 39.9 | | |
| Maximum | 5.0 | 10.5 | 52.7 | 36.8 | 83.2 | 3.6 | 233.4 | | |

[a]Single sample

violet radiation at 320 nm) reaches the bottom of the pond. The zooplankton community of Pond A1 at the time of sampling (August 2002) was composed of the fairy shrimps *Artemiopsis stefanssoni* (500 ind. $m^{-3}$) and *Branchinecta paludosa* (1200 ind. $m^{-3}$), the cladoceran *Daphnia middendorffiana* (1000 ind. $m^{-3}$), cyclopoids (75 ind. $m^{-3}$) and rotifers (14 000 ind. $m^{-3}$, 90% belonging to the genus *Synchaeta*).

**Sample collection and analysis.** Samples of the dominant crustacean zooplankton species collected in 17 water bodies were used for carotenoid and scytonemin pigment analyses, and samples of the dominant crustacean zooplankton species from 7 water bodies were used for MAA analysis. Samples of *Daphnia* spp. from 16 water bodies were analysed for melanin. Melanin was also determined from the fairy shrimps in Pond A1 and from a population of *Scapholeberis mucronata*, a black-coloured cladoceran that is often found in association with the neuston at the surface of northern ponds. In total, 12 different zooplankton species representing 31 populations of adults were collected. The species included cladocerans (Cladocera: *D. middendorffiana*, *Polyphemus pediculus*, *S. mucronata*), copepods (Copepoda: *Leptodiaptomus minutus*, *Hesperodiaptomus arcticus*, *Heterocope septentrionalis*, *Diaptomus pribilofensis* and an unidentified cyclopoid), fairy shrimps (Anostraca: *Artemiopsis stefanssoni*, *Branchinecta paludosa*, *Polyartemiella hazeni*) and tadpole shrimps (Notostraca: *Lepidurus arcticus*). Phytoplankton and benthic algae were sampled for carotenoids and scytonemin at 17 sites, while MAAs were measured only for Pond A1. Samples for total phosphorus (TP), DOC and chlorophyll *a* were collected at all sites and analysed as described by Rautio & Vincent (2006). The penetration of UV radiation was approximated from DOC concentrations using the relationships for high-latitude waters given by Laurion et al. (1997).

Zooplankton samples were obtained by horizontal trawls using a 250 µm mesh sized net attached to a long pole. Animals were transferred to GF/F-filtered water for approximately 30 min to eliminate any algal contamination, sorted by hand while still alive and stored frozen in Eppendorf tubes. Some of the individuals were reserved for dry weight measurement. All samples were collected in replicates, except where the low amount of material did not allow more than one sample. Number of individuals in the replicates varied between 1 and 540, depending on the size of the animals, the amount of material required for analysis and the availability of species.

Up to 4 l of water was collected for phytoplankton chlorophyll, carotenoid, scytonemin and MAA analyses. The samples were taken subsurface with acid-washed, 1 l Nalgene bottles, and filtered either in the field or within a few hours in the laboratory under low

vacuum pressure onto 25 mm diameter GF/F glass-fibre filters. The filters were frozen and subsequently transferred to a −80°C freezer until further processing. Mat cores for pigment and MAA analysis were gently taken with a 6 or 10 mm diameter sediment corer (a syringe with the end cut off). The top 1 mm of the core was sectioned with a blade, wrapped in aluminum foil and frozen until analysis.

Chlorophylls, carotenoids and scytonemin were analysed by HPLC according to Zapata et al. (2000). In the laboratory, zooplankton and mat pigments were extracted by grinding each sample for 3 min, following sonication twice for 30 s at 10 W in 2 to 4 ml of 90% acetone. The extraction was then incubated overnight in the dark at −20°C under argon gas. This protocol gave an optimal extraction for carotenoids and scytonemins in these 2 communities. The frozen phytoplankton filters were sonicated in 2 ml of 95% methanol. All the extracts were kept in darkness until injected into a Varian ProStar HPLC (Mulgrave, Australia) as described by Bonilla et al. (2005). Specific pigments were detected by diode-array spectroscopy (350 to 750 nm), and absorbance chromatograms were obtained at 384 nm for scytonemin and 450 nm for carotenoids, while chlorophylls were detected by fluorescence detection (excitation $\lambda$ = 440 nm; emission $\lambda$ = 650 nm). The identification and quantification of the pigments was based on commercial standards as detailed by Bonilla et al. (2005). The conversion factor for astaxanthin was applied to all peaks identified as astaxanthin-related. Astaxanthin, because of its 2 available OH groups, can make esters with fatty acids, and usually at least 2 peaks appear in chromatograms at the most non-polar end. These pigments were combined and referred to in the rest of this paper as astaxanthin. Similarly, the conversion factors for canthaxanthin and echinenone were applied to similar compounds and identified as canthaxanthin-like and echinenone-like in the zooplankton samples; these were combined and referred to here as canthaxanthin and echinenone. The concentration of unknown peaks was calculated by using the average conversion factor for all carotenoids. The same quantification procedure was used for a pigment closely resembling red-scytonemin. These 2 pigments were combined in the analyses and referred to here as scytonemin.

UV-absorbing substances with properties similar to MAAs from phytoplankton (on filters), algal mats (cores) and zooplankton were extracted in 25% methanol as described by Tartarotti & Sommaruga (2002) and quantified in a Cary-Varian spectrophotometer within the waveband from 220 to 400 nm. The MAA content, expressed as µg MAA per mg dry weight (DW) zooplankton) or per litre (phytoplankton) or per $cm^2$ (mats), was obtained according to:

$$C = D \times V \times 10^4 / E \times W$$

where $C$ is the concentration of MAAs in the sample, $D$ is the peak absorbance (~320 nm), $V$ is the volume of extract in ml, $E$ is the extinction coefficient and $W$ is the weight/volume/area of the sample in mg $l^{-1}$ $cm^{-2}$. A generic extinction coefficient of 120 was used as an approximate value for MAAs (Garcia-Pichel 1994).

The melanin extraction method was modified from Rautio & Korhola (2002). *Daphnia* spp. individuals from each site were placed in a test tube in 5 ml aqueous solution of 5 M NaOH and homogenised with an ultrasonic probe (Microson Ultrasonic Cell Disruptor) for 4 min. The tubes were heated to approximately 80°C for 2 h and were then left to cool. This was repeated twice. The extracts were cleared by centrifugation at 12 000 × $g$ for 10 min at room temperature, and the absorbance of the supernatants was measured spectrophotometrically at from 200 to 800 nm. Absorbance was converted to concentration by means of a calibration curve calculated from synthetic melanin (Sigma No. M8631).

**Data analysis.** Regional patterns in zooplankton pigment composition (carotenoids and scytonemins) were statistically analysed (multi-dimensional scaling) with the software Primer (Clarke & Gorley 2001). Data were fourth-root transformed prior to analysis, to avoid the strong impact of common pigments as recommended by Field et al. (1982). Differences in pigment structure among regions and communities were tested by non-parametric analyses of similarity (1-way ANOSIM). When significant differences were found, *a posteriori* pairwise tests were performed.

## RESULTS

### Temperature, UV regime and phototrophs

The subarctic sites in Quebec, Alaska and the Mackenzie River delta had similar temperatures (~14°C) and were up to 7°C warmer than the Arctic sites on Cornwallis and Ellesmere Islands (Table 1). TP varied markedly among sites, ranging from 0.5 to 53 µg $l^{-1}$. TP did not follow south–north or temperature gradients, and the highest TP concentrations were measured in lakes on Ellesmere Island. The polar desert sites on Cornwallis Island contained markedly lower concentrations of DOC than the other ponds. These water bodies had higher UV exposure than the more humic-rich ponds in other regions, with on average >70% of $UV_{320}$ reaching the bottom of the water body. Some ponds on Ellesmere Island were also highly UV exposed, but in general these sites were UV protected by their high DOC concentrations (Table 1).

At most sites the concentration of pigments in benthic mats was 2 to 3 orders of magnitude higher than that of phytoplankton. The benthic community averaged 114 mg chl *a* $m^{-2}$, representing >95% of the total autotrophic biomass (phytobenthos plus phytoplankton) per unit area for most of the sites (Table 1). The mats had a higher primary production per unit area than the plankton, consistent with the differences in chl *a* concentrations (Table 1). The pigment structure also showed differences between the mats and phytoplankton. In the phytoplankton, the carotenoids fucoxanthin, lutein and violaxanthin, and also chl $c_2$ and chl *b* suggested the dominance of chromophytes and chlorophytes, while the presence of scytonemin, zeaxanthin and canthaxanthin in benthic mats indicated the dominance of cyanobacteria. Scytonemin was detected in all benthic samples, with the highest scytonemin:chl *a* ratios at several Cornwallis Island sites. The degradation product red-scytonemin was detected at Cornwallis Island, northern Quebec and Ellesmere sites (averages of 1.1, 7.3 and 4.2 mg $m^{-2}$, respectively). MAAs were detected in all 7 mats analysed, but 2 sites did not have them in phytoplankton. Most phytoplankton had a concentration around 0.4 µg $l^{-1}$, but in 1 pond we measured up to 4.5 µg $l^{-1}$. In the mats, the MAA concentration varied between 0.1 and 2.2 µg $cm^{-2}$. Additional information on autotrophic pigment composition at these sites is given in the paper by Bonilla et al. (2009).

### Zooplankton UV photoprotectants

The total carotenoid concentration in zooplankton per unit biomass (µg of pigment per mg DW) ranged from 0.1 µg $mg^{-1}$, measured in the cladoceran *Polyphemus pediculus*, to 12.4 µg $mg^{-1}$ in the fairy shrimp *Artemiopsis stefanssoni.* Degradation products of scytonemin were detected in *Daphnia middendorffiana* (max. 0.02 µg $mg^{-1}$, which represents 2% of the total pigments) and in *Branchinecta paludosa* (max. 0.1 µg $mg^{-1}$ and 15% of the total pigments). MAAs were measured for 6 species, and the concentrations were highly variable both among and within species. Two fairy shrimps, *A. stefanssoni* and *B. paludosa*, differed markedly in their MAAs concentration, and had both the lowest (zero) and highest (37 µg $mg^{-1}$) values among all zooplankton taxa. Melanin was measured in 2 cladocerans and 2 fairy shrimp species, and was always found to be present in these taxa (Table 2).

In addition to the differences in total carotenoid concentration, the composition of specific carotenoids varied among species (Fig. 2). Calanoid copepods had high concentrations of astaxanthin (0.2 to 2.9 µg $mg^{-1}$). In all 4 species (*Leptodiaptomus minutus*, *Diaptomus pribilofensis*, *Heterocope septentrionalis* and *Hesperodiaptomus arcticus*) astaxanthin contributed 29 to 63% of the total carotenoids. The 3 fairy shrimps *Polyartemiella hazeni*, *Branchinecta paludosa* and *Artemiopsis stefanssoni* had canthaxanthin as their dominant carotenoid pigment (0.01 to 9.2 µg $mg^{-1}$), with values ranging from 32 to 67% of the total carotenoid concentration. The 3 cladocerans *Polyphemus pediculus*, *Scapholeberis mucronata* and *Daphnia middendorffiana* had primarily 2 to 4 pigments, including astaxanthin, canthaxanthin, zeaxanthin, fucoxanthin and echinenone, and no single carotenoid could be identified as a typical cladoceran carotenoid. The cyanobacterial pigment echinenone was the primary carotenoid in *Lepidurus arcticus*. The contribution of chl *a* to the pigment content of zooplankton was always small or negligible, indicating the absence of algae in their guts (no chl *a* or

Table 2. Interspecies comparison of the 3 main pigment groups in zooplankton. Concentration values (µg $mg^{-1}$ dry weight) are mean (±1 SE) for 2 to 10 replicates. Region: the geographical area where the species was encountered; Ind.: number of individuals in each replicate; MAAs: mycosporine-like amino acids; High Arctic: Cornwallis and Ellesmere Islands; –: not analysed. Species are ordered according to their carotenoid concentration

| Species | Region | Ind. | Pigment concentration (µg $mg^{-1}$ dry weight) | | | |
|---|---|---|---|---|---|---|
| | | | Carotenoids | Scytonemins | Melanin | MAAs |
| *Polyphemus pediculus* | N. Quebec | 100 | 0.1[a] | 0 | – | – |
| *Scapholeberis mucronata* | N. Quebec | 28, 100 | 0.4[a] | 0 | 68.4[a] | – |
| *Polyartemiella hazeni* | Alaska | 27 | 0.6 ± 0.4 | 0 | – | – |
| *Lepidurus arcticus* | Ellesmere | 1 | 0.9 ± 0.6 | 0 | – | – |
| *Daphnia middendorffiana* | All | 10–180 | 1.2 ± 0.4 | 0.02 ± 0.003 | 4.7 ± 0.9 | 1.0[a] |
| *Branchinecta paludosa* | High Arctic | 4–100 | 1.4 ± 0.5 | 0.06 ± 0.04 | 4.4[a] | 0 |
| *Leptodiaptomus minutus* | N. Quebec | 72–100 | 1.6 ± 1.0 | 0 | – | 5.1 ± 4.8 |
| *Diaptomus pribilofensis* | Alaska | 540 | 2.2[a] | 0 | – | – |
| *Heterocope septentrionalis* | Alaska | 30–56 | 3.2 ± 1.4 | 0 | – | – |
| *Hesperodiaptomus arcticus* | N. Quebec | 10–15 | 3.8 ± 2.2 | 0 | – | 0.02[a] |
| *Artemiopsis stefanssoni* | High Arctic | 3–350 | 4.3 ± 2.2 | 0 | 2.3[a] | 21.3 ± 8.5 |
| Cyclopoida | Cornwallis | 30 | – | – | – | 0.08 |

[a]Single sample

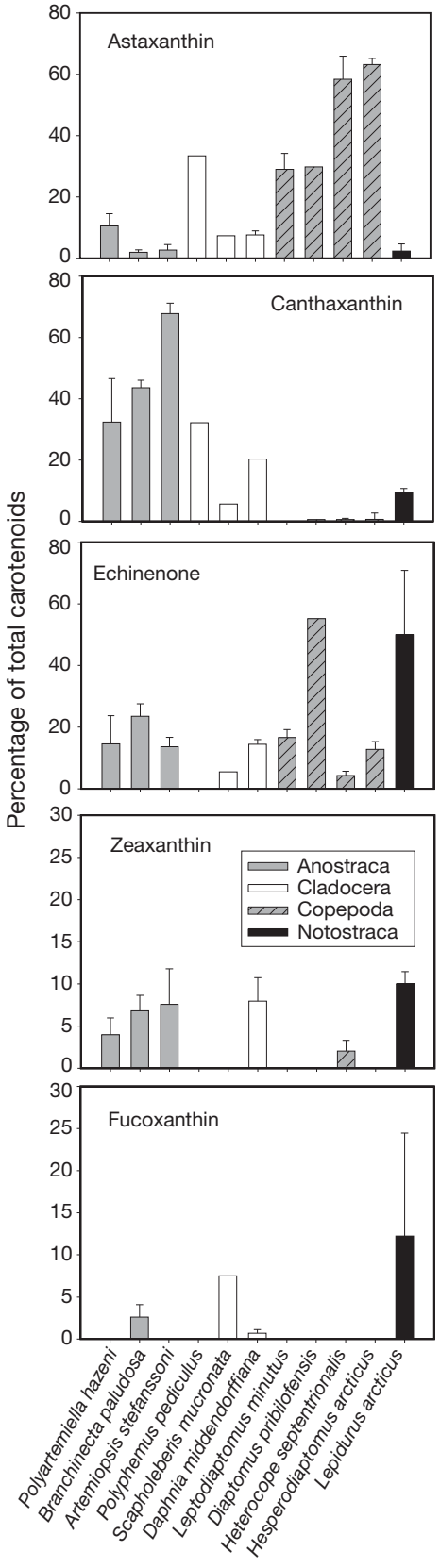


Fig. 2. Astaxanthin, canthaxanthin, echinenone, zeaxanthin and fucoxanthin in zooplankton as percentages of total carotenoids

its degradation products in >50% of samples analysed, <1% of all pigments in 77% of samples, highest prevalence of 7.9% in 1 sample of *S. mucronata*).

Zooplankton carotenoid composition differed between the arctic and subarctic geographical regions. For all carotenoids and scytonemin (13 identified, 18 unidentified) detected in zooplankton, there was a highly significant difference between subarctic (N. Quebec + Alaska) and arctic (Cornwallis Island + Ellesmere Island) zooplankton in their pigment structure (ANOSIM, $R = 0.34$, $p < 0.02$, Fig. 3). This division was mostly due to the different concentrations of canthaxanthin, astaxanthin and zeaxanthin between subarctic and arctic species (Fig. 3). These pigments explained 16.3, 14.3 and 13.3% of the dissimilarity, respectively. To test whether this difference was driven only by differences in species composition between subarctic and arctic sites, or whether there was an environmental effect on species, we examined the patterns in *Daphnia middendorffiana*, the only zooplankton species found in all geographical regions. Again, the subarctic and arctic populations of this species were significantly different in their pigment composition (ANOSIM, $R = 0.70$, $p < 0.02$). Nearly 60% of the difference between subarctic and arctic *D. middendorffiana* populations could be explained by the carotenoid pigments zeaxanthin, astaxanthin and fucoxanthin (29.1, 16.6 and 13.5%, respectively). The total concentration of carotenoids in *D. middendorffiana* was higher at the subarctic sites (average subarctic: 1.4 µg mg$^{-1}$; average arctic: 0.9 µg mg$^{-1}$), and this difference was statistically significant ($p = 0.013$).

### Pigments in phototrophic communities and zooplankton in Pond A1

The autotrophic biomass in Pond A1 in the high arctic polar desert was distributed between the sparse phytoplankton and biomass-rich benthic algal mats (Fig. 4), with the latter accounting for 98.9% of the total algal biomass, as measured by chl *a* concentration (72 mg m$^{-2}$ for mats and 0.2 mg m$^{-2}$ for phytoplankton). The HPLC chromatograms (Fig. 5) showed that the pigment composition also varied between these 2 algal communities. The benthic pigment composition indicated the dominance of cyanobacteria (scytonemin, echinenone, canthaxanthin, myxoxanthophyll, a glycoside resembling 4-keto-myxoxanthophyll and zeaxanthin). A compound that is likely to be red-scytonemin, a degradation product of scytonemin, was also detected in high concentrations (3.3 mg m$^{-2}$). Phytoplankton samples were dominated by lutein (0.3 mg m$^{-2}$), violaxanthin and chl *b*, indicating a dominance of chlorophytes in Pond A1; followed by crypto-

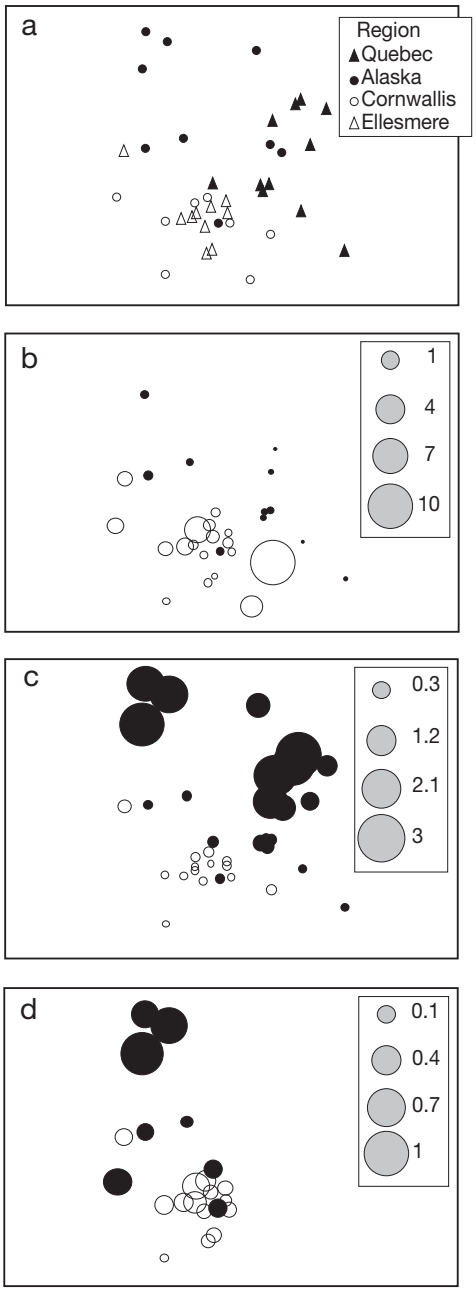


Fig. 3. (a) Two-dimensional plot (MDS ordination) showing the site distribution in the 4 regions according to zooplankton pigments for subarctic (filled symbols) and arctic (open symbols) regions. (b to d) Circle plots showing the relative importance of the 3 main carotenoids for the separation of concentrations (in µg $mg^{-1}$) at subarctic (black filled circles) and arctic (open circles) sites: (b) canthaxanthin, (c) astaxanthin and (d) zeaxanthin

phytes (alloxanthin, chl *b*), cyanobacteria (zeaxanthin) and fucoxanthin species (diverse chromophytes). In general, the carotenoids and accessory chlorophylls in the phytoplankton, with the exception of zeaxanthin, did not occur in the benthos.

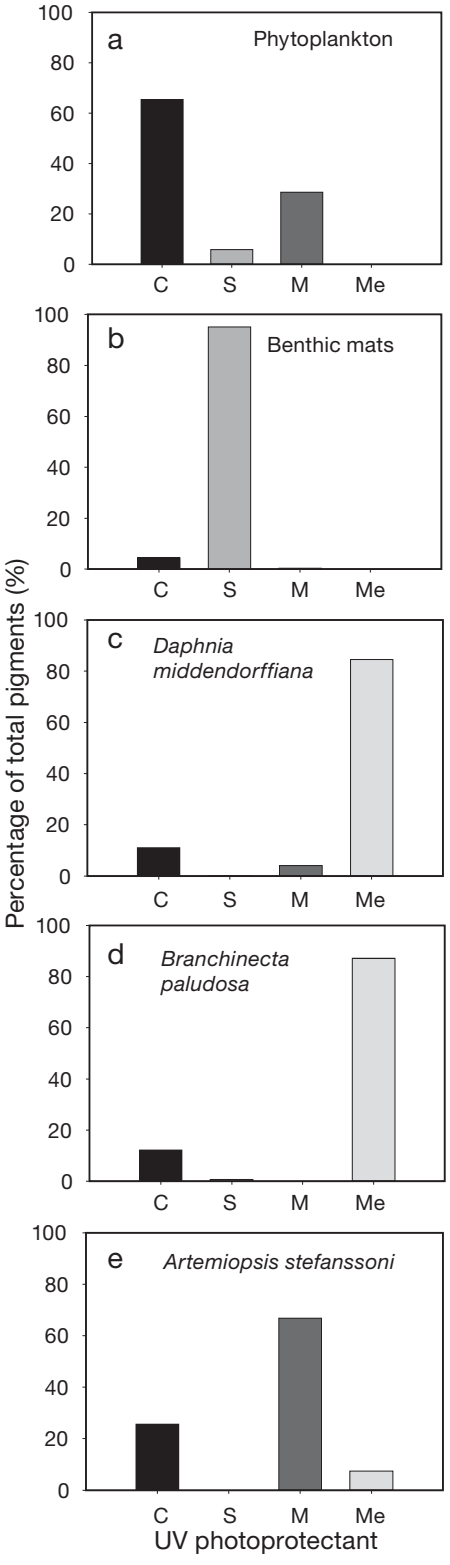


Fig. 4. Photoprotective compounds in Pond A1 autotrophs (phytoplankton and benthic mats) and zooplankton (*Daphnia middendorffiana*, *Branchinecta paludosa* and *Artemiopsis stefanssoni*). Each histogram shows the relative contribution of carotenoids (C), scytonemin (S), mycosporine-like amino acids (M) and melanins (Me) to the total mass of UV protectants

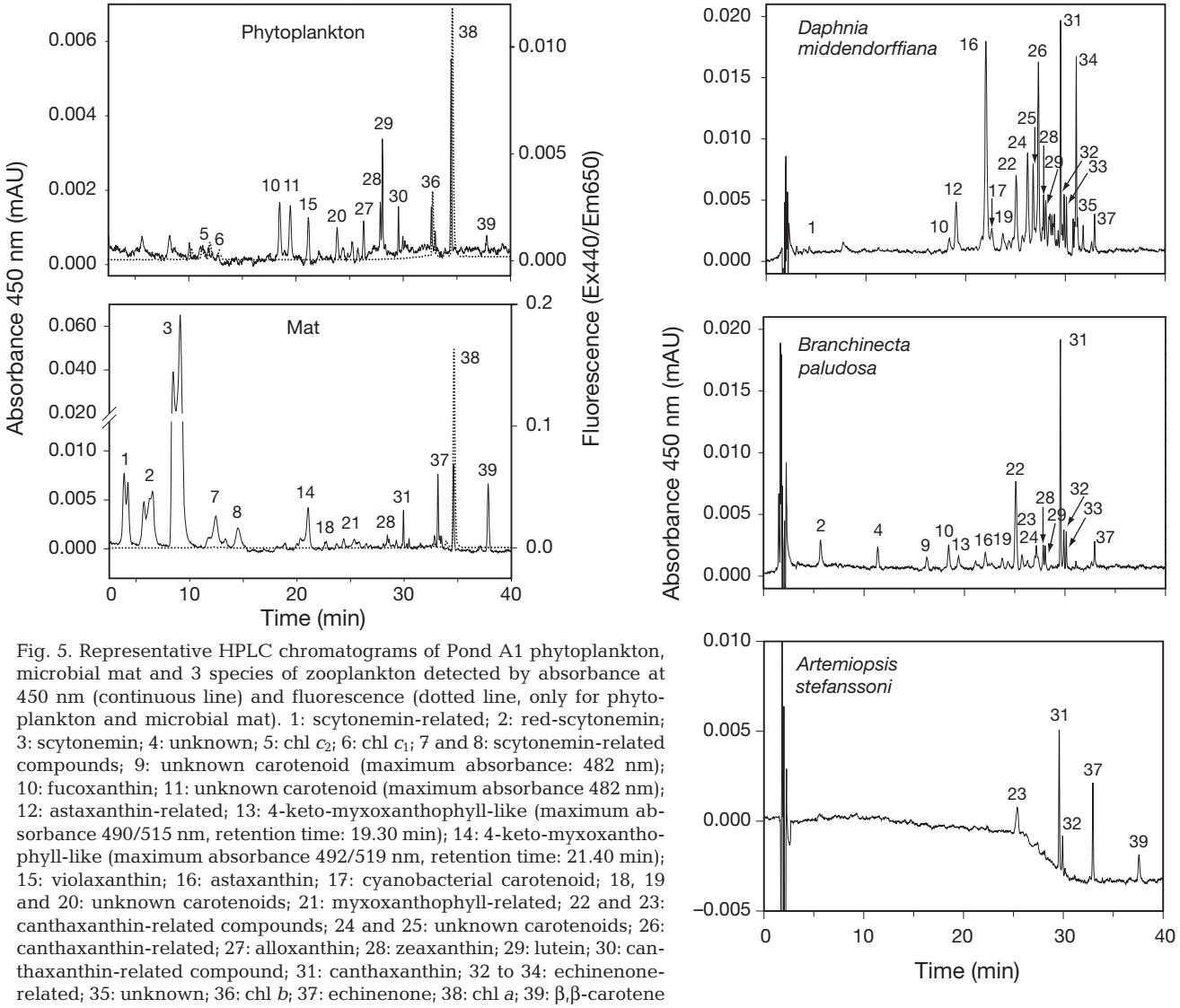


Fig. 5. Representative HPLC chromatograms of Pond A1 phytoplankton, microbial mat and 3 species of zooplankton detected by absorbance at 450 nm (continuous line) and fluorescence (dotted line, only for phytoplankton and microbial mat). 1: scytonemin-related; 2: red-scytonemin; 3: scytonemin; 4: unknown; 5: chl $c_2$; 6: chl $c_1$; 7 and 8: scytonemin-related compounds; 9: unknown carotenoid (maximum absorbance: 482 nm); 10: fucoxanthin; 11: unknown carotenoid (maximum absorbance 482 nm); 12: astaxanthin-related; 13: 4-keto-myxoxanthophyll-like (maximum absorbance 490/515 nm, retention time: 19.30 min); 14: 4-keto-myxoxanthophyll-like (maximum absorbance 492/519 nm, retention time: 21.40 min); 15: violaxanthin; 16: astaxanthin; 17: cyanobacterial carotenoid; 18, 19 and 20: unknown carotenoids; 21: myxoxanthophyll-related; 22 and 23: canthaxanthin-related compounds; 24 and 25: unknown carotenoids; 26: canthaxanthin-related; 27: alloxanthin; 28: zeaxanthin; 29: lutein; 30: canthaxanthin-related compound; 31: canthaxanthin; 32 to 34: echinenone-related; 35: unknown; 36: chl *b*; 37: echinenone; 38: chl *a*; 39: β,β-carotene

Table 3. Scytonemin and carotenoids as average percentage contributions to total lipid-soluble pigments in the phytoplankton, benthic mats and zooplankton of Pond A1. The zooplankton species were *Daphnia middendorffiana*, *Branchinecta paludosa* and *Artemiopsis stefanssoni*

| Pigment | Percent pigments | | | | |
|---|---|---|---|---|---|
| | Phytoplankton | Mat | *D. middendorffiana* | *B. paludosa* | *A. stefanssoni* |
| Scytonemin | 4.6 | 91.5 | 0.2 | 0.5 | 0 |
| Fucoxanthin | 10.4 | 0 | 0.1 | 0.1 | 0 |
| 4-ketomyxoxanthophyll | 0 | 0.3 | 0 | 1.1 | 0 |
| Violaxanthin | 8.8 | 0 | 0 | 0.6 | 0 |
| Alloxanthin | 2.6 | 0 | 0 | 0.5 | 0 |
| Zeaxanthin | 2.7 | 0.1 | 14.4 | 14.4 | 0 |
| Lutein | 19.9 | 0 | 0 | 3.0 | 0 |
| β,β-carotene | 0.7 | 0.5 | 0 | 0.2 | 0 |
| Chl *a* | 13.9 | 3.5 | 0 | 0 | 2.8 |
| Echinenone | 0.7 | 0.8 | 14.5 | 24.3 | 13.6 |
| Canthaxanthin | 2.5 | 0.4 | 45.7 | 44.9 | 70.2 |
| Astaxanthin | 0 | 0 | 9.0 | 0.5 | 0.3 |

The 3 dominant zooplankton species in Pond A1 were all heavily pigmented. The planktonic fairy shrimp *Artemiopsis stefanssoni* was the most notable with its bright pink colour, while the benthic fairy shrimp *Branchinecta paludosa* had more green-brownish shading and the cladoceran *Daphnia middendorffiana* was dark brown or black. All 3 species had abundant concentrations of carotenoids and other photoprotective compounds. *D. middendorffiana* had the highest melanin concentration; *A. stefanssoni* had a high content of MAAs and *B. paludosa* obtained part of its photoprotection from scytonemin (Fig. 4).

The concentration of total carotenoids in *Artemiopsis stefanssoni* (7.9 µg $mg^{-1}$) was 7 times higher than the values measured for *Daphnia middendorffiana* (1.0 µg $mg^{-1}$) and *Branchinecta paludosa* (1.1 µg $mg^{-1}$). Canthaxanthin was the main carotenoid in all 3 species, and in *A. steffanssoni* contributed >70% of total measured pigments (Fig. 5, Table 3). The concentrations of canthaxanthin, echinenone and zeaxanthin were high in *B. paludosa* (0.5, 0.3 and 0.2 µg $mg^{-1}$, respectively). The dominant carotenoids in *D. middendorffiana* were the carotenoids astaxanthin, canthaxanthin, echinenone and zeaxanthin (Table 3), which are commonly found in many animals.

## DISCUSSION

All of the zooplankton populations that we sampled in northern Canadian and Alaskan ponds were heavily pigmented. The existence of such a high pigment content in zooplankton is in agreement with previous studies that were restricted to specific pigment types in high-latitude communities (see Rautio et al. 2008 and references therein), and this biological characteristic likely offsets the high risk of UV exposure and damage in these low-CDOM waters (see Molot et al. 2004). A broad range of coloured and non-coloured UV photoprotectants were present in the 12 zooplankton species, including carotenoids, scytonemins, melanins and MAAs. The nature and concentration of these compounds varied among zooplankton taxa and between subarctic and arctic regions, suggesting there are differences in UV photoprotection among polar zooplankton. To our knowledge, this is the first time that all known photoprotective compounds have been quantified in individual species of zooplankton. This is also the first time that scytonemin, a cyanobacterial UV-screening pigment, has been detected in zooplankton, and the first time that MAAs are reported in high concentrations in the genus *Daphnia* and in fairy shrimps.

### Range of pigments and MAAs in high-latitude zooplankton

The occurrence of scytonemin is thought to be restricted to cyanobacteria (Garcia-Pichel & Castenholz 1991), but we detected it in 2 species of zooplankton, the cladoceran *Daphnia middendorffiana* and the fairy shrimp *Branchinecta paludosa*. Both of these species were obtained from lakes with abundant cyanobacteria mats where scytonemin was the dominant pigment. The occurrence of scytonemin in arctic zooplankton is the result of the ecosystem dominance of benthic cyanobacteria in many lakes and ponds in the Arctic (Vincent 2000, Bonilla et al. 2006, 2009), and the benthic feeding strategy of some high-latitude zooplankton species (Rautio & Vincent 2006, 2007). The cladoceran and the fairy shrimp likely ingested and absorbed scytonemin when grazing on the benthos or on resuspended benthic material. The absence of chl *a* in these species indicated that scytonemin was incorporated in the zooplankton tissue rather than being present only in the gut, where it would solely reflect the recent feeding history of the animal. The absorption maximum of scytonemin is in the UVA range, at around 380 nm, but it also significantly absorbs at lower wavelengths and is therefore an efficient broadband protection against both UVA and UVB (Garcia-Pichel & Castenholz 1991).

Carotenoids include over 600 natural lipid-soluble pigments that are synthesized by algae, bacteria, fungi and plants. We detected carotenoids in all species of zooplankton, with a total concentration ranging from 0.1 µg $mg^{-1}$ in the cladoceran *Polyphemus pediculus* to 12.4 µg $mg^{-1}$ in the fairy shrimp *Artemiopsis stefanssoni*. These were composed of 18 specific carotenoids, including alloxanthin, fucoxanthin, lutein, violaxanthin and zeaxanthin. However, most of these pigments were either present in low concentrations or were not detected at all, but were rather transformed to the principal animal keto-carotenoid (astaxanthin) or to echinenone and canthaxanthin (Goodwin 1984, Partali et al. 1985, Miki 1991), which were the most abundant carotenoids encountered in zooplankton. Echinenone and canthaxanthin in zooplankton may, hence, be original algal pigments, reflecting feeding on benthic cyanobacteria, but they could also be animal pigments converted from β,β-carotene, fucoxanthin, peridinin, or other phytoplankton carotenoid precursors (Goodwin 1984, Kleppel & Lessard 1992). Carotenoids provide little direct protection from short-wavelength solar radiation, but they mediate against UV damage by quenching the toxic reactive oxygen species that are produced during UV exposure (Hairston 1979a, Vincent & Neale 2000).

Melanins are the only photoprotective pigments that animals can synthesize without algal or plant precursors. They absorb mostly in the UV range and functionally act as a sunscreen, as well as scavenging reactive oxygen species that can damage cellular constituents, including DNA. Black eumelanin is a common pigment in artic *Daphnia* spp. (Hebert & Emery 1990, Hessen & Sørensen 1990, Hobæk & Wolf 1991, Rautio & Korhola 2002, Hansson et al. 2007), but has not been reported for other zooplankton. In the present study, we measured melanin in 4 species of zooplankton species (*Daphnia middendorffiana*, *Scapholeberis mucronata*, *Artemiopsis stefanssoni* and *Branchinecta paludosa*). Visually, melanin was mostly present in the dark dorsal carapace of *D. middendorffiana* and on the black ventral side of *S. mucronata*, reflecting the swimming position of these cladocerans and hence their exposure to solar radiation. The latter species is often found feeding on the neuston at the surface of northern ponds, where strong UV-screening protection may be especially advantageous. Melanin was also detected in 2 fairy shrimps *A. stefanssoni* and *B. paludosa*, although they lacked the characteristic dark colouration that is usually associated with melanised zooplankton. Some of the detected fairy shrimp melanin was probably eumelanin in the animal's eye and in the brown eggs that some of the individuals may have been carrying. Pheomelanin was most probably present in the fairy shrimp body. Pheomelanin is a yellow to red material elsewhere found in red hair and red feathers (Ozeki et al. 1995). It also absorbs in the UV range, and its separation from eumelanin was not possible with the method used here (Ozeki et al. 1995).

MAAs were detected in 5 of the 6 zooplankton taxa analysed, with values ranging from 0.02 µg $mg^{-1}$ in the calanoid *Hesperodiaptomus arcticus* to 37 µg $mg^{-1}$ in the pelagic fairy shrimp *Artemiopsis stefanssoni*. This is the first time MAAs have been reported in high concentrations in the genus *Daphnia* (1 µg $mg^{-1}$). The benthic fairy shrimp *Branchinecta paludosa* was the only species that did not have any MAAs, despite the presence of MAAs in the benthic mats that were potentially available to this species in its food. Worldwide, MAAs are the most commonly encountered UV-absorbing compounds in aquatic organisms and are found, for example, in UV-exposed phytoplankton and copepods (Sommaruga & Garcia-Pichel 1999, Tartarotti et al. 2001, 2004, Moeller et al. 2005, Hansson et al. 2007, Hylander et al. 2009b) and in arctic benthic and planktonic algae (Bonilla et al. 2005, Hylander et al. 2009b). MAAs have their absorption maxima in the UV range, between 310 and 360 nm, and provide protective screening against radiation before it penetrates the cell. Moeller et al. (2005) showed that the laboratory-raised copepod *Leptodiaptomus minutus* used MAAs as their main UV-photoprotective strategy, with contents ranging from 0.5 to ~2 µg $mg^{-1}$. This is within the same range of values that we measured for high-latitude *L. minutus*, although for some populations we measured MAAs of up to 10 µg $mg^{-1}$. The highest *L. minutus* MAA concentration was measured in the pond where the phytoplankton also had the highest MAAs among sites (4.5 µg $l^{-1}$). We detected even higher concentrations (up to 37 µg $mg^{-1}$) of MAAs in the fairy shrimp *A. stefanssoni*. The distribution of this species was restricted to the DOC-poorest ponds that were <1 m in depth, and the species was, therefore, highly exposed to UV radiation. Recently, Hylander et al. (2009b) reported a latitudinal pattern in copepod MAA concentration, with low concentrations in Subarctic and temperate copepods (1.0 and 1.4 µg $mg^{-1}$) compared to dry-temperate copepods (up to 58 µg $mg^{-1}$). The MAA concentrations in copepods in the present study were in generally at the low end of this range, but showed high variation between 0.02 and 10 µg $mg^{-1}$, depending on species and site, perhaps because ponds are more heterogeneous habitats than the lakes that were studied by Hylander et al. (2009b).

### Taxonomic influence on pigmentation

Zooplankton species often differ in their tolerance to UV radiation (Leech & Williamson 2000), which suggests that taxonomic differences may play a role in the degree of UV protection via screening and quenching compounds. We observed pronounced differences in pigment concentration among zooplankton species in communities that were apparently exposed to the same UV radiation regime. Copepods had astaxanthin as the main pigment in their body, and their concentration of total carotenoids was significantly higher than that of cladocerans (average ± SE: 2.6 ± 1.4 µg $mg^{-1}$ and 1.0 ± 0.5 µg $mg^{-1}$; $F = 8.8$, $p = 0.007$). This is in accordance with the recent study of Persaud et al. (2007), in which copepods had a total carotenoid concentration of 3.0 µg $mg^{-1}$, and cladocerans, 0.02 µg $mg^{-1}$. Several other studies have also reported higher carotenoid concentrations in copepods relative to cladocerans (Hairston 1979b, Partali et al. 1985, Hessen & Sørensen 1990, Hansson 2004) and have related this to variation in the susceptibility to different wavelengths, food compositions and availabilities. Species-specific differences have also been reported for MAAs. The cladoceran genera *Daphnia*, *Bosmina* and *Chydorus* appear to lack or have only a trace amount of MAAs, while copepods often have higher concentrations of these compounds (Tartarotti et al. 2001, 2004, Persaud et al. 2007). This pattern was also observed in the present study. Hansson et al. (2007) showed that copepods and cladocerans

use avoidance and protection strategies differently, which may explain differences in pigmentation. Copepods rely mainly on accumulating UV protectants, which accounts for their higher pigment concentration, whereas cladocerans also show behavioural responses (vertical migration). In the present study, however, most of the sites were <1 m deep and vertical migration was not observed.

Variability in the pigmentation at a finer scale of taxonomic resolution could also exist. The 3 fairy shrimp species in our study had highly variable concentrations of total carotenoids, ranging from low in *Polyartemiella hazeni* (0.6 ± 0.2 µg $mg^{-1}$) to intermediate in *Branchinecta paludosa* (1.4 ± 0.4 µg $mg^{-1}$) and to high in *Artemiopsis stefanssoni* (4.3 ± 2.3 µg $mg^{-1}$), but they were all dominated by canthaxanthin and also had similar proportions of other specific carotenoids in their bodies. Zagarese et al. (1997) showed similar differences in the UV tolerance of 3 species of *Boeckella* calanoids. These differences depended on the UV exposure in the animal's natural habitat and also on the use of different protection strategies (avoidance versus photoreactivation). Pigmentation alone may not, therefore, be a good measure of the animal's UV exposure or its tolerance to UV radiation, but should be used in combination with other indices of physiological status and behaviour.

### The role of feeding in photoprotection

The predatory cladoceran *Polyphemus pediculus* had the lowest concentration of carotenoids in the present study. It does not graze on phytoplankton, the dominant source of carotenoids to zooplankton, therefore explaining the low concentration of these compounds and further supporting the role of algal food in zooplankton photoprotection. The recent reports of Andersson et al. (2003) and Sommer et al. (2006) have also shown that phytoplankton community composition and biomass have strong effects on the production of carotenoids in calanoid copepods. Copepods that grazed on a diverse phytoplankton community dominated by chlorophytes, dinoflagellates and diatoms with thin silica frustules had the highest astaxanthin production (Andersson et al. 2003).

In Pond A1, phytoplankton represented 0.2% of the total phototrophic biomass and benthic biomass dominated the total standing stocks, yet carotenoid concentrations in the pelagic feeder *Artemiopsis steffanssoni* were an order of magnitude higher than those of *Branchinecta paludosa* and *Daphnia middendorffiana*, which grazed on phytobenthos (Rautio & Vincent 2006). It is likely that phytoplankton is a higher quality food than the coherent benthic mats, which are mixed with high amounts of detritus, therefore offsetting the effects of low biomass. However, as differences in pigmentation also result from species-specific abilities to assimilate carotenoids and from pigment transfers in the zooplankton body, single-species experiments would be required to accurately quantify the role of grazing in these zooplankton.

The benthic microbial communities, despite their likely lower quality and poorer accessibility, may still contribute to zooplankton photoprotection. Some zooplankton are able to graze directly on phytobenthos (Hansson & Tranvik 2003, Rautio & Vincent 2006), which gives them access to additional UV photoprotectants. We hypothesized that a benthic algal diet with cyanobacterial prevalence should be registered in the zooplankton by the presence of exclusively prokaryote pigments such as myxoxanthophylls and scytonemin (Bonilla et al. 2005). Our results supported this hypothesis, and some of the pigment analyses pointed to feeding on benthic mats; for example, the presence of 4-keto-myxoxanthophyll in *Branchinecta paludosa* (Fig. 5, Table 3) indicated that benthic filamentous cyanobacteria made up a part of the diet in this species. The presence of scytonemin in *B. paludosa* and *Daphnia middendorffiana* also implies that these species feed on benthic cyanobacteria.

To conclude, the present study indicates that freshwater zooplankton have multiple UV-protection strategies. The diverse sets of UV-screening compounds provide a broadband strategy for coping with the high irradiance present in clear arctic water bodies, with additional protection conferred by compounds that quench reactive oxygen species. A similar broadband strategy has recently been identified in the benthic microbial assemblages of high arctic ice shelves, where the biota face multiple stressors of high irradiance, variable salinity, desiccation and low temperatures (Mueller et al. 2005, Bonilla et al. 2009). Our results point to the importance of measuring multiple pigments and MAAs when evaluating the photoprotective strategies of zooplankton against UV radiation. These results also show the disparate trophic origin of carotenoids, and that some of these pigments may provide information on zooplankton feeding relationships.

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