# UV-B susceptibility and photoprotection of Arctic *Daphnia* morphotypes

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UV-B tolerance and susceptibility of high Arctic morphotypes of the *Daphnia pulex*/ *D. tenebrosa* complex were assessed by *in situ* experiments at Ny-Ålesund, Svalbard (79°N). Animals from local ponds were exposed to ambient light plus additional UV-B from lamps in a greenhouse facility. Taxonomic affinities did not appear as major determinants of UV susceptibility, but a major difference in UV-B tolerance was seen between morphotypes with pigmented carapaces and those without, the latter being far more susceptible. Assays on levels of carotene and the anti-oxidant enzymes catalase and superoxide dismutase did not reveal clear-cut differences between populations, and could not account for the higher tolerance in pigmented populations. Levels of glutathione transferase were higher in the transparent population, however. In the absence of blue light and UV, laboratory reared animals did not reconstitute their carapace melanization after moulting, indicating that short-wave light is the cue for melanin synthesis. Tests on melanized individuals and individuals of the same population reared indoors through 1–2 moults supported the major role of melanin for UV protection. Periods with high UV exposure during hatching of ephippia could induce shifts in morphotype or clonal dominance.

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## Introduction

Like all species in the high Arctic, freshwater zooplankton face short growing seasons and low temperatures. While low light levels constrain primary production for most of the year, the short summer is characterized by a great deal of light in the shallow, clear freshwaters. Though most localities are still ice-covered during the period with maximum solar irradiance and high albedo, and summers are typically foggy, high levels of short-wave radiation appear to be potentially of high ecological relevance for Arctic freshwater communities (Hebert & McWalter 1983; Hebert & Emery 1990; Hessen 1996). Changes in incident radiation could dramatically alter the productivity and community structure of these vulnerable ecosystems. There is recent evidence that, like the Antarctic "ozone hole", the Arctic and sub-Arctic stratospheric ozone layer has also decreased (Kerr & McElroy 1993; Madronich et al. 1995), causing increased levels of UV light, which could particularly affect biota in shallow ponds. Linked to drought and warming, reductions in dissolved organic carbon (DOC) – the major determinant of short-wave radiation levels in lakes (Scully & Lean 1995) – may impact UV attenuation in freshwaters (Schindler et al. 1997) and profoundly affect UV induced mortality in zooplankton (Williamson et al. 1996).

Inhabiting the shallow ponds and small lakes in the Arctic and sub-Arctic, the crustacean zooplankter *Daphnia* spp. comprise a species complex with astonishing clonal complexity and genetic variability (Weider, Beaton et al. 1987; Hobæk & Wolf 1991; Colbourne et al. 1998). Most remarkable is the co-existence of morphotypes with and without carapace melanization (Hebert & Emery 1990; Zellmer 1995; Hessen 1996). Highly light-exposed *Daphnia* populations

*Table 1.* Major properties and taxonomic affinities and pigmentation scores for *Daphnia* in the four localities. Approximate maximum depth given in m; UV-abs = spectrophotometrical UV absorbance at 273 nm; Tot. P = total phosphorus as $\mu g l^{-1}$. For pigmentation (Pig.), a score of 0 meant no visually detected pigmentation; 1 = weak pigmentation (shading) on dorsal part of head and antennae; 2 = clearly visible pigmentation on the dorsal carapace; 3 = pronounced dark dorsal pigmentation.

| Locality | Max depth | UV-abs | Tot. P | Pig. | Tax. affinity |
|---|---|---|---|---|---|
| 1 Brandal | 3.0 | 0.037 | 14 | 0 | *D. tenebrosa* |
| | | | | 2 | *D. tenebrosa* |
| 2 Solvatnet | 1.0 | 0.125 | 62 | 1 | *D. tenebrosa* |
| | | | | 2 | *D. pulex/pulicaria* |
| 3 Øtvretjern | 3.5 | 0.033 | 32 | 0 | *D. pulex/pulicaria* |
| 4 Nedretjern | 0.3 | – | – | 3 | *D. pulex/pulicaria* |

of various species and clones are dark-coloured (Luecke & O'Brien 1983; Wolf & Hobæk 1986), a property apparently linked to light exposure (Siebeck 1978; Hebert & McWalter 1983; Hebert & Emery 1990; Hessen 1996). Melanic clones differ genetically from non-melanic clones (Weider, Beaton et al. 1987; Hobæk & Wolf 1991) and are almost uniquely endemic to alpine localities or Arctic ponds. Within a region, these clones often occur in the clearest ponds and are replaced by non-melanic clones when vegetation cover increases or water transparency decreases (Hebert & Emery 1990; Hessen & Sørensen 1990; Hobæk & Wolf 1991).

Highly light-exposed zooplankton are commonly bright red, due to high tissue levels of carotenoids. Carotenoids play a dual role as physical light screens and major anti-oxidants. But while carotenoids help protect copepods from short-wave light (Hairston 1978, 1979; Ringelberg et al. 1984), they are apparently less important in Cladocera (Hessen & Sørensen 1990; Hessen 1994). Levels of other major anti-oxidants, or radical "scavengers" such as ascorbic acid, catalase (CAT), glutathione transferase (GST) and superoxide dismutase (SOD), have scarcely been reported for Cladocera; the presence of potentially UV absorbing compounds collectively labelled mycospirinelike amino acids (MAAs) has also been little studied.

Here we report the results from testing of UV susceptibility of various *Daphnia* populations, which were characterized genetically and scored for pigmentation and some major anti-oxidants.

# Material and methods

During late July and early August 1996 and 1997, *Daphnia* spp. were sampled from freshwater localities around Ny-Ålesund (79°N), Svalbard. Samples were stored on EtOH or frozen (liquid nitrogen) for later analysis of taxonomic affinities, pigmentation and selected anti-oxidants. Most localities are shallow (maximum depths ranging from ca. 0.3–3 m), oligotrophic and surrounded by sparse tundra vegetation. On the basis of a 1996 survey as well as previous data (Weider & Hobæk 1994; Hessen 1996), four populations were selected for a closer examination in 1997 (Table 1). Localities 1, 3 and 4 had low productivity and algal biomass (0.5–1.5 $\mu g$ Chl.a $l^{-1}$); locality 2 was somewhat influenced by surrounding bird colonies and had Chl.a levels of nearly 5 $\mu g$ Chl.a $l^{-1}$.

## Pigmentation scores

Pigmentation was visually examined and scored for carapace melanin on a scale from 0 (no pigmentation) to 3 (darkest pigmentation) (Table 1). Simple tests on the melanin synthesis under various light climates were performed by incubating 10 melanized individuals from locality 1 in dark bottles (wrapped in aluminium foil), glass bottles, quartz bottles with Mylar® film, and quartz bottles for 6 days in a shallow pond. Then pigments were visually scored for all individuals. A simpler test was performed in the laboratory, where pigmented individuals were kept for 4–5 days (through at least one moult) in dim light, and fed with the green algae *Selenastrum capricornutum*.

## Taxonomic affinities

Rough taxonomic affinities were assessed on populations 1 and 2 (both dark and transparent individuals), 3 (transparent), and 4 (dark). Genomic DNA was isolated from approximately five melanic and non-melanic individuals from each population. Whole daphniids were minced and digested overnight at 37°C in 1 ml of lysis buffer (1 M Tris, 5 M NaCl, 10% SDS and 0.5 M EDTA, pH 8.0, 0.01% b-mercaptoethanol) and 20 ml of proteinase K (10 mg/ml). Following incubation, the lysate was treated with DNase free RNase at 37°C for 1 hour. The samples were then extracted

once with 70% phenol/chloroform (Saveen biotech AB) and twice with chloroform. The DNA pellet was precipitated with 2 volumes of 96% ethanol, dried and resuspended in sterile $dH_2O$.

PCR amplification of a 603 bp segment of the mtDNA 12S region was performed using the following primers: 5′-ATGCACTTTCCAGTA-CATCTAC-3′ and 5′-AATCGTGCCAGCCGT-CGC-3′. A denaturation step at 95°C for 5 min was followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and extension for 1 min at 72°C. The PCR products were sequenced on a Vistra DNA Sequencer 725 using Vistra system Thermo Sequenase sequencing kit (Vistra DNA system, Molecular Dynamics and Amersham Life Science) with the primer 5′-GTGAAATTTTGTTGATAAGGTG-3′ labelled with Texas-red.

The DNA segment sequenced corresponds to nucleotides 179–773 of the *D. pulex* 12S rRNA gene (Van Raay & Crease 1994). The sequences were aligned to the 12S sequences of several species from the *pulex* and *longispina* group of the subgenus *Daphnia*, as well as two species from the subgenus *Ctenodaphnia*. The alignment of sequences was performed using the SeqApp 1.9a sequence editor (Gilbert 1992). Maximum parsimony analysis was conducted using PAUP 3.1.1. (Swofford 1993) with a branch and bound search and bootstrapping using 100 replications.

### *Anti-oxidant assays*

Levels of anti-oxidants were analysed for populations 1 (pigmented individuals only), 2, 3 and 4. Collected animals from net hauls were immediately frozen in liquid nitrogen and kept in a freezer (−80°C) until time of enzyme assays. 20–40 animals were homogenized in 1 ml of ice-cold 50 mM K-phosphate buffer pH 7.7 (SOD assay) containing 1 mM EDTA and 0.1% Triton X-100 (CAT and GST). Supernatants (1000 g for 20 min at 4°C) of homogenates were used directly as enzyme source. CAT activity was basically determined as described by Claiborne (1985) using 20 mM $H_2O_2$ as substrate. One unit (U) is defined as μmol $H_2O_2$ decomposed $min^{-1}$, at pH 7.0 and 30°C. GST activity toward CDNB was measured as described by Habig et al. (1974) at pH 6.5 in a thermostatically-controlled (30°C) Beckman DU 62-spectrophotometer. One unit is defined as nmol CDNB conjugated $min^{-1}$. SOD activity was assayed by inhibition of xantine/xantine oxidase generated reduction of nitroblue tetrazolium (Spitz & Oberley 1989). One unit is defined as the amount of protein producing 50% inhibition of the reaction. Carotenoids were determined according to Hairston (1978) and Hessen & Sørensen (1990). All determinations were performed in duplicate or triplicate. Protein concentrations were determined according to Bradford (1976), using dye reagent from Bio-Rad and with bovine serum albumin as standard.

### *UV-B tolerance*

UV-B tolerance tests were performed in Ny-Ålesund's greenhouse facilities. To compensate for loss of UV through the glass ceiling, additional UV light was provided by two 15 W Wilber-Lourmat lamps with peak intensity at 312 nm (range 280–380 nm). Relative doses were assessed by a Vilber-Lourmat VLX-3W Radiometer with peak sensitivity at 312 nm. To control for indoor light relative to ambient light, light was measured with a LICOR 1800 spectroradiometer.

Populations used for UV tolerance assessment were sampled by net hauls, brought immediately to the greenhouse, and were gently pipetted into 50 ml beakers (water from "own" locality). Only adults (females larger than minimal size for egg production) were used. For each test, 10 individuals were placed in each of the 50 ml beakers, which were covered with cellulose acetate filters (cut off at 290–300 nm) to screen off potential UV-C emitted by the lamps. Controls (1–3 for each population) were covered with a Mylar® sheet (cut off at 320 nm) for selectively screening of UV-B, but otherwise corresponded to ambient light. Both filter types were replaced after each experiment. Each population was exposed in triplicate for ca. 48 h with 12 h on/off for the UV lamps. Immobilized individuals (i.e. lying on the bottom) were counted every 6–12 h during the experiments.

Since experimental conditions (i.e. temperature, cloud cover, ambient irradiance) could vary somewhat within and among the various exposure periods, experimental efforts focussed on pairwise comparison between animals with different pigmentation. Three experimental runs were performed. The first tested pigmented animals from two localities, but where PAR light for photorepair was either provided (natural light) or excluded by placing the animals in a dark box after each exposure period. This experiment was performed under two slightly different light levels of UV-B

*Table 2.* Specific activities[1] (±SD) of catalase (CAT), glutathione transferase (GST), superoxide dismutase (SOD) and carotenoids (CAR) in *D. pulex* from four different ponds in the vicinity of Ny-Ålesund.

| Locality | Animal size[2] (μg dw $ind^{-1}$) | CAT (U $mg^{-1}$) | GST (U $mg^{-1}$) | SOD (U $mg^{-1}$) | CAR (μg $mg^{-1}$ dw) |
|---|---|---|---|---|---|
| 1 Brandal | 59 | 78.1 ± 6.2 (n = 3) | 69.6 ± 5.8 (n = 3) | 207 ± 10.8 (n = 3) | 0.48 ± 0.013 (n = 3) |
| 2 Solvatnet | 111 | 89.6 ± 5.0 (n = 2) | 72 ± 0.6 (n = 2) | 157 ± 13.6 (n = 3) | 0.24 ± 0.06 (n = 2) |
| 3 Øvretjern | 126 | 61 (n = 1) | 174 (n = 1) | 190 ± 19.8 (n = 3) | 0.25 (n = 1) |
| 4 Nedretjern | 143 | 111 (n = 1) | 90.9 (n = 1) | 155 ± 26.6 (n = 3) | 0.44 (n = 1) |

[1]Supernatant (1000 g) of whole body homogenates of 20–40 adult animals were used as enzyme sources. Carotenoid concentration was calculated by absorbance of ethanol extracts measured at 474 nm. Units of activities: Catalase (μmol $H_2O_2$ decomposed $min^{-1}$ mg $protein^{-1}$), GST (nmol CDNB conjugate $min^{-1}$ mg $protein^{-1}$), carotenoids (μg $mg^{-1}$ dw), SOD (amount of protein necessary to decrease the rate of NBT reduction to 50%).
[2]Average of at least 10 animals.

(integrated intensity 300–320 nm: 1.4 and 1.1 W $m^{-2}$). For the two other experiments, natural light and UV-B were provided. The second tested the difference between transparent and pigmented populations. For this test, transparent and pigmented individuals from locality 1, as well as the neighbouring transparent and pigmented populations from localities 3 and 4, respectively, were chosen. The third test was performed on pigmented individuals from locality 1 that were kept indoors for five days, passing through 1–2 moults and thus becoming transparent (they did not reconstitute their carapace melanization in the absence of short-wave radiation). These transparent individuals and their pigmented conspecifics from the pond were then compared. Admittedly, these individuals were not raised under identical conditions, but those individuals raised for 5 days under laboratory conditions produced larger broods than pond animals and there was no mortality in control chambers without UV exposure. Absence of UV light for 3 days may have deactivated various UV protective mechanisms, however.

## Results

The rough categorization of taxonomic affinities revealed two major groups, the *D. pulex*/*D. pulicaria* group and the *D. tenebrosa* group according to the new taxonomic designation (Colbourne et al. 1998). Our method was insufficient for a closer resolution, but judging from the observed differences of 1–2 mutations relative to published sequences, clonal richness was likely higher than detected by this first screening. Pigmented morphs were detected in both groups (Table 1).

The low ambient temperatures (9°C) were insufficient for most animals to complete a moulting cycle during the 6 day outdoor experiment; only a few carapaces were found, thus most animals had retained their initial pigmentation. In the dark bottle, 20% of the adult animals and all neonates had become hyaline, while none became so under the other light regimes: blue light may also be a sufficient stimulus for maintaining melanin synthesis. In contrast, all individuals placed in the lab (18°C) moulted within 2 days; some passed through 2 moults during the 5 day duration. All completely lost melanization after moulting.

The screening of major anti-oxidants did not reveal clear-cut differences for catalase (CAT) among populations 1, 2 and 3, but markedly higher levels were found in the highly pigmented animals of population 4 (Table 2). Levels of superoxide dismutase (SOD) in individuals from locality 1 were significantly higher (one-way ANOVA) from populations of localities 2 and 4 ($p < 0.005$ and $p < 0.05$, respectively), but not from locality 3, while for glutathione transferase (GST), the transparent population from locality 3 had more than twice as high enzyme activity as the other populations (174 vs. 70–91 units $mg^{-1}$). Levels of pooled carotenoids (CAR) were highest in the pigmented populations from locality 1 and 4, but were generally very low in all populations. Due to

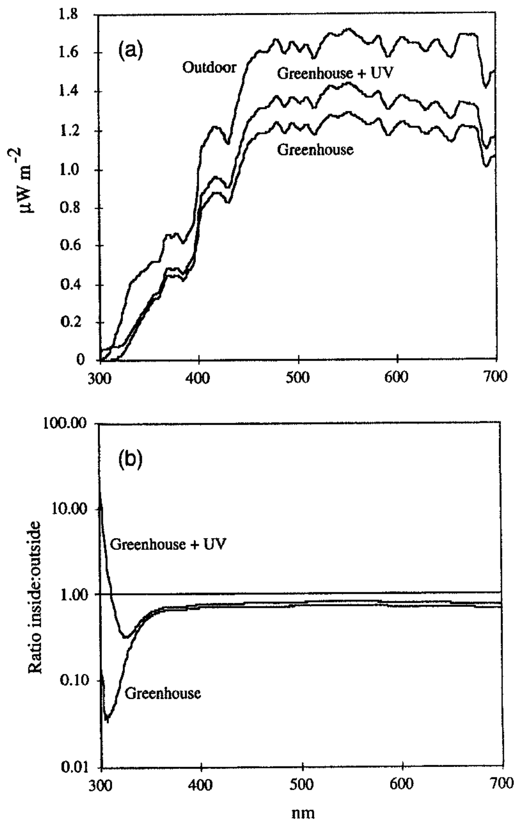


*Fig. 1.* (a) Spectral distribution and intensities over the 300–700 nm range measured under nearly identical conditions outdoor and in the greenhouse with and without additional UV from lamps. (b) Deviations from outdoor light is provided in lower panel.

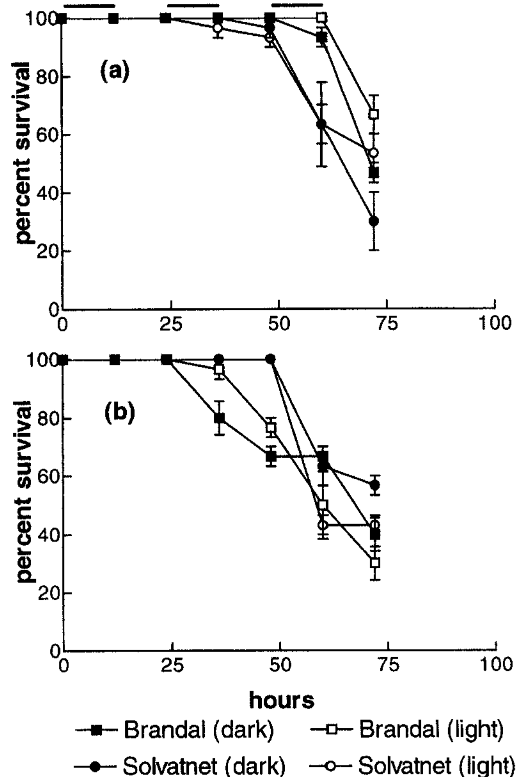


*Fig. 2.* Survival of pigmented individuals from localities 1 (Brandal) and 2 (Solvatnet) when kept under natural light or in the dark after irradiation periods. Integrated light intensities for PAR (400–700 nm) were ca. 3.4–3.6 $*10^2$ W $m^{-2}$ (inside greenhouse) in both experiments (a and b), whereas integrated UV-B (300–320) were 1.1 W $m^{-2}$ in (a) and 1.4 W $m^{-2}$ in (b). Periods with UV-B exposure indicated as horizontal lines above.

small sample sizes, statistical testing of differences among populations was performed for SOD only; a direct comparison would also be obscured by the fact that average size of individuals among populations varied from 59 to 143 μg dry weight $ind^{-1}$, a factor that could have influenced enzymatic specific activities (Borgeraas & Hessen unpubl. ms.). With the exception of GST, there seemed to be no consistent pattern with higher levels of anti-oxidants in the transparent population. In fact, for SOD and CAR, the highest levels were found among pigmented populations.

The light climate in the greenhouse was fairly similar to ambient (outdoor) light conditions for wavelengths >350 nm (Fig. 1a). For the range 315–350 nm, UV absorption by the greenhouse glass yielded slightly lower intensities inside than outside, while the lamp caused increasing deviations from outdoor UV towards 300 nm (Fig. 1b). The minor difference between the runs with and without lamps in the greenhouse was due to a minor time lag between the measurements, and thus small differences in total light intensities. Although the lamp emitted higher intensities for wavelengths <310 nm, outdoor integrated UV-B (300–320 nm) was still ca. 20% higher than under lamp exposure. While UV-A and light for photorepair in the greenhouse resembled outdoor conditions, the spectral qualities of the lamp deviated markedly for outdoor conditions in the low UV-B range. Typical values for integrated PAR light (400 –700 nm) ranged from 3.6 $*10^2$ W $m^{-2}$ (inside greenhouse) to 4.7 $*10^2$ W $m^{-2}$ (outdoor) at noon under a bright sky.

Survival of pigmented individuals from local-

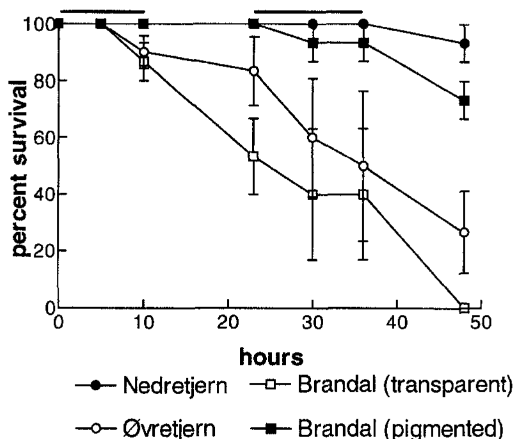


*Fig. 3.* Survival of pigmented and transparent individuals from loc. 1 (Brandal), as well as pigmented and transparent individuals from loc. 4 (Nedretjern) and 3 (Øvretjern), respectively. Integrated light intensities as for Fig. 2a.

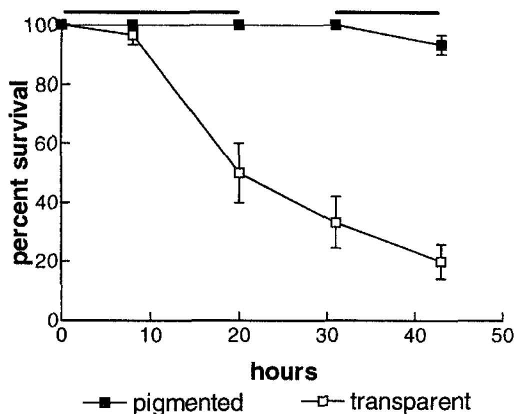


*Fig. 4.* Survival of previously pigmented animals that became transparent in the laboratory, and their pigmented conspecifics, both from loc. 1. Light intensities as for Fig. 2a.

ities 1 and 2 revealed no marked difference ($p > 0.5$, log rank test on survival for all tested pairs), whether they were kept in natural light or in the dark after irradiation periods (Fig. 2). Lower intensities improved survival somewhat. Comparing pigmented individuals and transparent individuals from locality 1, as well as pigmented and transparent individuals from localities 4 and 3, respectively, revealed marked differences in survival under UV exposure (Fig. 3). This was confirmed by the test with previously pigmented animals that became transparent in the laboratory, in contrast to their pigmented conspecifics not kept in the laboratory (Fig. 4). Pigmented animals from locality 1 had significantly higher survival than their transparent conspecifics ($p < 0.0001$, log rank test), and both pigmented and transparent individuals from locality 1 yielded significantly higher survival than the transparent individuals of locality 3. The low death rates of the strongly pigmented individuals of locality 4 did not allow for a conventional log rank test. Comparing last date survivors, by one-way ANOVA (Tukey Kramer), indicated that survival of this population was significantly higher ($p < 0.01$) than the hyaline populations of locality 1 and 3, but not significantly higher than the pigmented individuals of locality 1.

Susceptibility to UV among hyaline individuals was indicated by their vertical distribution. Among the strictly transparent population of locality 3, all animals were concentrated at or close to the sediment surface during bright days (hindering sampling by net hauls); animals were evenly distributed in the water column on cloudy days. Among pigmented animals from localities 1 and 2, the pattern was less clear, yet the main distribution at noon during bright sunshine was always deeper than on cloudy days, and the hyaline morphotype was always found at deeper layers during daytime. The heavily pigmented population from the shallow locality 4 was always evenly distributed through the 20 cm deep water column.

## Discussion

Our results suggested some variability in susceptibility between pigmented populations, but failed to show any strong effect of post-irradiation light repair, in contrast to the findings of Siebeck & Böhm (1994). The laboratory experiments demonstrated the lower UV-B susceptibility of melanized *Daphnia*, supporting the findings of Hebert & Emery (1990), Zellmer (1995) and Hessen (1996), and the prediction that heavily melanized individuals in the shallowest locality are most tolerant to UV (Fig. 3). These findings should be judged with some caution due to the low number of replicates. The pigmented animals from localities 1 and 2 showed fairly similar responses to the UV treatments, although *Daphnia* from the former location were slightly more pigmented. Locality 1 had lower algal biomass and higher transparency compared with locality 2 (cf. Hessen 1996). On the

other hand, this pond offered a larger depth refugium.

The role of UV light as a mediator of species or clonal replacements is not settled. In scenarios of increased UV radiation, owing to reduced DOC levels or reduced ozone layer, pigmented species or clones would probably have a competitive advantage over transparent relatives. Convergent evolution in many Arctic *Daphnia* lineages with regard to properties such as carapace melanization (Colbourne et al. 1998; Weider, Hobæk et al. 1999) renders the outcome of enhanced UV on species or clonal distribution hard to predict. The role of anti-oxidants and other means of UV protection also needs further scrutiny.

When released from exposure to shorter wavelengths, melanin production normally ceases, suggesting that melanin synthesis is counter-selected in the absence of UV. Repeated melanin synthesis may be energetically unfavourable since the melanin has to be resynthesized after each moult (Hebert & Emery 1990). Pigmented individuals seem to be competitively inferior to hyalines (Weider 1987; Hessen 1996). Specific melanin content may show strong clonal and seasonal variability. Hobæk & Wolf (1991) found that melanin constituted no more than ca. 0.03 $\mu g\ mg\ DW^{-1}$ in alpine *Daphnia longispina*, tenfold less than that Hebert & Emery (1990) reported for sub-Arctic *D. pulex*. Even with a maximum estimate of 0.3% melanin of body weight, this is minor relative to the total exuvia, which drain off nearly 10% of total body carbon per moult (Lynch et al. 1986). The actual costs of melanin synthesis may be more substantial.

Carapace melanization in cladocerans is restricted to alpine and Arctic populations. Increased heat absorption mediated by a dark carapace is one possible cause (Byron 1979), but is unlikely due to the effective heat transfer in these tiny organisms (Hairston 1981). Although there is accumulated evidence for the general role of melanin in UV protection, the extensive need for photoprotection in Arctic zooplankton is somewhat puzzling, since Arctic light is considerably less intense than at lower latitudes.

Low temperature could be one cause of UV-B induced cellular damage. The long days could add to this. While most harmful effects of UV-B are not temperature dependent, repair mechanisms could be. Nevertheless, there is no strong evidence for a decreased survival and anti-oxidant expression at low temperatures under UV stress (Borgeraas & Hessen in press). Continuous 24 hour solar exposure could be another cause of increased UV stress, although integrated midnight UV levels at this latitude are $<20\%$ of maximum intensities at noon.

Most high Arctic populations can only complete one generation of offspring from parthenogenetic eggs per year. There is an almost synchronous hatching of ephippia at the surface during ice-out, normally in mid- to late July but with strong interannual variability. The first generation grows until maturity during the first half of August; the second generation terminates the annual cycle by producing ephippia in early September. Early neonates are transparent and have a higher moulting rate compared to adults. Thus the period of ice melt and hatching of ephippia is not only the period with maximum potential solar irradiance, but also with maximum solar exposure. If hatching occurs during periods of clear sky and/or low ozone levels, this could determine the total annual production of *Daphnia*, and also induce shifts in species and clonal dominance.

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## References

Bradford, M. M. 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem. 72*, 248–254.

Byron, E. R. 1979: The adaptive significance of zooplankton pigmentation: a new hypothesis. *Abstr. Bull. Ecol. Soc. Am. 60*, 79.

Colbourne, J. K., Crease, T. J., Weider, L. J., Hebert, P. D. N., Dufresne, F. & Hobæk, A. 1998: Phylogenetics and evolution of a circumarctic species complex (Cladocera: *Daphnia pulex*). *Biol. J. Linn. Soc. 65*, 347–365.

Claiborne, A. 1985: Catalase activity. In R. A. Greenwald (ed.): *Handbook of methods of oxygen radical research.* Pp. 283–284. Boca Raton, Florida: CRC Press.

Gilbert, D. 1992: SeqApp version 1.9a. A multiple editor for Macintosh computers. Published electronically on the internet at www.fly.bio.indiana.edu.

Habig, W. H., Pabst, M. J. & Jakoby, W. B. 1974: Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem. 249*, 7130–7139.

Hairston, N. G. Jr. 1978: Carotenoid photoprotection in *Diaptomus kenai. Verh. int. Ver. Limnol. 20*, 2541–2545.

Hairston, N. G. Jr. 1979: The adaptive significance of color polymorphism in two species of *Diaptomus* (Copepoda). *Limnol. Oceanogr. 24*, 15–37.

Hairston, N. G. Jr. 1981: The interactions of salinity, predators, light and copepod color. *Hydrobiologia 81*, 151–158.

Hebert, P. D. N. & Emery, C. J. 1990: The adaptive significance of cuticular pigmentation in *Daphnia. Funct. Ecol. 4*, 703–710.

Hebert, P. D. N. & McWalter, D. B. 1983: Cuticular pigmentation in Arctic *Daphnia*: adaptive diversification of asexual lineages. *Am. Nat. 122*, 286–291.

Hessen, D. O. & Sørensen, K. 1990: Photoprotective pigmentation in alpine zooplankton populations. *Aqua Fennica 20*, 165–170.

Hessen, D. O. 1994: *Daphnia* responses to UV-light. *Arch. Hydrobiol. Beih. Ergebn. Limnol. 3*, 185–195.

Hessen, D. O. 1996: Competitive trade-off strategies in Arctic *Daphnia* linked to melanism and UV-B stress. *Polar Biol. 16*, 573–579.

Hobæk, A. & Wolf, H. G. 1991: Ecological genetics of Norwegian *Daphnia*. II. Distribution of *Daphnia longispina* in relation to short-wave radiation and water colour. *Hydrobiologia 225*, 229–243.

Kerr, J. B. & McElroy, C. T. 1993: Evidence for large upwards trends of ultraviolet-B radiation linked to ozone depletion. *Science 262*, 1032–1034.

Luecke, C. & O'Brien, W. J. 1983: Photoprotective pigmentation in a pond morph of *Daphnia middendorffiana. Arctic 36*, 365–368.

Lynch, M., Weider, L. J. & Lampert, W. 1986: Measurements of the carbon balance in *Daphnia. Limnol. Oceanogr. 31*, 17–33.

Madronich, S., McKenzie, R. L., Caldwell, M. M. & Björn, L. O. 1995: Changes in the ultraviolet radiation reaching the Earth's surface. *Ambio 24*, 143–152.

Ringelberg, J., Keyser, A. L. & Flik, B. J. G. 1984: The mortality of ultraviolet radiation in a translucient and in a red morph of *Acanthodiaptomus denticornis* (Crustaea, Copepoda) and its possible ecological significance. *Hydrobiologia 112*, 217–222.

Schindler, D. W., Curtis, P. J., Bayley, S. E., Parker, B., Beaty, K. G. & Stainton, M. P. 1997: Climate-induced changes in the dissolved organic carbon budgets of boreal lakes. *Biogeochemistry 36*, 9–28.

Scully, N. M. & Lean, D. R. S. 1994: The attenuation of UV radiation in temperate lakes. *Arch. Hydrobiol. 43*, 135–144.

Siebeck, O. 1978: Ultraviolet tolerance of planktonic crustaceans. *Verh. Int. Ver. Limnol. 20*, 2469–2473.

Siebeck, O. & Böhm, U. 1994: Challenges for an appraisal of UV-B effects upon plankonic crustaceans under natural radiation conditions with a non-migrating (*Daphnia pulex obtusa*) and a migrating cladoceran (*Daphnia galeata*). *Arch. Hydrobiol. Beih. Ergebn. Limnol. 43*, 197–206.

Spitz, D. R. & Oberley, L. W. 1989: An assay for superoxide dismutase activity in mammalian tissue homogenates. *Anal. Biochem. 179*, 8–18.

Swofford, D. L. 1993: Paup – a computer-program for phylogenetic inference using maximum parsimony. *J. Gen. Physiol. 102*, A9.

Van Raay, T. J. & Crease, T. J. 1994: Partial mitochondrial DNA sequence of the crustacean *Daphnia pulex. Curr. Genet. 25*, 66–72.

Weider, L. J. 1987: Life history variation among low-arctic clones of obligately parthenogenetic *Daphnia pulex*; a diploid-polyploid complex. *Oecologia 73*, 251–256.

Weider, L. J., Beaton, M. J. & Hebert, P. D. N. 1987: Clonal diversity in high-arctic populations of *Daphnia pulex*, a polyploid apomictic complex. *Evolution 41*, 1335–1346.

Weider, L. J. & Hobæk, A. 1994: Molecular biogeography of clonal lineages in a high-Arctic apomictic *Daphnia* complex. *Mol. Ecol. 3*, 497–506.

Weider, L. J., Hobæk, A., Colbourne, J. K., Crease, T., Dufresne, F. & Hebert, P. D. N. 1999: Holarctic phylogeography of an asexual species complex I. Mitochondrial DNA variation in Arctic *Daphnia. Evolution 53*, 777–792.

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 implication for plankton communities. *Limnol. Oceanogr. 41*, 1024–1034.

Wolf, H. G. & Hobæk, A. 1986: Ecological genetics of Norwegian *Daphnia*. I. Genetic difference between pigmented and unpigmented alpine pond populations. *Hereditas 104*, 193–198.

Zellmer, I. D. 1995: UV-B-tolerance of alpine and Arctic *Daphnia. Hydrobiologia 307*, 153–159.