

# Evidence of Sublethal Damage in *Daphnia* (Cladocera) during Exposure to Solar UV Radiation in Subarctic Ponds

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## Abstract

*Daphnia* was collected from five subarctic ponds which differed greatly in their DOC contents and, consequently, their underwater light (UV) climates. Irrespective of which *Daphnia* species was present, and contrary to expectations, the ponds with the lowest DOC concentrations (highest UV radiation levels) contained *Daphnia* with the highest eicosapentaenoic acid (EPA) concentrations. In addition, EPA concentrations in these *Daphnia* generally decreased in concert with seasonally increasing DOC concentrations.

*Daphnia* from three of the ponds was also tested for its tolerance to solar ultraviolet radiation (UVR) with respect to survival. *Daphnia pulex* from the clear water pond showed, by far, the best UV-tolerance, followed by *D. longispina* from the moderately humic and *D. longispina* from the very humic pond. In addition, we measured sublethal parameters related to UV-damage such as the degree to which the gut of *Daphnia* appeared green (as a measure of their ability to digest algae), and whether their guts appeared damaged. We developed a simple, noninvasive scoring system to quantify the proportion of the gut in which digestive processes were presumably active. This method allowed repeated measurement of the same animals over the course of the experiment. We demonstrated, for the first time, that sublethal damage of the gut precedes mortality caused by exposure to UVR. In a parallel set of experiments we fed UV-exposed and non-exposed algae to UV-exposed and non-exposed daphnids. UVR pretreatment of algae enhanced the negative effects of exposure to natural solar UV-irradiation in *Daphnia*. These UV-related effects were generally not specific to the species of *Daphnia*.

## Introduction

Ultraviolet radiation (UVR, 280–400 nm) has many harmful effects on aquatic organisms (see reviews by Vincent and Neale, 2000; Williamson et al., 2002), especially in shallow habitats, where organisms cannot escape into deeper water. Previous studies indicate that *Daphnia* (Crustacea), a circumpolar planktonic genus, is highly susceptible to solar UVR, even at relatively low radiation intensities (Zellmer, 1998, de Lange et al., 2000). In arctic and subarctic ponds, UVR is high during summer, especially during the 24 h daylight period. However, the attenuation of UVR is known to vary greatly amongst waterbodies (Arts et al., 2000) primarily due to differences in the concentrations of UV-absorbing chromophoric dissolved organic matter (CDOM). Previous studies have found increased survival of *Daphnia* to UVR exposure with increasing DOC concentrations (Rautio and Korhola, 2002).

UVR at low intensities may cause sublethal damage in *Daphnia* with possible consequences on reproduction and lifespan (De Lange and van Donk, 1997). In theory, the intestine of *Daphnia* is a likely target organ for UV damage due to its dorsal position. One indication of the UVR-vulnerability of *Daphnia*'s intestine is the melanization of the dorsal region of the carapace often found in field animals. Melanized *Daphnia* exhibit a significantly increased UVR-tolerance compared to transparent individuals (Hebert and Emery, 1990; Zellmer, 1995, De Lange et al., 2000).

If gut damage occurred as a result of UV exposure we would expect *Daphnia* to suffer from reduced digestion and/or assimilation of its food. In the low food environments typical of arctic and subarctic ponds such effects might result in malnutrition and contribute to the premature death of these animals. We tested the hypothesis that exposure to solar UVR

results in gut damage in two *Daphnia* species from three subarctic ponds with different UVR attenuation characteristics.

In addition to its direct effects on zooplankton, UVR may cause changes to the basic biochemistry and productivity of the phytoplankton community (Behrenfeld et al., 1995; Häder et al., 1995) with positive or negative effects on zooplankton grazers (Williamson et al., 2001). Sommaruga and Garcia-Pichel (1999) found positive effects when UV-treated algae were fed to grazers due to UV-protective substances (MAAs) incorporated by the grazer from their food. Alternatively, UVR may reduce food quality thereby indirectly affecting grazers. De Lange and van Donk (1997) fed UV-irradiated algae to *D. pulex* and observed that *Daphnia* produced fewer offspring.

We hypothesize that exposure of algae to solar UVR may reduce their quality as food for *Daphnia*. Quality of algae as food is positively correlated to sestonic lipid and fatty acid (FA) concentration and composition as well as other characteristics (e.g. size, shape, phosphorus content, etc.). Lipids are the preferred form of energy storage in daphnids (Arts, 1999) and it has recently been demonstrated that certain fatty acids are strongly correlated to growth rates of *Daphnia* in some lakes (Müller-Navarra et al., 2000; von Elert, 2002). Most of the lipids that *Daphnia* store are derived from their food (algae), since *de novo* lipid synthesis probably does not exceed 2% (Goulden and Place, 1990). Changes in lipid composition in algae may therefore severely affect grazers. Further, UVB has been shown, in the laboratory, to reduce the concentrations of some essential fatty acids (EFA) such as EPA (eicosapentaenoic acid: 20:5n-3) in algae (Goes et al., 1994; Wang and Chai, 1994). It has been suggested that the disproportionate reduction in polyunsaturated fatty acids (PUFA) such as EPA is due to the vulnerability of the multiple double bonds of PUFA to lipid photo-oxidation by free radical attack (Girotti, 2001).

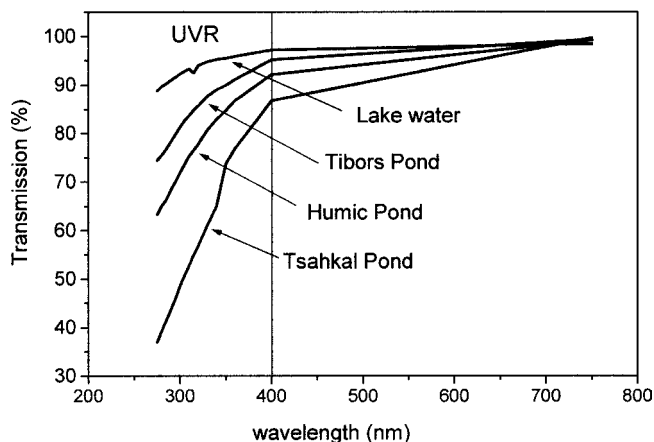


FIGURE 1. Spectral transmission (in %) through 1 cm of filtered (paper filter) water samples from the clear waters of Lake Kilpisjärvi, Tibors Pond, moderately colored Humic Pond, and very humic Tsahkal Pond.

Since most of the fatty acids found in *Daphnia* are incorporated directly from their diet (mostly algae) and, since higher UVB levels have been correlated with lower EPA levels in algae, we expected to find *Daphnia* with relatively lower EPA concentrations in ponds where algae are exposed to higher UV radiation levels (clear non-humic ponds). Here we make a preliminary assessment of whether such previously documented effects of UV on algal FA composition in the laboratory can be documented in wild *Daphnia* populations feeding on algae (food chain transfer) under natural, ambient, solar radiation levels.

We studied two species of *Daphnia* from three ponds in subarctic Finland. The ponds differed greatly in their dissolved organic carbon (DOC) content and water color thus creating very different underwater UVR regimes (Fig. 1). We used a noninvasive observational method to evaluate the effects of natural solar radiation on the digestive system of *Daphnia*. We tested whether pretreatment of food algae with UVR (indirect effects) and exposure of *Daphnia* to UVR (direct effects) affects the digestive system of *Daphnia*. In addition, we analyzed the total lipid levels and fatty acid composition in three *Daphnia* species from five different ponds. Our initial hypothesis was that concentrations of fatty acids (i.e. EPA) should be lower in *Daphnia* feeding on UV-exposed algae in the ponds with relatively lower DOC levels compared to levels in *Daphnia* from the more colored ponds.

## Methods and Materials

### SITE DESCRIPTION

*Daphnia* was collected from five ponds (Easy, Malla, Humic, Tsahkal, and Tibors Ponds) located in the immediate vicinity of the biological research station of the University of Helsinki in Kilpisjärvi, Finland (69°03'N, 20°50'E). Physical parameters of the three main study ponds are summarized in Figure 1 and Table 1. Tibors Pond is

TABLE 1

Selected physical and chemical parameters of Tibors, Humic, and Tsahkal Ponds (means and S.E. of data collected three to six times from 12 June to 3 July 2002)

	Tibors Pond	Humic Pond	Tsahkal Pond
DOC (mg L <sup>-1</sup> )	5.9 ± 1.8	7.9 ± 0.2	11.2 ± 0.54
Mean temperature (°C)	12.0 ± 2.0 S.E.	16.4 ± 2.5	13.4 ± 1.0 S.E.
Conductivity (µS)	69.0 ± 3.5	23 ± 0	13.5 ± 0.4

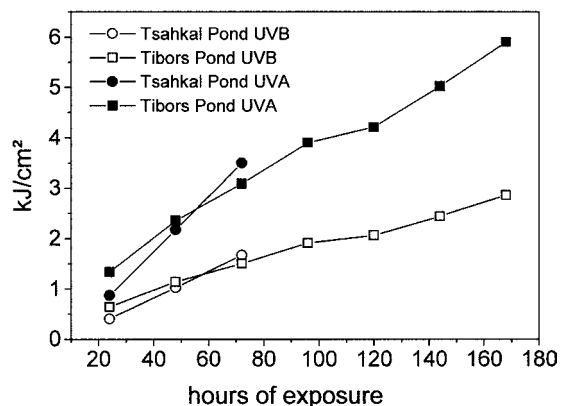


FIGURE 2. Cumulative UVA and UVB-doses at the surface of Lake Kilpisjärvi during the days of experiment measured from 0700 to 1900 h in 10-min intervals.

situated in a birch forest close to the edge of the treeline. It is an extremely shallow pond (~20 cm depth) with a single small (2 m<sup>2</sup>), deep ( $Z_{max} = 1$  m), depression on the north side. *Daphnia pulex* was only found in this depression. Water transparency was very high and there were very few aquatic macrophytes. Tsahkal Pond ( $Z_{max} = 0.8$  m) is located just above the treeline and has very few aquatic macrophytes and very brown water. *Daphnia longispina* was found throughout the pond. Humic Pond is situated just below the treeline. It is of moderate brown color, has many aquatic macrophytes and a depth of ~1 m. Here too, *D. longispina* is the most common cladoceran. *Daphnia longispina* is also found in Easy Pond, which is situated in the birch forest. It is very shallow (~50 cm depth) and has no aquatic macrophytes. Malla Pond is a clear water pond above the treeline. Aquatic macrophytes are only at part of the borders of the pond. Malla Pond is inhabited by *Daphnia umbra*.

### EXPERIMENTAL CONDITIONS AND TREATMENTS

Three experiments were performed, one with *D. pulex* from clear water Tibors Pond (25 June to 2 July 2002), one with *D. longispina* from humic water Tsahkal Pond (23–26 June 2002) and one with *D. longispina* from Humic Pond (11–15 June 2002). All experiments were run in 160 µm filtered Lake Kilpisjärvi water. This water is of very high transparency and only weakly attenuates UVR (Fig. 1). Cumulative UV doses during the experiments are provided in Figure 2.

### STOCK CULTURES

We cultured the unicellular green algae *Ankistrodesmus gracilis* (Chlorophyta), as a food source for the daphnids, in sterile medium (Zellmer, 1995) in 500-ml flasks. The cultures were placed inside a lab window and were thus exposed to natural sunlight without UV (midnight sun). Flasks were shaken several times per day.

### EXPERIMENTAL CULTURES

Two fresh treatment cultures (50 ml of media in 250-ml beakers) were started every day with the addition of 2-ml aliquots from the stock culture. Both beakers were placed in direct sunlight on a bench outside the lab. One cup was covered with WIPA foil (WIPA Technik, Tettang, Germany) which excluded UVB and most of the UVA. The control food containers were covered with acetate foil (Benn und Söhne, Hanau, Germany) which was transparent to UVR and visible wavelength. Both treatments therefore experienced different radiation regimes but were exposed to identical temperatures during the

experiments. Cultures were grown for 2 d with or without UV and, thereafter, fed to the daphnids according to the experimental treatments described below.

## DAPHNIA CULTURES

Fresh *Daphnia* specimens were caught with a dip net in the morning prior to each new experiment. Predators were immediately removed and the *Daphnia* were placed in a refrigerator (10°C) until the start of the experiment to avoid heat stress (room temperature in the lab was 25°C, whereas mean water temperature in Tsahkal Pond ranged from 9°C to 16.6°C, mean 14.5°C). Next, 10 vigorous adult *Daphnia* were added to each experimental container which was filled with 150 ml (i.e. 4-cm-deep water column) of lake water. Each experimental setup consisted of three replicates per treatment (10 animals per beaker, a total of 30 animals per treatment). *Daphnia* were fed equal quantities of algae (equivalent to 1.5 mg C L<sup>-1</sup>) and exposed to natural sunlight in a 10°C water bath. Ungrazed algae settled to the bottom. Thus, we considered these to be *ad libitum* feeding conditions. The containers were either covered with acetate foil (plus UV) or WIPA (minus UV) foil. The treatments were as follows:

- Control *Daphnia* (no UV) fed control algae (no UV)
- *Daphnia* exposed to UV and fed control algae (no UV)
- *Daphnia* exposed to UV and fed UV-exposed algae

Every evening, all *Daphnia* individuals were scored for the following parameters: survival and reproductive status, proportion of green intestine, and intestinal damage. Dead animals were removed. Data shown represent the means of the three replicates of each treatment.

**Green Intestine:** Roughly 75% of the intestine (foregut and midgut) usually appears bright green in actively feeding *Daphnia* due to the chlorophyll still present in the incompletely digested algae. The hindgut (~25% of the intestine) appears more yellowish or brownish due to pigment degradation as the algae are digested. We used a conservative scoring system to quantify changes in the digestive competency of *Daphnia* in the various treatments wherein a score of 1 or 0 was assigned to a *Daphnia* whose gut appeared entirely green or entirely brown, respectively. Intermediate stages were ranked 0.25, 0.5, or 0.75 depending on the extent of the green part of the gut.

**Damaged Intestine:** In some individuals the intestine appeared ruptured (i.e. green contents of the intestine could be seen leaking into the body cavity), completely or partly empty, or else had sharp bends or breaks. The intestine was scored as damaged if any of these signs were visible. This scoring method was quick, noninvasive and non-destructive. Repeated scoring of the same animals was therefore possible and proved a practical way to track changes in treatment groups over the course of the experiment.

After each scoring, the *Daphnia* were again fed leaving the algae enough time to settle during the night. Thus, UV-transmittance was not unduly altered due to suspended algae. Experiments were terminated when more than 50% of the initial *Daphnia* specimens died.

UV-radiation was measured with a calibrated, cosine-corrected UV-meter (X9, Giga-Hertz Optik, Munich, Germany,) with separate sensors for UVA and UVB. We continuously measured integrated UVA and UVB surface intensities from 0700 to 1900 h. Due to technical difficulties we were unable to record radiation intensities during the experiment conducted with Humic Pond *Daphnia*. However, skies were very clear on the first 2 d of experiment (and therefore solar radiation was high, comparable to the first 2 d of the experiment with Tibors Pond *Daphnia*) while on day 3 and 4, heavy cloud cover (80–100%) reduced radiation levels.

We used one-way ANOVAs (included in the Origin (5.0) program) to test for main effects between the three different treatments at each day in each of the experiments.

## LIPID AND FATTY ACID ANALYSIS OF DAPHNIA

*Daphnia* was collected between 13 and 17 June 2002 from each of the three main study ponds as well as between 25 June and 10 July 2003 from two additional ponds (Easy Pond and Malla Pond). Animals were transferred into clean cryogenic vials in the lab. We pooled 50 to 90 individual *Daphnia* from each pond to obtain sufficient tissue mass for lipid and FA analyses. The *Daphnia* were placed in a cryogenic freezer at –80°C after which they were freeze-dried and again stored at –80°C.

Fatty acid methyl esters (FAME) of *Daphnia* were obtained in a three-step process: extraction, derivatization, and quantification on a gas chromatograph (GC). Samples were extracted 3× by grinding freeze-dried tissue in (2:1 vol:vol) chloroform:methanol (Bligh and Dyer, 1959) and centrifuged at 4000 r.p.m. to remove nonlipid material (e.g. exoskeletons). From a final volume of 2 ml, duplicate, 200- $\mu$ l aliquots were dispensed into preweighed vessels which were dried and reweighed on a Cahn (Model C-30) electron balance with 1- $\mu$ g precision to provide a quantitative measure of total lipid content. The remaining extract (1.6 ml) was then transferred into a 5 ml Shimadzu vial (Sigma #27319U) and evaporated to dryness using nitrogen gas and stored at –80°C until derivatization.

The FA extracts were resuspended in 2 ml hexane prior to derivatization. Two ml of BF<sub>3</sub>-methanol (10% w/w) were added and vials were heated (70°C) for 2 h after which 1 ml each of water was added. The FAME-containing hexane-layer was carefully removed and placed into a 2 ml Kuderna-Danish receiving vial (Sigma #6-4689U). One ml hexane was then added to the original Shimadzu vial to extract the remaining FAME. This step was repeated once more to get the best extraction efficiency (90–95%). The FAME-hexane solution was evaporated to 2.0 ml using nitrogen gas and transferred to a 2 ml glass GC vial and stored in a –80°C cryogenic freezer prior to GC analysis.

FAME concentrations were quantified on a Hewlett Packard 6890 GC with the following configuration: splitless injection; column = Supelco (SP-2560 column) 100 m × 0.25 mm ID × 0.20  $\mu$ m thick film; Oven = 100°C (hold 1 min) to 240°C at 5°C min<sup>-1</sup>, hold for 38 min; carrier gas = hydrogen, 20 cm s<sup>-1</sup>; detector = FID @ 260°C; injector = 260°C; total run time = 67 min/sample. Three individual pure FA standards (20:2, 20:5n-3, and 22:6n3), were used to estimate the derivatization efficiency ( $\bar{x}$  = ~76%). A 37-component FAME standard (Supelco #47885-U) was used to identify and quantify (4-point calibration curves) FAME in the samples (unknowns) i.e. by comparing their retention times to those of the FAME standard. Results are reported as  $\mu$ g FAME/mg dry weight tissue.

## Results

Our first objective was to test whether *Daphnia* from ponds with different underwater radiation regimes varied in their tolerance towards solar UV. *Daphnia pulex* from clear Tibors Pond as well as *D. longispina* from moderately Humic Pond and from very humic Tsahkal Pond were exposed to solar radiation with and without UVR. Survival was markedly higher in *D. pulex* derived from clear Tibors Pond (Fig. 3A) despite the fact that highest UVA- and UVB-doses occurred during the first 48 h of the experiment (Fig. 2). Here, on day 3, 100% of the UV-exposed animals were still alive compared to 40% and 30% of the *D. longispina* from Humic (Fig. 4A) and Tsahkal Pond (Fig. 5A), respectively. Significant differences in mortality between treatments were already noticeable after 72 h in Tsahkal Pond *Daphnia* ( $P = 0.007$ ), while mortality of Tibors Pond daphnids remained very low in all three treatments up to day 6 (day 7:  $P = 0.0325$ ). Here control animals did very well until the end of the experiment (90% survival at day 8), while in *D. longispina* control treatments from the two other ponds, mortality increased after day 3.

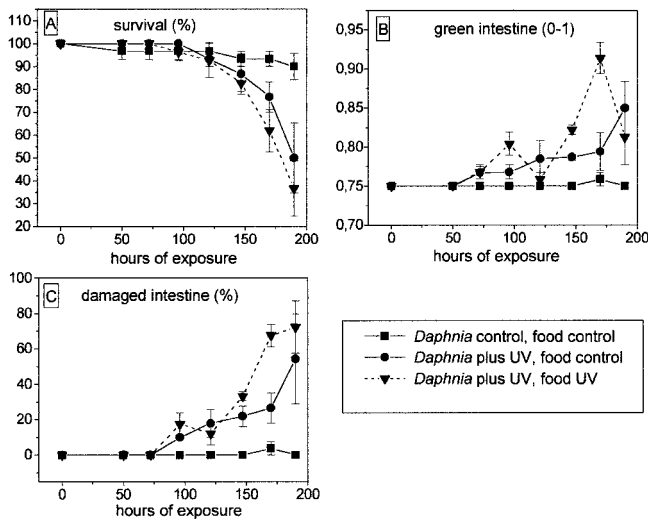


FIGURE 3. Measured parameters of *Daphnia pulex* from clear Tibors Pond during exposure to solar radiation with/without solar UV, fed *Ankistrodesmus gracilis* precultured in solar radiation with/without UV (means, S.E.). (A) Survival (%), (B) proportion of the gut that appeared green in color (0 = no green at all, 1 = entire gut is green), (C) animals with damaged intestine (% of live animals).

The quantification of the intestinal characteristics revealed differences between control and UV exposed *Daphnia*. In control *D. pulex* from clear-water Tibors Pond, the green portion of the gut remained constant at 75% over the course of the experiment (Fig. 3B). This compares favorably with the typical percentage found in *Daphnia* collected directly from this pond (75%, 26 June–July 2003, every 3 d, 12–25 animals). If *D. pulex* was exposed to solar UVR, the green portion of the intestine increased to 85% ( $\pm 3.4$  S.E.,  $P = 0.045$ ) of the entire gut system by the end of the experiment. In the Tsahkal Pond experiments (Fig. 5B) the proportion of green-colored intestine increased in UV-treated *Daphnia* (96.4%) compared to controls (79.6%) after 72 h of exposure; however, these differences were not significant. In contrast, *D. longispina* from Humic Pond showed no change in the relative length (%) of the green colored region of the gut on exposure to UV (Fig. 4B).

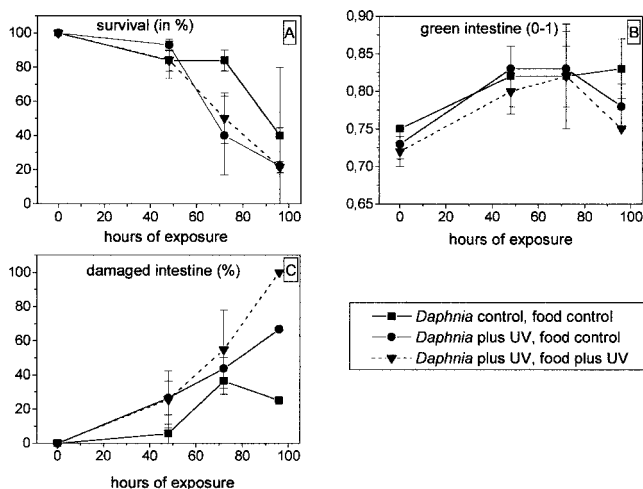


FIGURE 4. Measured parameters of *Daphnia longispina* from Humic Pond during exposure to solar radiation with/without UV, fed *Ankistrodesmus gracilis* precultured in solar radiation with/without UV (means, S.E.). (A) Survival (%), (B) proportion of the gut that appeared green in color (0 = no green at all, 1 = entire gut is green), (C) animals with damaged intestine (% of live animals).

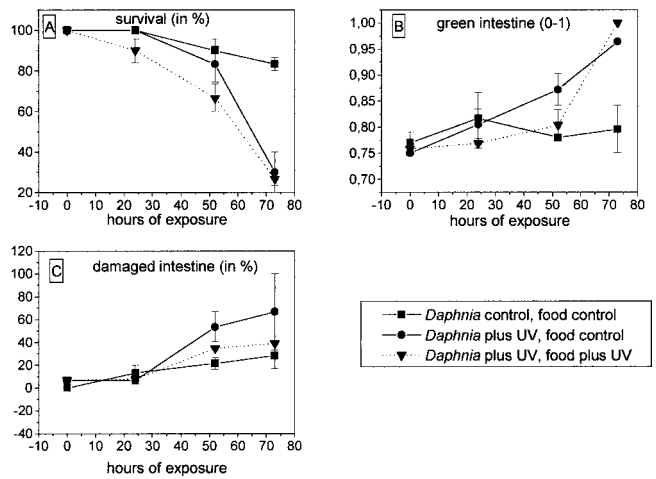


FIGURE 5. Measured parameters of *Daphnia longispina* from humic Tsahkal Pond during exposure to solar radiation with/without UV, fed *Ankistrodesmus gracilis* precultured in solar radiation with/without UV (means, S.E.). (A) Survival (%), (B) proportion of the gut that appeared green in color (0 = no green at all, 1 = entire gut is green), (C) animals with damaged intestine (% of live animals).

The next criterion was visible damage of the intestine. In *D. pulex* control animals from clear-water Tibors Pond no damage was observed throughout the course of the experiment (Fig. 3C). In contrast, the proportion of *Daphnia* with visible intestinal damage increased continuously in the UV-exposed group, reaching 54% ( $\pm 25.3$  S.E.,  $P = 0.099$ ) by day 8 in the animals fed control algae. *Daphnia longispina* from Humic and Tsahkal Ponds exhibited similar damage patterns (Figs. 4C, 5C). Intestinal damage in the control *Daphnia* was low (25 and 28.3%, respectively) but showed an increase in the UV-treated animals (66.7%,  $P = 0.55$ , Humic Pond and  $P = 0.33$ , Tsahkal Pond). Although neither of the differences is statistically significant due to high variability, the trends are suggestive.

Our second objective was to test whether UV-treatment of food algae alters the UV-tolerance of the *Daphnia*, and if so, whether *Daphnia* originating from the different habitats are affected to different extents. UV-treated food had a negative, but not significant, effect on survival in UV-exposed animals from Tibors Pond and Tsahkal Pond (Figs. 3A, 5A), but not on Humic Pond animals (Fig. 4A).

*Daphnia* originating from different ponds differed in their response to UV-treated food with respect to the intestinal parameters. In UV-treated *D. pulex* from clear Tibors Pond fed UV-treated food, the green gut portion of the intestine increased to a maximum of 92% (75% of control animals and 85% of UVR treated animals with control food) by day 7 (Fig. 3B). Here, the entire digestive tract remained the same bright green color as the algal food. However, on day 8, this value dropped to 81%. We infer that the intestines of these last few surviving *Daphnia* apparently had not suffered that much from the UV-treatment or the UV-treated food. In *D. longispina* from Humic Pond no difference of gut color was recorded between treatments (Fig. 4B). Similarly, there was no additional effect of UV-treated food with respect to green gut in *D. longispina* from Tsahkal to be seen in the few animals that were still alive after 72 h of experimental UV exposure (*Daphnia* with control food = 96%, those with UVR treated food = 100%, Fig. 5B).

The percentage of *Daphnia* with visible damage of the gut system was also affected by UV-treatment of the food algae. *Daphnia pulex* fed UV exposed algae exhibited the greatest degree of gut damage. 72% of UV-exposed *Daphnia* with UVR-treated food had damaged intestines on day 8 compared to 54% for UV-exposed *Daphnia* fed non-UV-exposed food ( $P = 0.53$ ) and to 0% of the control animals

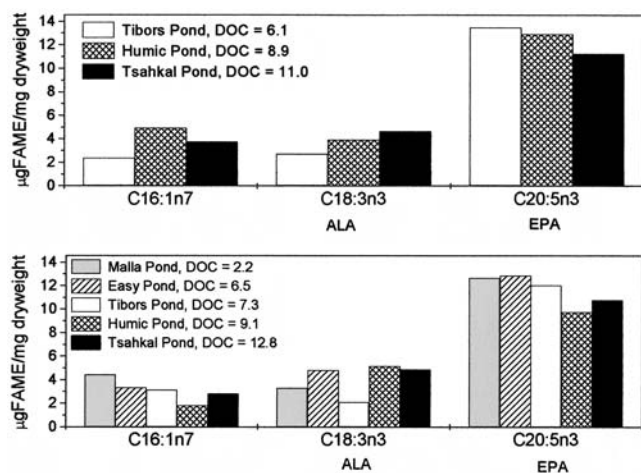


FIGURE 6. Concentrations ( $\mu\text{g}$  FAME/mg dry weight tissue) of three selected fatty acids (EPA, ALA and 16:1n-7) in *Daphnia* from five subarctic ponds. Top panel is for 2002 and bottom panel is for 2003. Note: *Daphnia pulex* was present in Tibors Pond, *Daphnia umbra* was found in Malla Pond, and *Daphnia longispina* was present in Easy, Tsahkal, and Humic Ponds.

without UV exposure ( $P = 0.016$ , Fig. 3C). Similarly, the percentage of animals with damaged guts in UV-treated *D. longispina* from Humic Pond increased to 100% with UV-treated food compared to 60% with control food ( $P = 0.55$ ) and 20% in non-UV-treated controls ( $P = 0.4$ , Fig. 4C). In contrast, the percentage of damaged animals in *D. longispina* from Tsahkal Pond decreased to 38.9% ( $\pm 20.0$  S.E.) with UV-treated food compared to those with control food ( $66.7\% \pm 33.7$  S.E.,  $P = 0.5$ , Fig. 5C). However, the degree of gut damage parameter was very variable and therefore the observed differences were not statistically significant.

We also examined the naturally occurring lipid levels and FA concentrations of *Daphnia* from the three main study ponds (Tibors, Tsahkal, and Humic) as well as two other ponds (Malla and Easy). *Daphnia pulex* from Tibors Pond (mean = 201.7  $\mu\text{g}$  DW/*Daphnia*) were much heavier than *D. longispina* from both Tsahkal Pond (mean = 83.7  $\mu\text{g}$  DW/*Daphnia*) and Humic Pond (mean = 86.0  $\mu\text{g}$  DW/*Daphnia*). However, *D. longispina* from Easy Pond weighed more (140.1  $\mu\text{g}$  DW/*Daphnia*) than the same species in Tsahkal and Humic Ponds. *Daphnia umbra* from Malla Pond were smaller and, consequently, weighed only 61.3  $\mu\text{g}$  DW/*Daphnia*. Tibors Pond *Daphnia* had slightly higher total lipid concentrations (17.6%) on a dry weight basis than *Daphnia* from Tsahkal (16.3%) and Humic (16.3%) Ponds however, total lipid concentrations of *D. longispina* from Easy Pond (22.4%) were the highest measured. *Daphnia umbra* from Malla Pond had the lowest lipid concentrations (11.3%).

*Daphnia pulex* in Tibors pond had higher concentrations of EPA than *D. longispina* from Tsahkal and Humic Ponds in both years (Fig. 6). We suggest that this difference may be more related to the particular species of algae present in the ponds and/or to the DOC concentration (i.e. underwater UVR regime) in the ponds rather than inter-specific differences in FA metabolism amongst the *Daphnia*. For example, in 2003, EPA concentrations were lowest in *D. longispina* from the two ponds (Humic and Tsahkal) with DOC concentrations greater than 9  $\text{mg L}^{-1}$  but all three *Daphnia* species (*longispina*, *pulex* and *umbra*) had higher EPA concentration from ponds where DOC concentrations were less than 9  $\text{mg L}^{-1}$ . In 2002, concentrations of a-linolenic acid (ALA; 18:3n-3), the precursor of EPA, were generally highest in *Daphnia* collected from ponds with the highest DOC concentrations (Fig. 6). In 2003, concentrations of 16:1n-7 were typically higher in *Daphnia* collected from ponds with the lowest DOC concentrations

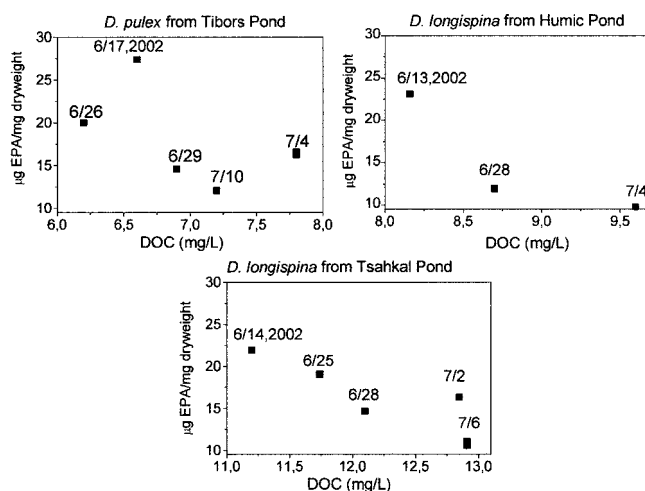


FIGURE 7. Seasonal changes in the EPA concentration ( $\mu\text{g}/\text{mg}$  dry weight) of *Daphnia* as a function of DOC concentration in Tibors, Tsahkal, and Humic Ponds in 2003. An additional data point from 2002 is also provided.

(Fig. 6). Finally, concentrations of EPA in *Daphnia* were highest at the beginning of the season, when DOC levels were highest in Tsahkal, Humic, and Tibors Ponds, and generally declined as the season progressed and DOC concentrations fell (Fig. 7).

## Discussion

Observations from previous field seasons suggested that the intestinal tract of UV-exposed *Daphnia* was affected well before finally mortality took place. Since these visible manifestations of damage were eventually followed by mortality we hypothesized that there might be a connection between changes in gut appearance and death. We developed a scoring system to quantify these changes and follow the trajectories of damage over time. We also hypothesized that *Daphnia* from ponds of varying UV-transmission due to different DOC-concentrations should differ in their UV-tolerance therefore expected differences in the exposure time needed to induce these changes.

One means of defense against UVR-damage in *Daphnia* is pigmentation. Several authors have shown that melanin increases UV-tolerance in *Daphnia* (Hebert and Emery, 1990; Zellmer, 1995; Hessen, 2001; Rautio and Korhola, 2002). *Daphnia longispina* from humic Tsahkal Pond and from Humic Pond appeared light brown, whereas *D. pulex* from clear-water Tibors Pond were red most likely due to carotenoids. None of the animals was dark brownish as would be expected in the case of intense melanin pigmentation. Similarly, mycosporine-like compounds (MAA) have been found to provide efficient UV-protection in algae as well as in grazers (Sommaruga and Garcia-Pichel, 1999). However, no MAAs were detected in *Daphnia* from Tsahkal or Humic Pond (Abele and Obermüller, unpublished).

Our first question was; does sensitivity to solar UVR differ in *Daphnia* in relation to the UV environment of their source habitat? In fact, we found highest mortality in *D. longispina* from the most humic pond (Tsahkal Pond, Fig. 5A) followed by *D. longispina* from Humic Pond (Fig. 4A). *Daphnia pulex* from the clear water pond showed by far the highest UV-tolerance (Fig. 3A). *Daphnia longispina* originated from habitats with high UV attenuation due to high CDOM concentrations. For these animals, experimental exposure in water from Lake Kilpisjärvi meant a dramatic increase in UV-intensity, whereas

TABLE 2

Mean percent ( $\pm$  S.D.) of *Daphnia* with filled brood pouches during the experiments

	<i>Daphnia</i> and food control	<i>Daphnia</i> UV, food control	<i>Daphnia</i> and food UV
<i>Daphnia pulex</i> Tibors Pond	90.2 $\pm$ 3.7	89.3 $\pm$ 7.3	86.9 $\pm$ 8.6
<i>Daphnia longispina</i> Humic Pond	98.8 $\pm$ 2.2	92.3 $\pm$ 9.5	85.5 $\pm$ 6.3
<i>Daphnia longispina</i> Tsahkal Pond	51.6 $\pm$ 2.1	59.3 $\pm$ 8.9	32.1 $\pm$ 23.1

*D. pulex* from Tibors Pond remained in similar UVR conditions. Changes of the light regime were further imposed by the limited depth (4 cm) of the experimental containers. Under natural conditions *D. longispina* remains at the bottom of the pond (50 cm water depth) under high radiation conditions (Rautio et al., 2003). The higher mortality in animals from humic waters therefore may indicate an adaptation of *Daphnia* to the lower UVR regime of their habitat. This is supported by data from Siebeck and Böhm (1994) who found greater UV-sensitivity in *Daphnia galeata* originating from a lowland lake compared to *D. pulex* from an alpine pond.

Since we found increased mortality due to UVR in both *Daphnia* species the next question was: what is the mechanism by which UVR affects the *Daphnia*? The high UV-tolerance of *D. pulex* allowed us to quantify intermediate stages of damage at daily intervals. We found the green midgut region of the daphnids expanded with increasing time of UV-exposure (Fig. 3B). This extension could be the result of increased filtration rates in UV-exposed animals. Unfortunately, we did not measure filtration rates in this study. However, in similar field experiments, de Lange et al. (2000) found that grazing rates of arctic *Daphnia middendorffiana* did not differ between UV-irradiated and control groups. It is more likely that our results reflect reduced digestion rather than increased filtering rates because we consistently found undigested green material in the hind guts of UV-treated *Daphnia*. This was not observed in control animals nor in freshly collected specimens. Their hind guts were always filled with brownish or grayish residues of digested material. In wild *D. pulex* from Tibors Pond the green portion of the gut extended to 75% of the gut system (as the controls) with only the last 25% being filled with digested food material during the entire sampling period.

*Daphnia longispina* from Tsahkal Pond followed the same pattern as described above for *D. pulex*, but in *D. longispina* from Humic Pond no change in the length of the green portion of the gut was found in UV-treated animals. However, variation between individuals was extremely high in this population. Consequently we suggest that this response is not species-specific but is common to daphnids exposed to stressful UVR conditions.

We recorded increasing numbers of *Daphnia* (from all three ponds) whose guts had breaks and ruptures as well as abnormal bends and/or a wrinkled appearance. This process culminated in completely green and, shortly thereafter, in completely empty intestines (Figs. 3–5C). Again, *Daphnia* from humic Tsahkal Pond and Humic Pond were affected faster than those from the clear water pond.

Due to the experimental design, UVR was intensified for the animals in comparison to their natural environment, especially for *Daphnia* from the two humic ponds. This explains the occurrence of the rather severe symptoms of damage; symptoms which are not commonly seen in wild populations. However, in summer 2003, when extreme drought and heat caused exceptionally low water levels in the ponds, gut damage (in 9.1% of the population) was observed even in wild *Daphnia* from Tsahkal Pond.

Since the gut in *Daphnia* is in a dorsal position it gets little shielding from other body tissues and thus may be an *a priori* target for vertically down-welling radiation. However, eggs or embryos in the brood pouch might serve as a shield protecting the underlying intestine from vertical radiation. In contrast, we found damage in the gut of UV-treated animals irrespective of filled brood pouches in up to 100 % of the animals (Table 2). This suggests that the shielding effect of the brood pouch may be less effective than expected.

The midgut of *Daphnia*'s intestine is likely to be the major site of digestion and absorption (Peters and de Bernardi, 1987). Consequently impairment of digestion and absorption of algae might theoretically follow the damage and could lead to a nutritional situation comparable to starvation. Zellmer (1996) showed in laboratory experiments that UV tolerance of *D. pulex* became reduced with prolonged food limitation within a week. Thus, a reduction in digestion and/or absorption of algal nutrients induced by direct exposure to UVR could further jeopardize the survival of *Daphnia* in the same way as food limitation reduces tolerance to UVR.

The phytoplankton in the three ponds is also exposed to ambient solar UVR. Natural UVR could cause changes in phytoplankton which, in turn, could affect the grazers. Several authors have reported changes in the biochemistry and/or anatomy of algae following UV-treatment (see Hessen, 2001). In our experiments animals were fed with *A. gracilis* which had been grown in either the presence or absence of solar UVR. We chose this species as food since we were not, at the time, able to successfully culture pond phytoplankton. In two of the three experiments mortality rates caused by solar UV-treatment of the animals increased if UV-irradiated food was supplied. Therefore, we suggest that exposure to UVR may have modified the algal food. The effect of UV-altered food on *Daphnia* survivorship was seen earlier in *D. longispina* obtained from the brown water Tsahkal Pond compared to those from *D. pulex* obtained from clear water Tibors Pond. The observed differences in response time might relate to the different UV intensities that the *Daphnia* populations were originally adapted to.

*Daphnia pulex* from clear water Tibors Pond fed UV-irradiated food showed a significant increase in the proportion of intestine with green color (presumably undigested algae) plus a higher incidence of gut damage upon prolonged exposure to UVR when compared to *Daphnia* fed control algae. While this effect was similar in *D. longispina* from Humic Pond, *D. longispina* from humic Tsahkal Pond showed a reduction in the number of individuals with damaged intestine when fed UV-treated food. In addition, the green part of the intestine was shorter compared to the UV-exposed control food animals, except for the very last day of the experiment, where survival was already extremely low. Thus, *D. longispina* from both ponds exhibited increased mortality due to direct UV-treatment and indirect UV-food treatment; however, they differed in the apparent susceptibility of their gut systems.

As exposure time increased, individual differences surfaced; in both species some *Daphnia* were seemingly unaffected by the treatments while others suffered greatly. The unaffected animals were those that survived until the end of the experiment and showed the least difference in gut damage and algal digestion (judged by color) compared to the control animals. One possibility is that there are genetic differences within populations of *Daphnia* modulating their ability to tolerate UVR stress. This is supported by the differences we found between the two populations of *D. longispina* with respect to the UV-related gut damage.

Lipids are the major form of energy storage in *Daphnia*. These compounds are largely obtained from the diet and enable *Daphnia* to resist periods of low food as well as providing them with the EFA (e.g. EPA) required for optimum growth and reproduction (see Demott and Müller-Navarra, 1997; Sundbom and Vrede, 1997). A recent study

(von Elert, 2002) suggested that ALA is readily converted to EPA (but not the reverse) suggesting that, although ALA is likely secondary in importance to EPA for *Daphnia*, ALA must also be considered an EPA for *Daphnia* under some circumstances (e.g. when ALA concentrations in the food are low).

Since EPA in algae have been shown to be sensitive to UVB radiation in the laboratory (Goes et al., 1994; Wang and Chai, 1994) we expected to see lower EPA concentration in *Daphnia* feeding on algae in the ponds with the lowest DOC concentrations. Contrary to our initial expectations we found that EPA concentrations were generally highest in ponds with the lowest DOC concentrations. These findings are similar to those in a recent study Tank et al. (2003) on the effects of solar UVR on biofilms (periphyton) in montane lakes where it was demonstrated that EPA concentrations were actually highest in biofilms grown in the least colored of the four study lakes.

In this study UV-effects on algal PUFA were not determined directly. Therefore we have no information on the FA composition of the algae ingested by *Daphnia* in either the irradiated food treatment nor how algae may have compensated for the higher UVR in the ponds with relatively lower DOC concentrations (i.e. in response to higher PAR). Thus, our observations must be interpreted with caution.

It is highly probable that the differences in FA observed in these *Daphnia* were produced by differences in the taxonomic composition of the algae present in the ponds. Further, although lipid synthesis rates in *Daphnia* are known to be very low (Goulden and Place, 1990), it is also possible that the observed differences in ALA and EPA concentrations were at least partially accounted for by species-specific differences in the biochemistry of lipid synthesis and metabolism amongst the three *Daphnia* species. However, we also note that, irrespective of *Daphnia* species, the three ponds with the lowest DOC concentrations contained *Daphnia* with the highest EPA concentrations (Fig. 6) and EPA concentrations generally decreased in concert with seasonally increasing DOC concentrations (Fig. 7). One explanation might be that *Daphnia* are feeding more heavily on EPA-rich diatoms in the ponds with relatively lower DOC concentrations. It has been suggested, for example, that high PAR intensities are a major stimulus for lipid production (Wainman et al., 1999). Thus, it may be that the higher underwater PAR intensities in clear water environments such as Tibors and Malla Ponds offset the detrimental effects of UVB on long chain PUFA for high light-adapted species like diatoms. Thus, it is possible that, even though some of the PUFA in algae are degraded (e.g. in diatoms) by UVB, in balance more PUFA are produced as a response to the higher underwater PAR availability. Alternately, diatoms may simply be relatively more abundant in ponds with higher light levels. In support of this hypothesis, concentrations of 16:1n-7, a FA thought to be associated with diatoms (Napolitano, 1999) were highest in the three ponds with DOC concentrations less than 9 mg L<sup>-1</sup> in 2003 (Fig. 6B). More effort is clearly required to sort out the relative ecological effects of UVR on the FA composition of algae and *Daphnia* under varying solar irradiance regimes.

We suggest that phytoplankton as well as *Daphnia* may be affected by UVR in subarctic ponds. Temporal fluctuations in UVR-intensity, temperature, ice-on and ice-off times vary tremendously on a year-to-year basis in subarctic ponds. In addition, 10°C fluctuations within 24-h periods occur as well as dramatic changes in UV-intensity due to the variable cloud cover. Also, DOC concentrations can vary greatly on a year-to-year, week-to-week, and even on a day-to-day basis. For example, the predominantly sunny weather in summer 2002, coupled with an extremely early start to the ice-free season and unusually low precipitation led to low water levels (decreased depth refuge), DOC degradation (e.g. Tsahkal Pond 6 June 2002 = 13.1 mg L<sup>-1</sup>, 2 July 2003 = 10.9 mg L<sup>-1</sup>), high temperatures (maximum of 22, 25.5, and 14°C in Humic, Tsahkal, and Tibors Ponds, respectively) and high UVR intensities. *Daphnia* in these ponds have to compensate

and adapt to both direct (UVR, temperature) and indirect (via food) stresses. Thus, the ultimate manifestation(s) of UV impacts on *Daphnia* in nature will depend on several interactive (Williamson et al., 2002), and often synergistic, environmental factors (i.e. UVR-intensity and temperature).

Our results suggest that *Daphnia* may be harmed by UVR damage to their intestinal system long before UVR-induced mortality occurs, presumably resulting in malnutrition with death as the final outcome. On a community level, such sublethal damage might well affect a population due to individual changes in growth, reproduction and offspring survival and the ability to deal with modified food qualities. The broader question of whether stress tolerance of *Daphnia* could be species-specific or differ on an individual basis, enabling specialized *Daphnia* species or individual clones or populations to survive in these habitats requires further research.

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