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> > (EFFect of temperature on the fatty acid composition and temporal trajectories of Fatty acids in starving Daphnia pulex (crustacea, Cladocera) BY: C. Schlechtriem, M. Arts, I. Zellmer NWRI Contribution No: 05-186

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# Effect of temperature on the fatty acid composition and temporal trajectories of fatty acids in starving *Daphnia pulex* (Crustacea, Cladocera)

C. Schlechtriem, M.T. Arts and I.D. Zellmer

# Abstract

5-186

Poikilothermic organisms accumulate highly unsaturated fatty acids (HUFA) in their lipids at reduced temperatures to maintain cell membrane fluidity. In this study we investigated the effect of temperature on temporal trajectories of fatty acids of starving *Daphnia pulex* cultured on a HUFA-free diet. *D. pulex* populations were maintained for one month at 22°C and 11°C and were fed the chlorophyte *Ankistrodesmus falcatus*. We observed "trophic upgrading" of  $C_{18}$  fatty acid precursors to eicosapentaenoic acid (EPA; 20:5n3) and arachidonic acid (ARA; 20:4n6) in *D. pulex*. We showed that long term exposure to cold temperature causes a significant increase in EPA. HUFA such as ARA and EPA are highly conserved during starvation. Therefore, *D. pulex* has the biosynthetic capacity to adjust and to maintain the content of HUFA required to survive at low temperatures.

# NWRI RESEARCH SUMMARY

# Plain language title

Effect of temperature and starvation on the lipid composition of a keystone and ubiquitous planktonic organism

# What is the problem and what do scientists already know about it?

Global warming represents a serious threat to the integrity of aquatic ecosystems. However, the extent of the threat has yet to be clearly elucidated. One concern for poikilothermic organisms (organisms that have an internal body temperature that changes depending on the conditions in the environment) relates to the fatty acid composition of their membranes. In such animals it is suspected that the degree of unsaturation of their fatty acids (i.e. the number of energy containing double bonds) generally decreases as temperatures increase.

## Why did NWRI do this study?

Arctic ecosystems are expected to be some of the most impacted ecosystems on this planet in terms of the effects of global warming. The water flea (*Daphnia*) is one of the most important animal plankton species that inhabits the millions of shallow depressions (ponds) in the Arctic. This organism provides an essential link between algae and fish. It not only provides food for fish but also increases the nutritive value of the food that it eats (trophic upgrading). This valuable ecological service may in fact be compromised by enhanced water temperatures and answering this question was one of the primary goals of this study.

### What were the results?

Daphnia grown at higher temperatures not only had less lipids overall but the degree of saturation of their fatty acids decreased. This means that at higher temperatures their ecological service role in the ponds is diminished because they are less able to upgrade shorter-chain precursor fatty acids to the longer-chain, more energy rich, highly unsaturated fatty acids. We also demonstrated that during starvation some fatty acids were particularly conserved suggesting that these compounds could be good candidates for bioindicators; i.e. to provide insights into the condition of fieldcaught Daphnia in the Great Lakes and other waterbodies.

# How will these results be used?

These results will help us to understand the impacts of climate change on the availability of essential fatty acids in temperature sensitive ecosystems. They also point us to the most likely candidate fatty acids to be used in future bioindicator development work.

# Who were our main partners in the study?

Martin-Luther University, Institute of Plant Physiology, Halle, Germany, Great Lakes Fisheries Commission.

Effet de la température sur la composition en acides gras et les trajectoires temporelles des acides gras dans des populations de *Daphnia pulex* (Crustacea, Cladocera) affamées

C. Schlechtriem, M.T. Arts et I.D. Zellmer

### Résumé

Les organismes hétérothermes accumulent les acides gras polyinsaturés à longue chaîne (AGPLC) dans leurs lipides à des températures basses pour maintenir la fluidité de la membrane cellulaire. Dans cette étude, nous avons étudié l'effet de la température sur les trajectoires temporelles des acides gras de populations de *Daphnia pulex* affamées et cultivées avec un régime sans AGPLC. Des populations de *D. pulex* ont été conservées durant un mois à 22 et 11 °C, et ont été alimentées avec le chlorophyte *Ankistrodesmus falcatus*. Nous avons observé une « élévation trophique » des précurseurs d'acides gras C<sub>18</sub> vers l'acide eicosapentanoïque (EPA; 20:5n3) et l'acide arachidonique (ARA; 20:4n6) dans *D. pulex*. Nous avons montré qu'une exposition à long terme à des basses températures entraîne une augmentation importante de l'EPA. Des AGPLC comme l'ARA et l'EPA sont largement conservés durant la période de non-alimentation. Par conséquent, *D. pulex* a la capacité biosynthétique de s'ajuster et de maintenir la concentration nécessaire en AGPLC pour survivre aux basses températures.

# Sommaire des recherches de l'INRE

### Titre en langage clair

Effet de la température et de la non-alimentation sur la composition en lipides d'un organisme planctonique ubiquiste clé.

# Quel est le problème et que savent les chercheurs à ce sujet?

Le réchauffement planétaire est une menace grave pour l'intégrité des écosystèmes aquatiques. Cependant, l'étendue de la menace n'a pas encore été clairement établie. L'une des préoccupations concernant les organismes hétérothermes (organismes dont la température interne varie selon les conditions de l'environnement) a trait à la composition en acide gras de leurs membranes. On croit que, dans ces animaux, le degré d'insaturation de leurs acides gras (c.-à-d. le nombre de double liaisons porteuses d'énergie) augmente généralement avec la température.

# Pourquoi l'INRE a-t-il effectué cette étude?

On s'attend à ce que les écosystèmes arctiques soient parmi les écosystèmes de cette planète les plus sensibles au réchauffement planétaire. Le cladocère (*Daphnia*) est l'une des plus importantes espèces zooplanctoniques habitant les millions de dépressions peu profonde (étangs) de l'Arctique. Cet organisme établit un lien essentiel entre les algues et le poisson. Non seulement il sert d'aliment au poisson, mais il augmente également la valeur nutritive des aliments qu'il ingère (élévation trophique). Cette fonction écologique précieuse pourrait en fait être compromise par l'élévation de la température de l'eau, et l'un des principaux but de cette étude était de répondre à cette question.

### Quels sont les résultats?

Les *Daphnia* cultivés à des températures plus élevées contenaient généralement moins de lipides, et le degré de saturation de leurs acides gras était également plus bas. Cela signifie qu'aux températures plus élevées leur rôle écologique dans les étangs diminue en raison de leur moins grande capacité de remplacer les précurseurs d'acides gras à chaîne courte par des acides gras polyinsaturés à chaîne longue plus riches en énergie. Nous avons également montré que, durant la période de non-alimentation, certains acides gras étaient particulièrement conservés, ce qui porte à croire que ces composés pourraient être de bons candidats pour servir de bioindicateurs, c.-à-d. pour permettre de connaître la condition de *Daphnia* prélevés sur le terrain dans les Grands Lacs et d'autres masses d'eau.

# Comment ces résultats seront-ils utilisés?

Ces résultats nous aideront à comprendre les impacts du changement climatique sur la disponibilité d'acides gras essentiels dans les écosystèmes sensibles à la température. Ils nous orientent également vers les acides gras les plus probables comme candidats à utiliser dans les travaux futurs sur l'élaboration de bioindicateurs.

# Quels étaient nos principaux partenaires dans cette étude?

Université Martin-Luther, Institut de la physiologie des plantes, Halle (Allemagne); Commission des pêcheries des Grands Lacs.

# Food chain effects of sublethal ultraviolet radiation on subarctic *Daphnia pulex* – a field and laboratory study

I. D. Zellmer<sup>1</sup>, M. T. Arts<sup>2</sup> and Vladimír Šustr<sup>3</sup>

With 5 figures

Abstract: In shallow subarctic ponds Daphnia and their algal food are often exposed to solar ultraviolet radiation (UVR). It was previously demonstrated that direct exposure of Daphnia to UVR may result in sublethal damage to Daphnia's intestinal system. This led to the hypothesis that, as consequences of such exposures, Daphnia may be experiencing a situation similar to starvation. We examined, in controlled experiments, the indirect effect of UVR-treated food algae on Daphnia pulex which, themselves, were also exposed to UVR. We specifically tested whether exposure of D. pulex to solar UVR affects food transport and enzymatic digestion. As expected, the UVR-exposed food and UVR-treated Daphnia combination produced the strongest effects on intestinal damage, and mortality. Some of these effects, as well as the grazerrelated effects, were similar to those observed during starvation. The total activities of digestive enzymes (amylase and cellulase) were somewhat reduced in UVR treated D. pulex, but the function of enzymes is not seriously damaged as is clear from the increasing trend of protein-specific activities. We conclude that, in some ways, Daphnia undergoing sublethal UVR exposures may be experiencing a condition similar to what they would experience under very low food or even fasting conditions.

Key words: UV radiation, Daphnia, enzymes, sub-Arctic ponds, chronic stress.

### Introduction

Ponds reflect changes in environmental conditions very quickly due to their shallowness and small volume. Thus, phyto- and zooplankton inhabiting such

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ecosystems are exposed to rapid changes in their physical (i.e. solar radiation, temperature, DOC dissolved organic carbon, pH) as well as biochemical environment. In high latitude ponds physical and chemical forcing of certain variables are even more extreme than for ponds at lower latitudes compelling biota to cope with sudden and drastic changes in their environment. Two of the most variable factors in high latitudes ponds are temperature and solar radiation. As a consequence of decreases in stratospheric ozone concentrations increases in solar ultraviolet radiation (UVR, 290-400 nm) have been recorded in some regions (PERIN & LEAN 2004). UVR is the most energetic and most harmful part of the solar spectrum and shows a maximum in early spring in northern latitudes. The attenuation of UVR in the water column depends mainly on the concentration of the chromophoric portion of DOC (ARTS et al. 2000). The shallowness of many subarctic ponds and their low DOC concentrations allows UVR to penetrate to the bottom of most ponds. Thus, the biota living in these ponds must tolerate at least some exposure to solar UVR especially in spring and early summer when many pond animals are reproducing and solar UVR levels are high.

Species in the genus Daphnia are herbivores that play a key role in subarctic pond systems by providing a critical link between primary producers and secondary consumers (invertebrate predators, fish and humans). Several investigators have examined the direct effect of UVR on Daphnia demonstrating for example, that melanization of Daphnia's carapace increases UVR-tolerance (HEBERT & EMERY 1990, ZELLMER 1998) and that Daphnia employs avoidance strategies such as migration to the bottom of the pond to avoid high radiation conditions (RAUTIO et al. 2003, LEECH & WILLIAMSON 2001). Many experimental and field studies use mortality as the endpoint to assess the effects of UVR on biota, however, sublethal damage may affect the fitness and reproductive success of Daphnia populations long before outright mortality occurs and, in more subtle, but equally important, ways. For example, in high latitudes sublethal solar UVR may damage Daphnia's intestine before mortality occurs (ZELLMER et al. 2004). Evidence of this form of UVR-induced damage led to the hypothesis that gut-damaged Daphnia may suffer from malnutrition comparable to starvation (ZELLMER et al. 2004). Hence, in the current study we addressed the following questions: a) which parts of the digestive system are affected by UVR?, b) is food transport through the gut affected?, c) is the food digestion (i.e. enzyme activity) process affected? In order to answer these questions we compared UVR-treated with control D. pulex as well as fasting versus fed D. pulex.

The second part of our study focussed on the indirect effect of UVR on the nutritional status of D. *pulex* due to UVR-induced alterations in their food (algae). Since most algae cannot precisely control their depth within the water column and are more subject to wind-induced mixing they are likely to be

even more exposed to the harmful effects of UVR than the more mobile grazers are. Several authors reported changes in the morphology and/or biochemistry of algae due to UVR-treatment (VINCENT & NEALE 2000). UVRprotecting substances (e.g. mycosporine-like amino acids) which are produced by some phytoplankton in the presence of UVR (LAURION et al. 2002) have been shown to be retained in copepods (TARTAROTTI et al. 2001) but such adaptations have not been reported in *Daphnia*. Changes in polysaccharide, protein and/or lipid content (ARTS & RAI 1997) as well as fatty acid (FA) composition (ZELLMER et al. 2004) following exposure to UVR may affect the nutritional status of algae.

Thus, we tested whether UVR-treated food algae modify the sublethal parameters examined in UVR-treated *D. pulex* compared to animals fed control food. Our goal was to gain insights into the sublethal effects that solar UVR may have on the planktonic food chain in subarctic ponds.

# Material and methods

### Site description

All field experiments were conducted with *Daphnia pulex* collected from Tibors Pond in June 2003 and June 2004 in the immediate vicinity of the biological research station of the University of Helsinki in Kilpisjärvi, Finland (69° 03' N, 20° 50' E). This pond is situated at the edge of the tree line and is, in general, extremely shallow (~ 20 cm depth) with a single small  $(2 \text{ m}^2)$ , deep  $(Z_{max} = 1 \text{ m})$  depression; the only region in this



Fig. 1. Radiation transmission (%) through 1 cm of water from Lake Kilpisjärvi and Tibors Pond. Scanning performed with a Bio 50 Spectrophotometer by Cary. Insert: Transmission through acetate and WIPA foils.

pond where D. pulex was found. Water transparency was high (Fig. 1) and there were very few aquatic macrophytes.

### Algal cultures

The green algae Ankistrodesmus gracilis (Chlorophyta) was used as food source for the daphnids and was grown in sterile medium (as in ZELLMER 1995) in 500 ml flasks. For the field experiments the cultures were placed inside a lab window and were thus exposed to long hours (midnight sun) of natural sunlight without UVR. Flasks were shaken several times per day. Two algae cultures (50 ml of media in 250 ml beakers) were started every day. Both beakers were covered with filters (UVR-absorbing WIPA foil, WIPA Technik, Tettnang, Germany, or UVR-transmitting acetate foil, Clarifoil, England, Fig. 1) and were placed in direct sunlight on a bench outside the lab. Both treatments (with or without UVR) therefore experienced different radiation regimes but were exposed to identical temperatures during the experiments. Cultures were grown for 2 d and thereafter fed to the daphnids according to the experimental treatments described below (for details see ZELLMER et al. 2004). For the laboratory experiments aliquots were taken from the stock cultures of A. gracilis and Scenedesmus obliquus growing in exponential phase, centrifuged and resuspended in boiled and filtered (folded paper filters, Schleich and Schuell, Germany) lake water (Lake Wallwitz, 51° 35' N, 11° 55' E) before feeding of surplus food quantities to stock cultures of Daphnia. For the life table experiments, an aliquot was taken from the stock culture three times a week, centrifuged and resuspended to an optical density of 1.1 at 750nm (equivalent to 1.5 mg C L<sup>-1</sup>). For both treatment cultures (with or without UVR), 2 ml (1.4 µg DW) of the suspension were added to 50 ml of medium each into 200 ml plastic bags (Whirlpak, Neolab, Heidelberg, Germany). Both Whirlpaks were aerated and placed in a light bank with white light only. Three times a week, at 2d intervals, the Whirlpak bag of the UVR-treatment was placed horizontally for 20 min under two Q-Panel bulbs (Pausch Messtechnik, Haan, Germany; intensity: UVB =  $295 \,\mu W^{-1}$  $cm^{-2} s^{-1}$ , UVA = 52.1  $\mu$ W<sup>-1</sup> cm<sup>-2</sup> s<sup>-1</sup>) covered with acetate foil to remove any UVC radiation. Thereafter the bag was returned to the light bank (recovery). Thus after 7d the algae experienced three 20 min UVR treatments in total followed by 24 h of recovery each time. Following this 7d period algae were centrifuged and resuspended in purified lake water to an optical density of 1.1 at 750 nm (equal to 1.6 µg DW ml<sup>-1</sup>).

### Daphnia cultures

For the field experiments *D. pulex* were caught in the morning prior to the experiment. 50 vigorous adult *D. pulex* were added to each experimental plastic container (150 ml lake Kilpisjärvi water, pre-filtered through 160- $\mu$ m sieve, 4 cm deep water column). Four replicate containers were added for each treatment resulting in a total of 200 *Daphnia* per treatment. *D. pulex* were fed equal quantities of *A. gracilis* (equivalent to 1.5 mg C L<sup>-1</sup>) and exposed to natural sunlight in a 10 °C water bath for 5 or 8 d. Ungrazed algae settled to the bottom of the containers and thus were considered to represent *ad libitum* feeding conditions. The containers were either covered with acetate foil (plus UVR) or WIPA (minus UVR) foil.

In the laboratory, stock cultures of D. pulex were maintained in glass jars (15 °C, dim white light) in lake water (Lake Wallwitz), which had been boiled, filtered (folded paper filters, Schleich and Schuell, Germany) and stored in a cold room (7°C) until use. To start the life table experiment gravid D. pulex were separated from the stock cultures in glass jars filled with purified lake water and fed A. gracilis or S. obliquus depending on the experiment. Released neonates of D. pulex were removed within 48 h and evenly distributed in 4 plastic jars (200 ml) with 150 ml of purified lake water each to a density of 50 neonates per beaker. Since we used neonates from a general population of gravid females, biases as a result of genetic differences between the treatment groups are unlikely. No attempt was made to isolate a specific genotype (clone) of D. pulex in these experiments in order to provide a more realistic simulation of a natural pond situation, i.e. where multiple clones of daphnids co-occur. Food quantity provided was 0.27 µg DW per Daphnia every other day (for details see ZELLMER et al. 2004). From the age of 3d, UVR-daphnids were irradiated 3 times per week for 10 min (or 15 min or 20 min in the food transportation experiments) under the same two Q-panel bulbs as above. The beakers were covered with acetate foil to remove any UVC radiation. It was previously determined that this dose rate did not cause acute mortality and was thus considered sublethal.

### Treatments and parameters measured

The following treatments were tested: 1) *Daphnia* not exposed to UVR fed algae not exposed to UVR 2) *Daphnia* not exposed to UVR fed UVR-exposed algae 3) *Daphnia* exposed to UVR fed algae not exposed to UVR and 4) *Daphnia* exposed to UVR fed algae exposed to UVR.

In the field experiments every evening and every 2d in the laboratory experiments all Daphnia individuals were scored for the following parameters under a stereo microscope (25-25× magnification): survival (%), intestinal damage (any changes in form or structure of the intestinal tube, i. e. bends, breaks or changes in diameter were scored as damage) and an estimate of the length of the green-colored portion of the intestine. In Daphnia's intestine roughly 75 % (foregut and midgut) usually appears bright green in actively feeding animals 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. We assumed that the green part of the intestine represented the largely undigested mass of algae, thus providing a rough measure of the digestive competency of D. pulex (ZELL-MER et al. 2004). This scoring method was fast and non-invasive. Animals appeared not to be stressed heavily by it since survival in the controls (no UV-treatments) was very high. Dead animals and neonates were removed.

After scoring, the *Daphnia* were returned to the containers and fed providing the algae enough time to settle during the night so as not to unduly alter the UVR-transmittance the next day due to suspended algae.

#### **Enzyme analysis**

Enzyme analysis on *D. pulex* was conducted immediately after the termination of the experiment at the field station. Enzyme activities were measured in whole body homogenates of groups of *Daphnia* individuals (n = 11 and 15 animals for the  $\alpha$ -amylase and cellulase assay, respectively). *D. pulex* were isolated from original pond water or the experimental containers by pipette. Water was filtered out using a plastic mesh and the mesh with animals was dried out carefully to remove residual source water. The animals were then placed into either 3.5 ml ( $\alpha$ -amylase assay) or 2.5 ml (cellulase assay) of cold sterile phosphate (Britton-Robinson) buffer pH = 8 (pH = 7 for cellulase assay) and homogenized in a glass homogenizer cooled by a surrounding ice bath. The homogenate was centrifuged at 6000 g for 7 min and the supernatant was taken for activity measurements at 30 °C after 1 h ( $\alpha$ -amylase assay) or 24 h (cellulase assay). Total soluble protein content was measured in 100 µl sub-sample of homogenate using the Bradford reagent (SIGMA, product No. B6916).

 $\alpha$ -Amylase assay: enzyme activity was measured using the tablet S-test (STU, Bratislava, Slovakia) with specific chromolytic substrate (URBÁŠEK & STARÝ 1994). One ml of supernatant was added to 50 mg of chromolytic substrate and a drop of toluene was added as a bactericide. This mixture was incubated at 30 °C for 1 h. Incubation was finished by adding 2 ml of acetone solution (900 ml of H<sub>2</sub>O, 100 ml acetone, 10 g Na<sub>2</sub>CO<sub>3</sub>). After centrifugation (6000 g, 7 min) the light absorbance of the final colored supernatant was measured at 620 nm. The results are expressed in mg of decomposed substratum per hour per individual (per mg of soluble protein).

Cellulase assay: enzyme activity was measured using carboxymethyl cellulose (CMC) as a substrate and assaying for the final concentration of glucose as the reaction product (ŠUSTR & CHALUPSKÝ 1996). A 0.5 ml aliquot of supernatant was incubated with a 0.5 ml suspension of CMC (0.1g of CMC in 5 ml of phosphate buffer). A drop of toluene was added as a bactericide. This mixture was incubated at 30 °C for 24 h. Incubation was finished by adding 0.3 ml of the incubation mixture to 1 ml of trichloracetic acid (50 g L<sup>-1</sup>). After centrifugation 0.2 ml of the supernatant was added to 1 ml of glucose-oxidase reagent for enzymatic determination of glucose (GLUCOSE GOD-250, Lachema, Czech Republic). Absorbance at 492 nm was measured after 30 min incubation at room temperature (about 22 °C, 30 min at 15-25 °C is sufficient safely for oxidation of all glucose in the sample according to the manufacturer).

# Determination of the food transportation rate through *Daphnia*'s intestine

To test whether the rate of food transportation through the gut is affected by UVRtreatment of *D. pulex* and/or its food we measured the time until control or UVRtreated *S. obliquus* passed through the entire intestine of UVR-treated or control *Daphnia*. In this experiment we chose three different UVR-treatments for the *Daphnia*: 10 min, 15 min and 20 min of UVR-irradiation for the last 5 d before the start of the grazing experiments. At an age of 13 d, *Daphnia* were transferred into a yeast suspension for 2 d. In this way the entire gut of these animals was filled with cream-colored yeast cells. The *Daphnia* were then rinsed in fresh culture water and transferred into a S. obliquus solution (OD<sub>750 nm</sub> = 0.68). At 5 min intervals Daphnia were checked under the microscope and the length of gut occupied by algae (as shown by the advancing green color) was determined. Daphnia were allowed to graze for 30 min and were then returned to the beakers with the yeast solution. After 2 h (following which their intestines were again completely filled with cream colored yeast cells) the grazing experiment was repeated with UVR-treated S. obliquus. Each treatment had between 2 and 3 replicates with 20 animals each. Due to the high mortality in Daphnia from the 20 min UVR treatment this portion of the experiment had only 1 replicate.

UVR-radiation was measured with a calibrated, cosine-corrected UVR-meter (X9, Giga-Hertz Optik, Munich, Germany) with separate sensors for UVA and UVB. During the field experiments we continuously measured integrated UVA and UVB surface intensities from 7 a.m. to 7 p.m. On sunny days, the UVB maximum was 0.22 mW cm<sup>-2</sup> s<sup>-1</sup> and the UVA maximum was 0.04 mW cm<sup>-2</sup> s<sup>-1</sup>.

We used one-way ANOVAs (Origin ver. 5.0) to test for main effects between the three different treatments on each day of each of the experiments. Enzyme activity data were tested using a non-parametric Kruskal-Wallis ANOVA (STATISTICA ver. 6.0).

### Results

#### Direct effect of UVR on *Daphnia pulex*

The effects of UVR-exposure on *Daphnia pulex* were similar between the field and the laboratory experiments (Figs 2 and 3). UVR treatment of *D. pulex* resulted in increased mortality, an extension in the length of the green-colored section of the intestine and an increase in gut damage compared to control *D. pulex*.

The transportation rate of algae through the intestine of the *D. pulex* increased with increasing UVR-exposure (Fig. 4). In control animals (no UVR)  $80 \% (\pm 28.3 \%; SD)$  of the animals had completely green intestines after 10 min., which was recorded already after 5 min in *D. pulex* with the longest (20 min, no SD available) UVR-exposure. In addition, 12.5 % (no SD available) of the UVR-treated animals did not ingest any algae during the first 10 min of grazing in comparison of  $3.3 \% (\pm 4.7; SD)$  of the control animals.

Digestive enzymes activity of *D. pulex* exposed to solar UVR for 8 d in the field experiment is shown in Fig. 5 (left panel). Three different parameters were tested: the total activity of  $\alpha$ -amylase (mg ind<sup>-1</sup> h<sup>-1</sup>) and cellulase ( $\mu$ M ind<sup>-1</sup> h<sup>-1</sup>), the body protein content as a measure of biomass ( $\mu$ g ind<sup>-1</sup>) and the protein specific activity (mg mg<sup>-1</sup> h<sup>-1</sup>) or ( $\mu$ M ind<sup>-1</sup> h<sup>-1</sup>) as a measure of the biomass related  $\alpha$ -amylase and cellulase activities respectively. The total enzyme activity in UVR-exposed compared to control animals showed a decreasing trend. The body protein content tended to be lower in UVR-treated *D*, *pulex* compared to the controls, too. In contrast to this, the protein-specific ac-



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Fig. 2. Clockwise from top left; survival (%), mean index (range 0 to 1) representing the length of the green-colored portion of the intestine (see Methods) and % Daphnia pulex with damaged guts. D. pulex from Tibors Pond exposed to solar radiation with-/without UVR, fed Ankistrodesmus gracilis cultured with/without solar UVR in Finland (July 2003). Means and S.E. of 4 replicates with 50-70 D. pulex.

tivity of digestive enzymes showed a tendency to increase, however due to the small number of replicates the statistical analysis failed to find significances (Kruskal-Wallis test, p > 0.2).

Change of enzyme activity in fasting control animals was not identical to that of UVR-exposed animals (Fig. 5 right panel). Although starvation of *D. pulex* (no UVR-treatment) resulted in a decreasing trend of body proteins as was the case in UVR-treated animals, total enzyme activities of fasting *D. pulex* showed a non-significant increase (Kruskal-Wallis, p > 0.2) accompanied with non-significant increase of protein specific activity (Kruskal-Wallis, p > 0.24 or 0.12 in  $\alpha$ -amylase and cellulase respectively) after 5 d of starvation.

# Indirect effect of UVR-treated food on Daphnia pulex

While UVR-irradiation of *D. pulex* caused direct effects on the animal's physiology, indirect effects may also be caused due to modifications in the digestibility and/or quality of UV-exposed food. The life-table experiments in the laboratory and in the field (Figs 2 and 3) showed no effect of UVR-treated algae on the survival of *D. pulex*. However, if *D. pulex* were repeatedly exposed to UVR, UVR-treated food resulted in higher mortality compared to control



Fig. 3. Clockwise from top left; survival (%), mean index (range 0 to 1) representing the length of the green-colored portion of the intestine (see Methods), % Daphnia pulex with damaged guts, and % D. pulex with partly empty guts. D. pulex were exposed to standardized radiation treatments in the laboratory with/without UVR and were fed Scenedesmus obliquus cultured under standardized radiation with/without UVR. Means and S.E. of 50 D. pulex.

food in both experimental sets. In contrast, the parameters characterizing the intestine of UV-treated *Daphnids* were not additionally affected by UVR-treated food.

The gut passage rate of UVR-treated algae through the intestine of control *D. pulex* and *D. pulex* which had received increasing doses of UVR-irradiation is shown in Fig. 4 b. There are clear differences in the gut passage rate of control algae (Fig. 4 a). In control animals, the non-UVR-algae were transported very quickly through the entire gut. After 15 min of grazing, in 90% ( $\pm$  14.1% SD) of the control *D. pulex* the gut was completely filled (100%) with green algae and in 6.7% ( $\pm$  9.4% SD) it was filled to 75%. At the same time, only 15.6% ( $\pm$  13.3% SD) of the control animals showed a completely filled gut if UVR-treated algae were fed, yet in 78% ( $\pm$  13.3% SD) of the animals 75% of the intestine was green (Fig. 4 b). Even 30 min of grazing only increased the percentage of animals with a completely green gut to 13.3% (no SD available) if UVR-treated food was provided 90% ( $\pm$  14.1% SD) in the control algae group after 15 min. This situation was modified if the *Daphnia* themselves had been UVR-exposed. As was recorded for the control-algae experiment, direct UVR-treatment of *D. pulex* accelerated the transport of UVR-treated algae



Fig. 4. Food transport through the intestine of *Daphnia pulex*. D. pulex had been exposed to standardized radiation treatments with/without UVR prior to the experiment. a) Scenedesmus obliquus with no UV-pre-treatment was provided as food source. b) S. obliquus with UV-pretreatment was provided as food source.

through the intestinal tube. With increasing UVR-exposure time of the Daphnia, the percentage of D. pulex with totally green guts increased and the time in which this stage was reached was shortened. In addition, the rate at which



### Fig. 4 b.

food was transported from the midgut (75%) of the gut green) to the hind gut (100%) of the gut green) became more rapid with increasing UVR-treatment of the *Daphnia*. This was true for all UVR-treatments of the daphnids.

The percentage of *D. pulex* which did not ingest any algae was much higher in *D. pulex* exposed to high UV doses (20 min) (80% (no SD available) after 5 min of grazing, Fig. 4 b) compared to *D. pulex* exposed to a lower dose of UVR (0% in all shorter UV-treatments after 5 min of grazing).



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Fig. 5. Total and protein-specific activity of digestive enzymes and body protein content of *Daphnia pulex* exposed to UVR (left) and fasting (right). Left y-axis refers to total activity and to body protein content in cellulase, right one refers to protein-specific activity and to body protein content in amylase. DkAuv = control *Daphnia* fed on UVR treated algae, DuvAk = UVR treated *Daphnia* fed on control algae, DuvAuv = UVR treated *Daphnia* fed on UVR treated *Daphnia* fed on UVR treated *Daphnia*, S. E.

Feeding of UVR-treated algae resulted in a decrease of body protein content as well as reduced total enzyme activities of both cellulase and amylase in D. pulex after 8d (Fig. 5 left panel). However, due to the low numbers of replicates only the decrease in total cellulase activity was significant (Kruskal-Wallis, p < 0.044). The protein-specific activity of digestive enzymes remained untouched showing rather an increasing trend (Kruskal-Wallis test, p > 0.2, Fig. 5 left panel). Feeding of UVR-exposed algae enforced the decrease of total amylase activity, but reduced the decrease of body proteins as well as the increase of protein-specific activity of amylase in UVR-exposed D. pulex.

As was stated above, fasting control *D. pulex* also showed a decreasing trend of body proteins (Fig. 5 right panel). In contrast to the both direct and indirect UVR-treatments, the total enzyme activity showed a non-significant increase (Kruskal-Wallis, p > 0.2) in fasting animals accompanied by a non-significant increase in protein specific activity (Kruskal-Wallis, p > 0.24 or 0.12 in amylase and cellulase, respectively) after 5 d of starvation.

# Discussion

Planktonic organisms in ponds are exposed to varying doses of solar UVRradiation with only limited possibilities to escape. Many studies have described the detrimental effects of solar UVR on zooplankton if animals were exposed in shallow water (e. g. ZELLMER 1995, 1998, ZELLMER et al. 2004, LEECH & WILLIAMSON 2001, RAUTIO & KORHOLA 2002). UVR-induced changes to the biochemistry and productivity of phytoplankton have also been reported (BEHRENFELD et al. 1995, VINCENT & NEALE 2000). However, the fact that, in nature, UVR-exposed algae are also grazed by UVR-exposed herbivores, enhancing the possibility of food chain effects, has not often been examined. In this study we examined whether the direct effect of UVR on *D. pulex* is further modified by an interactive effect of UVR on the algal food.

Despite the differences in spectral composition and intensity between solar UVR and artificial UV sources some of the results of field and standardized laboratory experiments are comparable. In both approaches repeated sublethal UVR-irradiation of D. pulex resulted in increased mortality (Figs 2, 3). Also, in field studies, UVR-induced mortality has recently been shown to be preceded by damage to the intestine of D. pulex (ZELLMER et al. 2004). The results of our laboratory experiments confirm these findings: UVR-treated D. *pulex* showed an increase in the length of the green-colored portion of the gut, increased percentages of D. pulex with damaged guts and increased mortality compared to control animals with no UVR-treatment (Fig. 3). If D. pulex were fed on UVR-treated algae these negative effects increased significantly suggesting a food chain effect of UVR, both under field and laboratory conditions. In order to get a more detailed view of the impact of UVR on the digestive process of D. pulex we studied food transportation through the gut, amylase and cellulase enzyme activity in UVR-treated and control animals, both with control food and UVR-treated food.

The rate at which algae is transported through the gut of D. pulex determines the time available for digestion and assimilation. Control animals fed control food provided a reference for "normal" gut passage time (Fig. 4 a). If control D. pulex instead were fed UVR-treated algae, food transportation basically stopped once the midgut was filled with algae (Fig. 4 b). Even after 30 min of grazing the proportion of D. pulex with entirely filled guts did not exceed 13.3 % (compared to 90 % after 15 min with control food). The midgut has been identified as the major site of absorption (PETERS 1987). Here food is solubilized and the solutes are absorbed by the epithelium lining the intestine. Our results indicate a longer retention time of UVR-treated algae in this part of the digestive system of D. pulex compared to individuals fed control algae. Several authors have reported reduced digestibility of UVR-exposed algae (VAN DONK & HESSEN 1995, VAN DONK et al. 2000) and a high percentage of

undigested cells passing intact through the gut. Longer retention times in the midgut may counteract this phenomenon (but see BOERSMA & WILTSHIRE 2006).

The direct effect of UVR-treatment on D. pulex with respect to food transportation through the gut was tested by exposing them repeatedly to three different doses of UVR (10, 15 or 20 min) prior to the grazing experiment (Fig. 4 a). UVR-irradiation of D. pulex resulted in an increasing, dose-dependant, percentage of animals with an entirely filled green gut compared to controls. In addition, as in control D. pulex, the time needed to fill 100 % of the gut with green algae was shorter with increasing UVR-exposure of the animals. This reduction in residence time of algae in the midgut in UVR-treated D. pulex is likely to affect the digestion and assimilation process negatively. These conclusions are reinforced by the increased percentage of UVR-treated animals with damaged guts or guts that showed partly or even completely empty intestines. All of these observations suggest a malfunction in the digestive process which should lower the nutritional benefit of the food to D. pulex. Daphnia exposed to UVR may therefore suffer from some degree of malnutrition comparable to the early stages of fasting animals. Observations on fasting animals, not exposed to UVR, also showed higher percentages of animals with damaged and empty guts compared to fed animals, resembling those effects of the UVR-treated animals (ZELLMER, unpubl.).

Enzymes such as cellulase and amylase play an important role in the digestion of storage and structural polysaccharides contained in algae cells. The cellulases in invertebrates are believed to originate predominantly from symbiotic bacteria or from ingestion of microbial enzymes but may also be produced by the animals (WILDISH & POOLE 1970). Transmission electron microscopy of *Daphnia magna* intestines did not reveal a gut flora and the cellulose utilization demonstrated in individuals of this species feeding on sterile food (SCHOENBERG et al. 1984). However, while evidence is lacking at present, microbial symbionts in microcrustacea cannot be ruled out (SCHOENBERG et al. 1984).

Our experiments show that protein content in the body is reduced if *D. pulex* or their food had been UVR-treated (Fig. 5, left panel). This situation corresponds to malnutrition leading to reduction of growth and using of some body proteins as energy source (Fig. 5, right panel). The total activity of both enzymes were somewhat reduced, but overall enzyme function is not seriously damaged as is clear from the increasing trend of protein-specific activity. The UVR changes are similar to those induced by short-term starvation with the difference, that the decrease of total digestive enzyme activity was not observed after starvation. It may be expected that digestive enzyme production and secretion in continuously feeding invertebrates, such as *Daphnia*, did not vary due to short-term starvation similarly to soil detritivorous oribatid mites

(HUBERT & ŠUSTR 2001), unlike discontinuous feeders as exemplified by blood sucking insects whose enzymes may be produced on demand (CHAP-MAN 1985). The increase of protein-specific enzyme activity may be interpreted as a reaction compensating energy malnutrition by more efficient digestion of usually permanently available food. The second possible explanation is that the amounts of digestive enzymes are less reduced due to malnutrition than other body proteins.

In summary, our results demonstrate that solar UVR-irradiation on planktonic organisms of shallow habitats may have complex effects on plankton at different levels in the community. Primary producers as well as grazers are directly affected by radiation which does not necessarily kill the organisms. Even at the low UV doses used in the laboratory experiments we observed the same changes in *D. pulex* as were recorded in the field experiments. In addition, we showed that the effects of UVR on *D. pulex* are enhanced if both the zooplankter and its food are exposed to UVR. We showed that this damage occurs at different levels of the digestive process with food related effects only (food transportation rate) as well as food and grazer related effects (gut damage and enzyme activity). The grazer related effects were similar to those determined in fasting animals. Thus, sublethal UVR treatment may to *D. pulex* result in comparable situations as low food or even fasting conditions, depending on the degree of damage caused in the animal.

Earlier we showed, in standardized laboratory experiments (ZELLMER 1996), that the reproductive output as well as reproductive success in the UVR-stressed daphnids is reduced. Such effects influence the population on a community level even if acute mortality due to the UVR-stress does not occur. In the natural pond populations of Daphnia from sub-arctic ponds of Lapland gut damage has only rarely been observed during routine sampling during the last 7 years with the exception of 2003, an extremely hot and dry year (ZELL-MER, unpubl.). In 2003 water levels were extremely low allowing UVR to penetrate to the bottom of the ponds. In mid July, 10% of the sampled animals showed the same symptoms of gut damage as had been recorded in the experimental animals described here. Therefore, D. pulex appear to be able to counteract the damages caused by UVR to a certain degree if the exposure time is limited perhaps because repair mechanisms can be brought into play. Enzymatic activity such as amylase and cellulase activity seem to be relatively insensitive to UV stress imposed here and we conclude that they may therefore not be suitable biomarkers of UVR stress in this context.

We conclude that increasing levels of UVR especially in early spring when DOC levels are low and many species are reproducing may affect the biotic communities of shallow subarctic ponds at different levels of the food chain and in subtle ways possibly leading to shifts in community structure to more tolerant species or genotypes.

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