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UV radiation and low calcium as mutual stressors for *Daphnia*

Abstract—The cladocerans *Daphnia magna* and *D. tenebrosa* were exposed to daily 6-h ultraviolet (UV) radiation along a gradient of ambient Ca in the medium (0.5, 1.0, 5.0, and 10 mg Ca L⁻¹) at nearly constant conductivity. Integrated irradiance over 300–400 nm was 35.95 W m⁻², corresponding to maximum outdoor intensities during midsummer. *D. magna* was most susceptible to UV radiation, but the UV susceptibility for both species increased significantly with decreased Ca content. Ca uptake (⁴⁵Ca) was apparently not influenced by short-term (3 h) UV exposure; thus, although lipid peroxidation and membrane damage are likely mechanisms for impaired ionic uptake, the overall role of low Ca may be reduced stress tolerance. While general conclusions on other species and taxa are premature, the data indicate that water hardness could be a major determinant to UV susceptibility among Ca-demanding zooplankton species like *Daphnia*.

Calcium (Ca) is an essential element to crustaceans and other groups of invertebrates with a calcified exoskeleton. Ca deficiency in soft-water localities may be a major determinant to success and distribution of benthic crustaceans (Sutcliffe 1978; Jussila 1997). There is also recent evidence that Ca deficiency could act as an important determinant of zooplankton community structure (Tessier and Horwitz 1990; Hessen et al. 1995). Laboratory studies with *Daphnia magna* suggest that this species has a lower threshold of 0.5–1.0 mg Ca L⁻¹ for survival and that growth in juveniles that have the highest specific Ca requirements may be subsaturated <10 mg Ca L⁻¹ (Alstad et al. 1999, Hessen et al. 2000).

The major sources of Ca for crustaceans are water and food, with water being the most important in zooplankton (Cowgill et al. 1986). The potential role of Ca deficiency will surely be most relevant for soft-water localities. Such lakes may show a scattered distribution, depending on local geology, but may also cover larger geographical areas like the Canadian Shield lakes (Neary and Dillon 1988) and major parts of Scandinavia. A national survey of 1,500 Norwegian lakes revealed that median Ca concentration was 0.9 mg Ca L⁻¹, 90% of the localities had less than 2.5 mg Ca L⁻¹ and almost no localities exceeded 5 mg Ca L⁻¹ (Skjelkvåle et al. 1997). Reduction in the long-term inputs of anthropogenic sulfate in previously acidified areas may lead to further decreased weathering rates and thus decreased inputs of Ca to watersheds (Likens et al. 1996).

Correspondingly, ultraviolet (UV) radiation is among the physical factors that may constrain zooplankton production and that may affect community composition in surface waters (Siebeck and Böhm 1994; Hessen 1996; Williamson et al. 1999). A number of physiological attributes, as well as

water quality parameters, could influence zooplankton susceptibility to shortwave radiation. Large-scale changes, such as climatic warming and acidification, which both cause reduced levels of dissolved organic matter and thus increased UV attenuation, would pose a mutual stress on zooplankton (Schindler et al. 1996; Williamson et al. 1999).

Judging from the above-cited evidence for Ca deficiency in *Daphnia*, we wanted to test whether Ca deficiency could add to the detrimental effects of UV radiation. One likely mechanism could be impaired efficiency for Ca uptake under UV radiation owing to UV-induced oxidation of membrane fatty acids, causing lipid peroxidation (Fuchs and Packer 1991). If basic water quality parameters such as hardness would yield highly different UV susceptibility, this should call for a more differentiated view of potential damage caused by increased UV exposure in different localities. In this study, rather than elaborate on the potential of direct physiological interactions between UV radiation and Ca, we assess whether these major environmental factors could work as mutual stressors.

Methods—Two assays were performed with UV exposure of *Daphnia magna* and *D. tenebrosa* along gradients of ambient Ca. The stock cultures were reared in Elendt M7 medium according to the OECD guideline (OECD 1997). Whereas the *D. magna* clone originated from a rock pool population and had been kept in the laboratory for years, the *D. tenebrosa* clone was raised from a shallow and highly UV-exposed arctic population (Brandallaguna at Svalbard, cf. Hessen et al. 1999) 2 months prior to the experiments. Taxonomic affinities of *D. tenebrosa*, a member of the formerly labeled “Arctic *D. pulex* complex” was revealed by 12 C mtDNA sequencing (Hessen et al. 1999). The Elendt M7 medium was made by adding salts and vitamins to distilled water. The Ca gradient was made by regulating the additions of CaCl₂·H₂O to the medium, keeping other elements unchanged. Nominal concentrations were 0.5, 1.0, 5.0, and 10.0 mg Ca L⁻¹ (0.013, 0.025, 0.13, and 0.25 mM). pH was 7.80 ± 0.20, adjusted by additions of HCl or NaOH. Care was taken not to change ionic strength of the medium, and the gradient of Ca additions over the range 0 to 10 mg Ca L⁻¹ only yielded minor differences in ionic strength and conductivity. Ionic strength ranged from 50 to 55 × 10⁻⁴ M and conductivity ranged from 221–273 μS cm⁻¹ over this gradient. Thus, effects caused by differences in ionic strength or animal osmoregulating ability would supposedly be minor. All experiments were performed at constant temperature (18 ± 1°C) at dim, blue-white light (<30 μmol quanta m⁻² s⁻¹). Real concentrations of Ca were measured

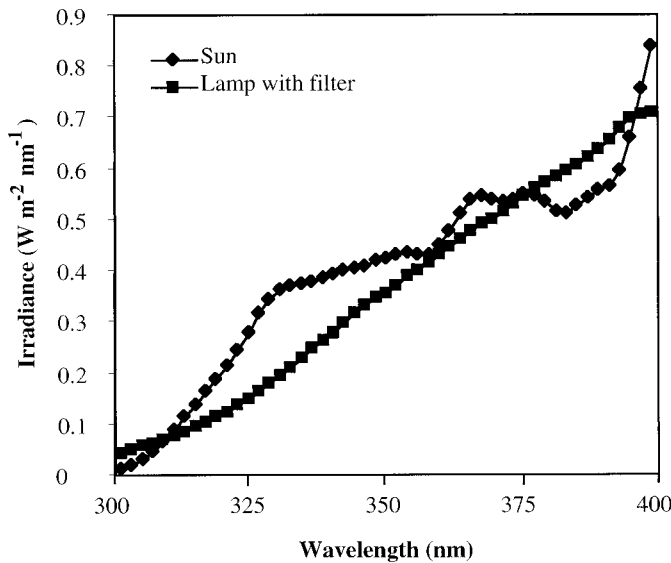


Fig. 1. Irradiation from xenon lamp with filters over 300–400 nm compared with solar irradiation at midsummer, noon (67°N).

at the start and end of all experiments and deviated less than 10% from nominal concentrations (Hessen et al. 2000). Ca content was analyzed on a flame atomic absorption spectrophotometer (Varian SpectrAA 10). A nitrous oxide/acetylene flame was used, and a 5,000 $\mu\text{g ml}^{-1}$ KCl solution was added to avoid ionization of Ca.

Adult individuals (~ 10 d old) were placed in their respective media for 7 d and fed *Selenastrum capricornutum* in surplus prior to the experiments. For all experiments, a total of 40, 10-d-old animals of the same age were kept at the respective Ca concentration for 7 d, meaning that all individuals had passed through at least one molt in that medium. To avoid contamination of Ca from food, a dense culture of *Selenastrum* was washed twice with Ca-free medium and frozen in small batches at -20°C . The 40 individuals were then distributed as following: two ($\times 10$) individuals for UV exposure in 150-ml quartz bottles, 10 individuals for unirradiated control, and 10 individuals for analysis of Ca content. This procedure was repeated three times for every Ca concentration, meaning six ($\times 10$ individuals) for UV exposure, three ($\times 10$ individuals) for control, and three ($\times 10$ individuals) for Ca analysis for every Ca concentration. For analysis of Ca concentration, the animals were rinsed in distilled water before they were dried at 60°C for 24 h. After cooling in a desiccator, dry weights were recorded to the nearest 0.1 μg with a Mettler ME 30 electrobalance. Digestion was performed in 50% HNO_3 at 120°C for 30 min, and each sample contained 10 animals. Ca was measured as described above.

UV light was provided with a 100-W Xenon lamp (AMKO model 02-A1020). By use of 93- μm cellulose acetate filters, the intensity and spectral quality of this lamp gave a close fit to midsummer outdoor irradiance, as revealed by a LICOR 1800 spectroradiometer (Fig. 1). Integrated irradiance over 300–315 nm was 1.096 and 1.092 W m^{-2} for filtered light and sun, respectively. The transition from UV-A to UV-B is set at 315 nm. For the 300–400-nm

Table 1. Time \times medium Ca concentration interactions in repeated measures analysis of variance model testing the effect of Ca concentrations in the medium on mean survival (%) of *D. magna* and *D. tenebrosa*.

	Wilks lambda	Approximate <i>F</i>	Number (df)	Density (df)	<i>P</i>
<i>D. magna</i>	0.0073	7.0917	24	38.305	<0.0001
<i>D. tenebrosa</i>	0.0063	3.4499	36	27.319	0.0006

range, corresponding intensities were 35.95 and 39.82 W m^{-2} , whereas standard integrated irradiance for PAR (400–700 nm) was somewhat lower for the lamp (282 vs. 350 W m^{-2}). The animals were exposed for 6 h each day, and the number of survivors were examined (not removed) until mortality was almost total for all Ca concentrations. No mortality was observed in the controls. To avoid contamination from Ca in the food, no food was added during the experimental period. Effect tests on survival under UV exposure, as related to Ca concentrations, was done by a repeated measures analysis of variance (ANOVA) model.

A test on Ca uptake was performed to control for an eventually impaired Ca uptake in UV-exposed individuals. Radioactive ^{45}Ca (Amersham ces 3, 200 mCi mmol^{-1} Ca) was added to final concentrations of 100,000 counts per minute (CPM) ml^{-1} to each of 6 quartz bottles with standard medium of 10 mg Ca L^{-1} . In a first set of experiments, 25 large (3.2–3.5 mm) individuals of *D. magna* were added to each of the bottles, and three bottles were exposed to UV radiation for 3 h under similar doses as those described above. The other bottles served as unexposed controls. From each bottle, five individuals were harvested after 1 and 3 h (under irradiation), and then at 9 and 24 h after irradiation was initiated. A second assay was performed with a clutch of 6-d-old (~ 2 mm) animals with synchronized molting cycle. In this assay, 50 individuals were added to each bottle, and 10 animals were harvested as described above, except that the first postradiation sample was taken after 6 h. Animals were carefully rinsed in cold medium and sorted individually into eppendorff vials. The animals were then digested as described above prior to counting in a 1500 Tri-carb Liquid Scintillation Counter on the ^{14}C preprogrammed window settings. This did not allow for use of internal standards but was adequate for comparison among treatments since counting efficiency was high ($>95\%$) and similar for all samples. Relative uptake of Ca was normalized to activity in the medium.

Results—Survival under UV exposure was strongly reduced with decreasing Ca concentrations for both species (Fig. 2). For *D. tenebrosa*, there was 20% mortality after 6 h exposure at 0.5 mg Ca L^{-1} , and an almost complete mortality had occurred 24 h after terminated UV exposure. A subsequent delay in mortality occurred at increasing Ca levels. A similar, yet even more pronounced effect was seen for *D. magna*, where survival periods after exposure were less than for *D. tenebrosa*, but again losses were clearly related to ambient Ca. Significant effects of Ca on survival under

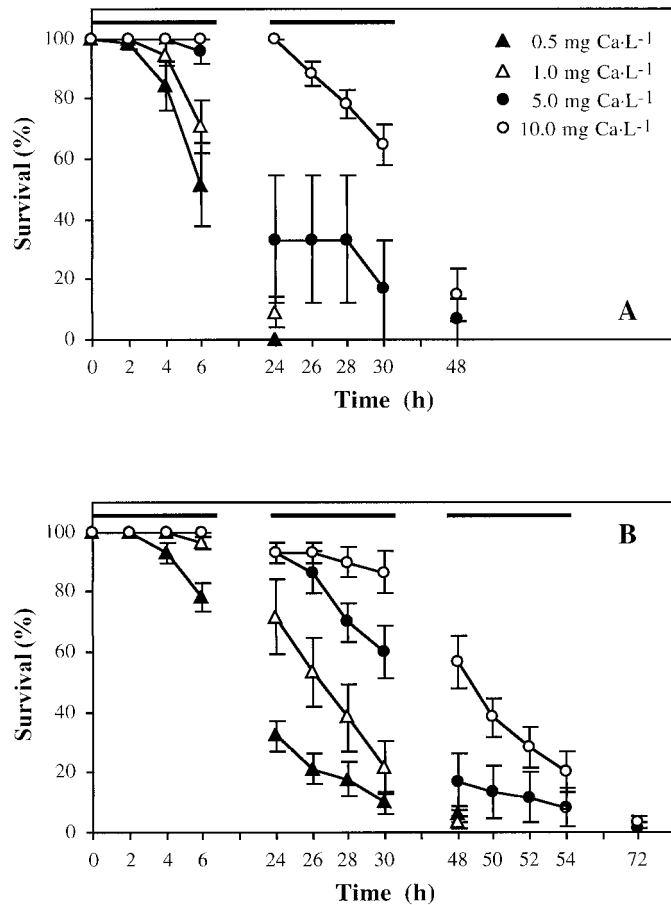


Fig. 2. Mean survival (\pm SE) of (A) *D. magna* and (B) *D. tenebrosa* reared in media with different Ca concentrations (nominal values) after different times of UV exposure. Each point represents the mean of six bottles with 10 animals in each bottle. Exposure periods given by bold line.

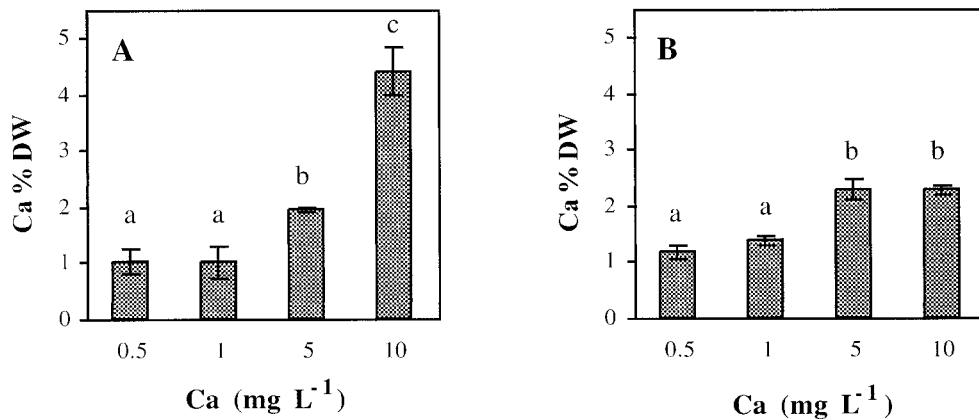


Fig. 3. Mean Ca percentage of dry weight (\pm SD) in (A) *D. magna* and (B) *D. tenebrosa* incubated for 7 d in media with different Ca concentrations (nominal values). $n = 3$ samples, each containing 10 animals for each Ca concentration. Different letters above figure bars denote significant ($P < 0.05$) differences between treatments (Tukey-Kramer HSD). Data for *D. magna* is obtained from Alstad et al. (1999).

UV radiation ($P < 0.001$, ANOVA) was found for both species (Table 1). No mortality was observed in the controls over a 3-d period. The specific Ca content decreased significantly with decreasing Ca in the medium for both species (Fig. 3) from approximately 2% Ca of dry weight (DW) at 5 mg, to 1% Ca of DW at 0.5 and 1 mg Ca L⁻¹. At 10 mg, *D. magna* obtained far higher levels of Ca compared with *D. tenebrosa*, however.

UV radiation did not impair Ca uptake in any clear manner (Fig. 4). ⁴⁵Ca uptake in adult (20–25 d) and juvenile (6 d) individuals of *D. magna* did not differ systematically between controls and exposed individuals. Not even uptake of ⁴⁵Ca under direct exposure (1 and 3 h) was clearly impaired by UV radiation; in fact for adults, slightly higher activities were recorded from 3 h onward relative to controls. The adults covered a wider size span and did not have a synchronized molting cycle. Radioactivity increased steadily over a 24-h period, yet with lower time-specific uptake after 9 h. Very few molts were observed over the 24-h period among the adults. For the juveniles with synchronous molting cycles, Ca uptake was low for the first 6 h (the premolt period), and the radioactivity increased strongly after 24 h when >80% of the animals had molted.

The somewhat different susceptibility between *D. tenebrosa* and *D. magna* could be due to different tolerance to the experimental conditions but most likely reflects a real difference in susceptibility to UV radiation. Whereas the *D. magna* clone had not experienced any UV exposure for its numerous generations in culture, *D. tenebrosa* was recently inoculated from a highly UV-exposed locality. Siebeck and Böhm (1994) recorded major differences in UV susceptibility between nonmigrating *D. pulex* from a shallow, alpine pond and a migrating population of *D. galeata* from a deeper lowland lake. Such differences in evolutionary or short-term physiologically adaptations could also cause different UV susceptibility between *D. magna* and *D. tenebrosa*. The main message is, however, that there was a strong mutual stress of UV radiation and low Ca for both species. Low Ca in itself may indeed pose constraints on *Daphnia* performance.

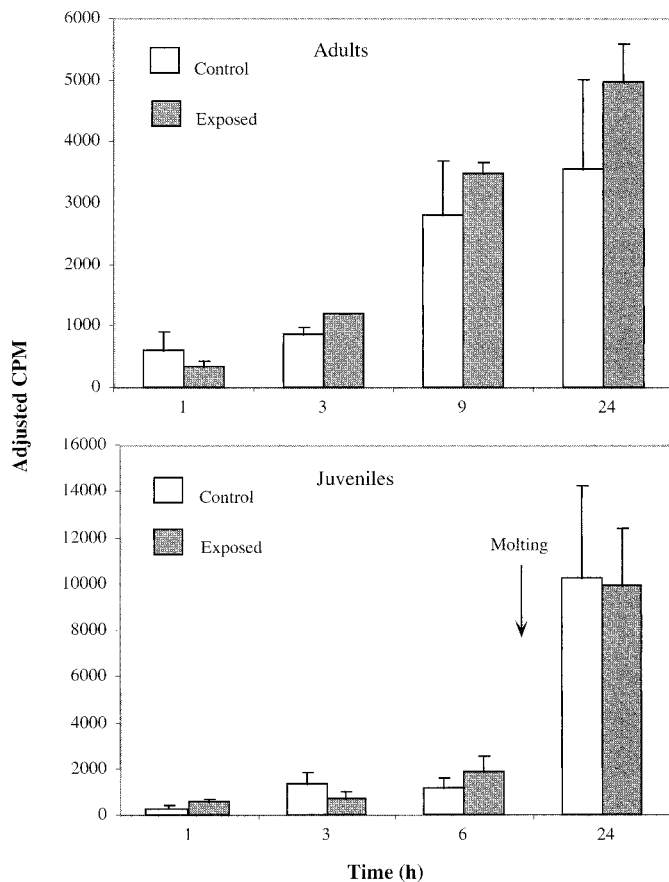


Fig. 4. Uptake of ^{45}Ca as CPM adjusted for number of individuals and radioactivity in the medium. UV exposure lasted 3 h. Mean activity \pm SD for three replicates for adults (3.2–3.5 mm) and juveniles (\sim 2 mm) of *D. magna*.

D. magna approach a lower threshold for survival at 0.5 mg Ca L $^{-1}$, and optimal Ca concentrations seem to exceed 10 mg Ca L $^{-1}$ (Alstad et al. 1999, Hessen et al. 2000). Yet no mortality was recorded in the absence of UV exposure. The causes for the increased UV susceptibility at low Ca concentration is not clear. *Daphnia* exposed to lethal doses of UV radiation frequently survive until molting, when animals show signs of swallowing and osmotic disorder that could indicate membrane damage. UV radiation produces reactive oxygen species (ROS) inside organisms (Hideg and Vass 1996). Interaction between excited sensitizers and triplet oxygen produces ROS like singlet oxygen, hydrogen peroxide, or superoxide, which in turn can lead to oxidation of membrane fatty acids, resulting in lipid peroxidation, oxidation of proteins, and DNA damage (Fuchs and Packer 1991). Judging from the assays with radioactive Ca, uptake of ionic Ca over membranes is not impaired by short-term UV exposure, however.

The observation that Ca uptake in intermolt was only minor compared to postmolt Ca uptake (Fig. 4, bottom) indicates that there was no significant intermolt medium–exoskeleton exchange of Ca. However, because very little Ca is stored in *Daphnia* during molting (Alstad et al. 1999), the postmolt Ca uptake constitutes almost all intermolt body Ca.

Ca content was significantly reduced at low ambient Ca. A major part of crustacean Ca uptake is by active transport (Marshall et al. 1964, Flik et al. 1994). If active transport constitutes a larger part of Ca uptake at low ambient Ca concentrations, shortage of surplus energy might explain the lower Ca content of *Daphnia* molting at low ambient Ca, as well as their increased susceptibility to UV exposure. The apparently higher demands for Ca in *D. magna* could also explain the somewhat higher UV susceptibility of this species.

Although the mechanisms may be manifold, the overall conclusion stands firm: low Ca and UV radiation may act as strong mutual stressors for *Daphnia*, meaning that low levels of Ca increase the likelihood for UV damage. The response of other members of the *Daphnia* family, as well as other zooplankton taxa, remains to be tested. Judging from the high number of northern localities that have low levels of Ca and also commonly have low levels (<2 mg L $^{-1}$) of DOC, we believe that this may be a major determinant of UV susceptibility for Ca-demanding species over large geographical areas.

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Size-dependent visual predation risk and the timing of vertical migration in zooplankton

Abstract—Zooplankton commonly exhibit diel vertical migration (DVM), descending from food-rich surface waters during the day. If DVM is a tradeoff between avoiding size-selective visually hunting predators and maximizing energy gain, smaller bodied prey should enter surface waters earlier and leave later than larger, more visually conspicuous organisms. Conventional sampling technologies lack the temporal resolution to test this prediction. Here, we report on the first test of this prediction using a new submersible optical-acoustic imaging system capable of resolving the timing of migration of the euphausiid crustacean *Euphausia pacifica* Hansen. Smaller bodied animals consistently ascended as much as 30 min earlier and descended up to 45 min later than adults. The timing of vertical migration reflects how the size-dependent risk of attack by visual predators alters the tradeoff between feeding and predator avoidance, supporting the predator-avoidance hypothesis for DVM.

The risk of attack by planktivorous fish increases with ambient light level and prey characteristics affecting visibility such as body size, morphology, pigmentation, mobility patterns, and gut contents (O'Brien 1987; Ryer and Olla 1999). Large-bodied and highly pigmented zooplankton are disproportionately vulnerable to visual predators and are subject to heavy mortality when fish are abundant (Hrbáček 1962; Brooks and Dodson 1965; Brodeur 1998). Therefore, morphological or behavioral characteristics that result in a reduction in the intensity of visual predation on zooplankton should confer a gain in fitness.

Many of the behaviors exhibited by zooplankters, such as reduced feeding during daylight, ontogenetic vertical migrations, seasonal diapause, and diel vertical migration (DVM), can reduce the risk of attack by predators (Ohman 1988; Verity and Smetacek 1996). Although the proximate stimulus for DVM is thought to be diel changes in ambient light intensity (Ringelberg 1995), the adaptive significance of the behavior has been controversial. After scrutiny of alternative hypotheses, the present consensus is that DVM typically concerns predator avoidance (reviewed in Lampert 1989, 1993). DVM is generally thought to minimize spatiotemporal overlap with visually hunting predators in food-rich surface strata during daylight hours, but the behavior can also be reversed to avoid encounter with carnivorous predators, which are themselves migrating to avoid visual predators (Ohman 1990). By performing DVM, migrants avoid surface strata at times when they are most vulnerable to predators. The principal costs associated with this reduction in mortality are the energetic costs of migration and the decreased potential for population growth through part-time residence in relatively low-food, deeper strata (Ohman 1990).

The predator-avoidance hypothesis for DVM can be framed in the context of selection between habitats in which rates of energy gain and mortality differ. If DVM is primarily a mechanism by which organisms balance the conflicting requirements of maximizing net reproduction and predator avoidance, the behavior should vary with vulnerability to