ESCAPE FROM UV THREATS IN ZOOPLANKTON:
A COCKTAIL OF BEHAVIOR AND PROTECTIVE PIGMENTATION

LARS-ANDERS HANSSON,1,3 SAMUEL HYLANDER,1 AND RUBEN SOMMARUGA2

1Institute of Ecology/Limnology, Lund University, Lund SE-223 62 Sweden
2Laboratory of Aquatic Photobiology and Plankton Ecology, Institute of Ecology, University of Innsbruck, Technikerstrasse 25, Innsbruck 6020 Austria

Abstract. In order to avoid environmental threats, organisms may respond by altering behavior or phenotype. Using experiments performed in high-latitude Siberia and in temperate Sweden, we show for the first time that, among freshwater crustacean zooplankton, the defense against threats from ultraviolet radiation (UV) is a system where phenotypic plasticity and behavioral escape mechanisms function as complementary traits. Freshwater copepods relied mainly on accumulating protective pigments when exposed to UV radiation, but Daphnia showed strong behavioral responses. Pigment levels for both Daphnia and copepods were generally higher at higher latitudes, mirroring different UV threat levels. When released from the UV threat, Daphnia rapidly reduced (within 10 days) their UV protecting pigmentation—by as much as 40%—suggesting a cost in maintaining UV protective pigmentation. The evolutionary advantage of protective pigments is, likely, the ability to utilize the whole water column during daytime; conversely, since the amount of algal food is generally higher in surface waters, unpigmented individuals are restricted to a less preferred feeding habitat in deeper waters. Our main conclusion is that different zooplankton taxa, and similar taxa at different latitudes, use different mixes of behavior and pigments to respond to UV radiation.

Key words: carotenoid; copepod; Daphnia; lakes; limnology; mycosporine-like amino acid; phenotypic plasticity; pigment; trait compensation; ultraviolet radiation; UV radiation; zooplankton.

INTRODUCTION

In natural ecosystems, organisms are faced with fluctuations in predation, competition, and environmental threats like ultraviolet (UV) radiation, and these can act within both temporal and spatial dimensions. These threats can give rise to behavioral responses that may serve to maximize fitness, for example, running or swimming away, as do gazelles on the savannah or small fish in schools. Alternatively, organisms may respond phenotypically, i.e., protecting themselves from the threat by altering their morphology. Such morphological protection may either be constitutive, i.e., present throughout the organism’s lifetime, or induced when needed (Tollrian and Harvell 1999, Brönmark and Hansson 2000).

Among aquatic organisms such as zooplankton, predation defenses include spine formation (e.g., rotifers; Stemberger and Gilbert 1987), helmet and neck teeth formation (many cladocerans; Tollrian 1995), as well as diel vertical migration (Lampert 1989). Environmental threats, such as ultraviolet radiation (UV), can induce defenses both in the form of behavioral responses, e.g., vertical migration (Rhode et al. 2001), or phenotypic responses, such as accumulation of photoprotective compounds (Goodwin 1986, Hebert and Emery 1990, Shick and Dunlap 2002, Tollrian and Heibl 2004). We here use the term phenotypic as a physiological response in pigmentation.

Stratospheric ozone reduction is threatening aquatic ecosystems via an increase in ambient UV radiation (Madronich et al. 1998), and in addition, recent studies indicate a 10% increase, from 1983 to 2003, in UV radiation fluxes reaching north temperate regions due to alterations in cloudiness (Josefsson 2006). The main concern with UV radiation is that it produces DNA damage, and current levels of UV radiation at temperate latitudes are associated with increased zooplankton mortality (Zagarese et al. 1994, Leech and Williamson 2000). To handle this, zooplankton commonly synthesize photoprotective compounds that function either as sunscreens or as scavengers of photo-produced radicals (Hairston 1976, Hessen 1994, Vinebrooke and Leavitt 1999). Among copepods, such pigments are carotenoids and mycosporine-like amino acids (MAAs; Hairston 1976, Sommaruga and Garcia-Pichel 1999, Hansson 2000, 2004). For cladocerans, on the other hand, melanin is the most important photoprotecting compound, but highly melanized specimens are almost exclusively found in arctic or high-altitude environments (Hebert and Emery 1990; see Plate 1).
Among herbivorous zooplankton, competition for food is often intense, and usually the greatest quantity, although not necessarily the highest quality (Williamson et al. 1996), of algal food is close to the surface, but this is also where UV intensities are highest. Thus, zooplankton are faced with a trade-off situation where the best area for feeding is also the most dangerous with respect to UV environment. Accordingly, behavioral responses to UV have been reported among zooplankton as well (Rhode et al. 2001, Williamson et al. 2001, Alonso et al. 2004). Since colored or chromophoric dissolved organic matter attenuates UV radiation efficiently (Morris et al. 1995, Tartarotti et al. 2001), depth can often serve as a refuge from UV radiation. Generally, vertical migration is seen as a predator response (Lampert 1989), but this behavior has also been reported in lakes without predatory fish; thus, UV may be a prompt for vertical migration (Williamson et al. 2001). This response by *Daphnia* has been demonstrated in both laboratory and field studies (Hessen and van Donk 1994, Storz and Paul 1998, Leech and Williamson 2001, Rhode et al. 2001); but with respect to copepods, reports on behavioral responses are few and contradictory (Aarseth and Schram 1999, Boeing et al. 2004, Hansson 2004). Although zooplankton diel migration is a predator-avoidance behavior, it seems to be primarily induced by light:dark cycles. Zooplankton relaxed their diel migratory behavior both at constant darkness (Loose 1993) and in natural systems with constant daylight (Hansson et al. 2007), irrespective of whether predators were present or not.

Both behavioral and morphological responses may be categorized as preventative measures against UV damages. A third possibility for zooplankton to avoid such damage is photoenzymatic repair of damaged DNA (Zagarese and Williamson 1994, Rocco et al. 2002, Macfayden et al. 2004). This mechanism includes an enzyme (photolyase) that repairs damaged nucleotides by using energy from visible light (Todo et al. 1993). Overall, photoenzymatic repair as well as responses in pigmentation and behavior may function as complementary traits (Dewitt et al. 1999, Rundle and Brand 2001) and constitute a cocktail of tactics to reduce the threat from ultraviolet radiation.

In the present study, we focus on the preventative responses in behavior and phenotype (pigments) with the aim of providing a combined view of when, where, and how much different zooplankton use such responses when challenged with UV radiation. We also aim to experimentally test whether zooplankton can switch between protective strategies when exposed to UV. We performed our studies in controlled laboratory experiments at two different latitudes: in subarctic Siberia with high UV-radiation levels, and temperate Sweden where the UV threat is modest. We appraised the choices the zooplankton made with respect to phenotypic and behavioral responses, i.e., in their way of utilizing and combining the cocktail of available protection alternatives.

### METHODS

Experiments were conducted in similar laboratory set-ups at two different locations—a high-latitude, high-ambient-UV-radiation site (Eastern Siberia, latitudes from 65° to 77° N; 6–18 August 2005) and a temperate site (Lund, southern Sweden, 55.67° N, 13.5° E; 31 October to 21 November, 2005). For the zooplankton, cylindrical UV opaque white plastic containers (5 L; height 0.19 m, diameter 0.21 m) were illuminated by fluorescent lamps (UV-A-340; Q-Panel, Cleveland, Ohio, USA) mounted 0.1 m above the whole set of containers. These lamps are commonly used to simulate solar radiation in the wavelength range 300–340 nm (Fig. 1; Hansson 2004, Macfayden et al. 2004). In one treatment, UV radiation was restricted by use of Plexiglas (Röhm GS 233; Röhm, Darmstadt, Germany), effectively cutting off radiation below ~370 nm, i.e., in the UV-B range (Fig. 1). In the second treatment, UV radiation, down to a wavelength of 270 nm, was admitted by use of UV-transparent Plexiglas (Röhm GS 2458; Fig. 1). The transmittance of the two types of Plexiglas was quantified by attaching a piece of the Plexiglas in the cuvette of a spectrophotometer (Beckman DU 800; Beckman, Fullerton, California, USA) and scanning
the transmittance at wavelengths between 250 and 500 nm. Both treatments allowed visible light to pass through (photosynthetically active radiation [PAR] 400–700 nm; Fig. 1). The water of each experimental aquarium was aerated, and every second day the animals were fed algal cultures: *Scenedesmus* sp. and *Peridinium inconspicuum*, in the Swedish experiment, and an algal mixture from local lakes in the Siberian experiment. The experiments were run at temperatures as close to ambient as possible, i.e., around 16°C in Siberia and 20°C in Sweden. Fish predators were present in the lakes from which the zooplankton originated.

**Sweden**

Each container was filled with 4 L of filtered water (55-μm mesh size) from a small lake (Dalby quarry) in southern Sweden. The quarry is about 10 m deep and has an absorption coefficient at 320 nm of 2.0 and a 1% attenuation depth of 1.56 m at that wavelength. We added to each container 300 individuals of the calanoid copepod *Eudiaptomus gracilis* from the quarry and 160 individuals of *Daphnia pulex* (L.) from a nearby pond. The animals were exposed either to a UV treatment (UV; n = 4; Plexiglas letting both UV and visible light through) or to a visible-light treatment (V; n = 4; Plexiglas letting only visible light through; Fig. 1). UV radiation was produced by eight 36-W fluorescent lamps (55–717 light : dark cycle, corresponding to the day length at that time of the year. This gave a daily integrated UV-A radiation of 175 kJ m⁻² d⁻¹ (natural range: 127–568 kJ m⁻² d⁻¹). The corresponding UV-B radiation in the experiment was 8.0 kJ m⁻² d⁻¹. Thus, both UV-A and UV-B were about twice as high in the Siberian as in the Swedish experiment.

**Behavioral response**

In order to quantify the zooplankton avoidance behavior due to UV radiation, two vertical lines (90 mm apart and extending from surface to bottom) were predrawn before the start of the experiment on the inside wall of each container. At 70 mm depth a horizontal line was drawn, which together with the vertical lines formed an H shape. At the end of the experiments, just before the final sampling, we visually counted the number of copepods and *Daphnia* above a depth of 70 mm within a 90 mm wide sector. These counts provided an estimate of the portion of zooplankton occurring above the 70 mm line. Counting was first performed with the Plexiglas lid on and then five seconds after carefully removing the Plexiglas lid. In this way, the response to UV by individuals previously exposed to UV radiation (UV treatment) and previously not exposed (V treatment) could be compared. Within these five seconds, any behavioral response was finished and no further reaction was ever recorded. Behavioral responses are expressed as portion of the population in surface waters (above a depth of 70 mm) before and after the Plexiglas lids were removed. The behavioral study took about 60 seconds for each container and was performed during daytime.

**Pigment responses**

The pigments copepods are known to accumulate are mainly mycosporine-like amino acids (MAAs) and carotenoids, whereas cladocerans mainly use melanin (Hairston 1976, Hebert and Emery 1990, Sommaruga and Garcia-Pichel 1999). Although MAAs are not pigments sensu strictu (since, to our eyes, they are not colored), we will refer to them here as pigments. At the start and at the end of each experiment (10 days in the Siberian experiment and 21 days in the Swedish), we collected 10–30 copepods for carotenoid analysis, 30 copepods for MAAs, and 30 *Daphnia* for melanin from each container. Animals of approximately the same size were used, and egg-bearing females were always excluded from pigment analysis. Animals were measured at 4× magnification (Olympus SZ 40; Olympus, Center Valley, Pennsylvania, USA) before freezing. Daphnids were measured from center of eye to base of spine and copepods were measured from top of head to end of furca. Laboratory cultures of *Scenedesmus* sp. and *Peridinium inconspicuum* (30 mL) were filtered onto GF/F filters for MAA analysis (only Swedish samples). All samples were kept at −80°C (~30°C during transport from Siberia to Sweden) until analysis.

Samples for carotenoid analysis were put in glass scintillation vials (25 mL) with 3.5 mL ethanol (95%)
and were disrupted using an ultrasonic converter (Heat Systems model CL4; Heat Systems, Farmingdale, New York, USA; 30 seconds on ice) followed by extraction at room temperature for 12 h in the dark. The samples were centrifuged for 5 min at 6900 m/s². Quantification of the extracted red pigment in the supernatant was performed with a Beckman DU 800 spectrophotometer at 474 nm, which is the absorption peak for common carotenoids in copepods, i.e., carotenoid and its esters (Hasson 2000, 2004). Absorbance of the samples was also scanned from 350 to 700 nm. No peaks were observed at the absorption maximum of chlorophyll (665 nm), indicating that gut evacuation had been effective and that chlorophyll did not interfere with the results. The concentrations of pigments were normalized to dry mass: In the Swedish experiment, these were calculated from published relationships that gave data relating length to dry mass for *Eudiaptomus gracilis* (Bottrell et al. 1976). For the Siberian species, no published regression was found; instead, a regression for *Leptodiaptomus minutus*, a congeneric species, was used (Antonsson 1992).

MAAs were extracted in 25% methanol and analyzed by high-performance liquid chromatography (HPLC; Tartarotti and Sommaruga 2002). The MAA content was calculated from HPLC peak areas and molar extinction coefficients (Tartarotti et al. 2001 and references therein); these MAAs were normalized to dry mass in the same manner as for carotenoids. Filters with phytoplankton were analyzed in the same way as for zooplankton, with the exception that the extraction volume was 2 mL.

Melanin was analyzed according to Hebert and Emery (1990) and Hobaek and Wolf (1991). Samples were extracted in 1 mL 1 mol/L NaOH for 16 h at 100°C in the presence of H₂O₂ (10 μL, 3% aqueous solution) and then centrifuged at 77,000 m/s² for one minute. Quantification of the extracted pigment in the supernatant was performed with a Beckman DU 800 spectrophotometer at 350 nm. The blank was 1 mol/L NaOH with 10 μL H₂O₂ (3% aqueous solution). The absorbance was related to concentration by a reference curve made from melanin extracted from *Sepia officinalis* (SIGMA, M2649-100MG; St. Louis, Missouri, USA) in 1 mL 1 mol/L NaOH with 10 μL H₂O₂ (3% aqueous solution). The concentration of all pigments were normalized to dry mass calculated from published relationships of length to dry mass for *Daphnia* spp. (Bottrell et al. 1976).

Differences among treatments were tested with Student's *t* test using SPSS (version 11.0.1 for Macintosh). When necessary, data were log-transformed to meet assumptions for the test.

**RESULTS**

In the Siberian experiment, *Daphnia* showed a higher mortality in the UV treatment compared to the control (*t*₁₂ = 6.002, *P* < 0.001). The mortality rates of copepods, however, did not differ between treatments in either the Swedish or the Siberian experiment (*t* test; *P* > 0.06). In the Swedish experiment, *Daphnia* population size increased, but there was no significant difference between control and UV treatments (*t*₆ = 0.37, *P* = 0.70). In both the Swedish and the Siberian experiments, there were no significant between-treatment differences in size of *Daphnia* or copepods (*t* test, *P* > 0.18).

Copepods, in either Siberia or Sweden, showed no behavioral response to UV (*t* always < 1.325, *P* > 0.20; Fig. 2). The natural levels of carotenoids, which also constituted the initial values in our experiments, were almost twice as high among Siberian animals compared to Swedish (7.29 and 4.49 μg·mg⁻¹·dry mass [DM], respectively). In the V treatment, where UV radiation was blocked from reaching the animals, the carotenoid levels decreased by ~1 μg/mg DM in both Siberian and Swedish experiments, corresponding to a decrease of 14% and 22%, respectively (Fig. 2). In the UV treatment, on the other hand, mean carotenoid contents increased by about 3% and 10% in Siberia and Sweden, respectively (Fig. 2). Differences in carotenoid contents between treatments were highly significant in both experiments (*t*₁₂ = 2.459, *P* = 0.034 in Siberia; *t*₆ = 6.744, *P* < 0.001 in Sweden; Fig. 2).

Five different MAAs were identified: mycosporine-glycine, shinorine, porphyra-340, and the isomers palynthine and usujirene. In addition, two unknown substances, absorbing at 320 and 332 nm, were found in some samples. Initial (ambient) MAA values were 0.046 and 0.450 μg/mg DM in Siberia and Sweden, respectively. The tendency that we observed for MAA in Siberian animals was the same as for carotenoids (Fig. 2). But in the Swedish experiment, the MAA content increased in both treatments; still, the increase was higher in the UV treatment than in the V treatment (*t*₆ = 14.866, *P* < 0.001; Fig. 2).

The experimental food algae, *Scenedesmus* sp., contained either no or only trace amounts of MAAs; *Peridinium inconspicuum*, on the other hand, contained six different MAAs and the same unknown substances as the copepods. Pooled samples from V and UV treatments showed that *Daphnia* had no or only trace amounts of MAAs and carotenoids, probably derived from gut contents (data not shown).

In contrast to copepods, *Daphnia* showed strong behavioral responses when re-exposed to UV radiation in the V treatments (no UV radiation), in both Siberia and Sweden (*t*₁₂ = 5.158, *P* = 0.002; and *t*₆ = 4.473, *P* = 0.018, respectively; Fig. 3). In the UV treatments, however, the animals showed no reaction when the Plexiglas lid was removed (Fig. 3). Initial (ambient) amounts of the pigment melanin in Siberian *Daphnia* was more than 100 times higher than for Swedish *Daphnia* (means 0.0290 and 0.0002 μg/mg DM, respectively; Fig. 3). Siberian *Daphnia* reduced their melanin content in both the V treatment (mean reduction, 41%) and the UV treatment (mean reduction, 28%). In
Sweden, however, *Daphnia* increased their melanin content, although both the ambient values and the increases were very low and close to detection limits (Fig. 3). Differences in melanin changes between treatments were not significant for any of the experiments ($t$ always $< 1.8, P > 0.05$).

**DISCUSSION**

To avoid UV damage, zooplankton have the opportunity to use different strategies including phenotypic changes, such as an increase in photoprotective pigments or an escape behavior, such as vertical migration. They
may also use photoenzymatic repair of DNA as a post-damage measure (Zagarese and Williamson 1994, Rocco et al. 2001). Furthermore, they may opt to switch between strategies or select an optimally blended cocktail of available protection strategies. We show here that UV defense in zooplankton is a plastic system that includes both photoprotective pigmentation and behavioral escape mechanisms. Phenotypic plasticity is a key factor for success of organisms, especially where environmental variables, such as UV radiation and predation risk, vary spatially and temporally. It may also be crucial that the time lag is short from the detection of a risk until the defense is fully induced and that a costly defense is reversed once the risk is reduced (Tollrian and Heibl 2004, Gabriel et al. 2005). In our study, for example, the strong plasticity is shown by *Daphnia* from subarctic Siberia reducing their UV protecting pigmentation by 40% when released from exposure to UV radiation. Moreover, the time lag for reduction in pigmentation was short (about 10 days), indicating that reversible photoprotective pigmentation can be advantageous in a fluctuating environment, a notion also suggested by models on plastic responses (Gabriel et al. 2005). Moreover, the fast and strong response upon removal of the UV threat indicates that pigmentation is costly, a suggestion in line with previous results showing that unpigmented morphs of *Daphnia* had a population doubling time that was twice as fast as pigmented morphs (Hessen 1996). When again exposed to UV (after having reduced their pigmentation), the Siberian *Daphnia* in our study showed a strong behavioral response by rapidly (within seconds) swimming downward. In the Swedish experiment, however, there was no reduction in melanin content, probably because the amounts of pigment were already extremely low. In turn, this suggests that *Daphnia* at different latitudes have different propensities to produce melanin, either due to different rates of production or to different genetic capabilities of melanin production (see Plate 1). Considerable differences in melanin concentrations among species and even between different clones have been recorded (Hebert and Emery 1990). The response has been shown to be stronger among zooplankton previously exposed to variable UV and predation regimes (Tollrian and Heibl 2004). Melanized *Daphnia* are more resistant to UV radiation, but notably they have a lower growth rate compared to transparent conspecifics, suggesting a trade-off between cost of melanin synthesis vs. UV protection (Weider 1987, Hessen 1996). The evolutionary advantage of melanization seems to be the ability to utilize the whole water column during the daytime; transparent *Daphnia* are restricted to a less preferred feeding habitat in deeper waters (Hebert and Emery 1990, Zellmer 1995). Even though *Daphnia* from low- and high-latitude sites (Sweden and Siberia, respectively) showed different abilities to produce melanin, they both responded behaviorally to UV by downward migration. Copepods, on the other hand, showed no, or very limited, avoidance behavior to UV exposure in either Sweden or Siberia. This is in accordance with recent results showing that UV radiation may influence vertical migration of *Daphnia*, and that copepods are less behaviorally responsive to UV radiation (Hansson 2004, Leech et al. 2005).

*Daphnia* thus seem to rely on a combination of avoidance and pigmentation, whereas copepods almost exclusively rely on photoprotective compounds for their UV defense. Ambient pigment concentrations in copepods, as observed in our study, suggest that a mixture of MAAs and carotenoids served as the important photoprotective compounds among Swedish copepods; but in Siberia, carotenoids alone were the photoprotectant for copepods. This difference, however, may have been a result of different MAA contents in food algae. Bloom-forming algae, especially dinoflagellates such as *Peridinium inconspicuum*, which occurred in the Swedish food, are known to contain large amounts of MAAs (Jeffrey et al. 1999); but other groups of algae, such as diatoms and cyanobacteria, may also contain those protective compounds (Liu et al. 2004, Carreto et al. 2005).

In both the Siberian and Swedish experiments, copepods had high pigmentation levels in the UV treatments and showed no difference in mortality between V and UV treatment in either experiment.

---

**Plate 1.** There is enormous phenotypic plasticity in pigmentation among zooplankton, illustrated here by non-pigmented *Daphnia* from temperate Sweden and a strongly pigmented (melanin) individual from subarctic Siberia, where the UV threat is considerably higher than in temperate lakes. Photo credit: L.-A. Hansson.
Even though *Daphnia* were able to maintain melanization when exposed to UV in the Siberian experiment, they showed an increased mortality upon UV exposure; contrastingly, in the Swedish experiment *Daphnia* mortality was low. Moreover, the extremely low pigment levels in the Swedish *Daphnia* restricted the animals to the deepest parts of the aquaria—that is, they would, in a natural system, have been excluded from utilizing the food present in surface waters. The importance of this niche exclusion in natural systems is unknown, but at high UV intensities it likely constitutes a considerable drawback for unpigmented individuals and a fitness gain for individuals with sufficient UV protection. On the other hand, pigmented zooplankton are more susceptible to predators than less-pigmented individuals (Byron 1982, Hansson 2004). For example, freshwater copepods show considerable plasticity in pigmentation by incorporating high amounts of carotenoids during periods with high UV risk and reducing the levels when the predation risk, e.g., fish, increases (Hansson 2004). Studies by Moeller et al. (2005), furthermore, indicate that copepods (*Leptodiaptomus minutus*) have the ability to switch between different pigments (carotenoids and MAAs). Behavior and phenotypic responses to UV radiation may be viewed as mechanistically independent traits. Although copepods and *Daphnia* use both traits, they differ in the proportion they rely upon each of them, suggesting that they use complementary trait compensation sensu Dewitt et al. (1999).

In conclusion, we show here that UV defense among zooplankton is a plastic system based on both phenotypic and behavioral responses. Upon UV exposure, the organisms are able to shift between defenses; *Daphnia* seem to rely on a combination of avoidance and pigments, whereas copepods rely almost exclusively on photoprotective compounds. In aquatic ecosystems, organisms are faced with a multi-threat situation where cues for detection of UV and predation risk are important. We show here that different zooplankton species face a similar threat situation by blending the cocktail of phenotypic and behavioral responses in different ways.

**Acknowledgments**

The study was funded by the Swedish Research Council (VR) to L.-A. Hansson, and analyses of MAAs were done within the framework of the project FWF 16559-B06 to R. Sommaruga. Logistic support was given by the Swedish Polar Research Secretariat. Bruce Jeffs kindly provided linguistic corrections, and Larisa Samchyschyna made the species determinations. Two anonymous reviewers made valuable comments on an earlier version of the manuscript.

**Literature Cited**


