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# Responses in pigmentation and anti-oxidant expression in Arctic *Daphnia* along gradients of DOC and UV exposure

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*Responses in carapace melanization and expression of the major anti-oxidant catalase (CAT) and glutathione transferase (GST) in Arctic Daphnia were assessed in enclosures along a gradient of dissolved organic carbon (DOC). This gradient was created by adding freeze-dried humic matter to 2 $m^3$ UV-transparent enclosures, yielding final nominal concentrations of 1, 2.5, 5 and 10 mg C $l^{-1}$. The UV attenuation was strongly affected by additions of DOC, and attenuation coefficients at 320 nm increased from 3.0 in the control to approximately 3.5 and 11.0 $m^{-1}$ in the 1 and 10 mg DOC treatments respectively. Most Daphnia showed pronounced carapace melanization, and the absorbance of short-wave radiation through the carapace was strongly related to the degree of melanization. Nevertheless, the different UV climate in the enclosures did not cause any short-term adaptation in Daphnia pigmentation over a 3 week period. The levels of CAT and GST were assessed over time in the control and at 10 mg DOC. These enzymes displayed opposite patterns, with somewhat lower activities of CAT at low DOC (control) relative to 10 mg DOC, while the opposite was found for GST. There was also a significant negative correlation between CAT and solar irradiation for GST in both bags, while no effects were found for GST.*

## INTRODUCTION

Tundra ponds and high-Arctic fresh waters may be highly exposed to ultraviolet radiation (UV-R) owing to their shallowness and low levels of dissolved organic carbon (DOC), yet there may be a pronounced inter-site variability as well as a intra-site seasonality in humus DOC (Hobbie, 1996; Hessen, 1996). This renders the biota of these localities susceptible to UV-R, and this may be further accentuated by current trends in Arctic ozone depletion (Rex *et al.*, 1997). DOC offer a highly efficient UV-R protection for aquatic phyto- and zooplankton however (Williamson *et al.*, 1996; Hessen and Færøvig, 2001), and for aquatic organisms, the run-off and concentrations of terrestrially derived DOC could be far more important than current changes in ozone. Global warming may have profound effects on the levels of allochthonous DOC in lakes and ponds. Increased temperature and reduced precipitation have caused reductions of DOC in North American lakes (Schindler *et al.*, 1996; Curtis and Schindler, 1997), and past climatic changes have caused major transitions in DOC-flux and thus changed UV regimes in northern lakes (Pienitz and Vincent, 2000). While ozone depletion will yield increased input of UV-B, a reduction in DOC will cause increased penetration, not only of UV-B but also of UV-A. The effects on fresh waters of permafrost thawing and reduced snow-cover of the Arctic are not easily predicted. Baseline information is thus needed not only to gain knowledge on how plankton production related to light and temperature in the Arctic, but also how the productivity is modified by DOC.

For most high Arctic ponds and lakes, members of the *Daphnia* species complex [a diverse multi-clonal species complex; (Colbourne *et al.*, 1998; Weider *et al.*, 1999)] are the dominant planktonic species. Its adaptation to high solar irradiation seems to be manifested in Alpine and Arctic localities as a carapace melanization, i.e. apparently the most UV-transparent localities are inhabited by

melanized clones, whereas those with a somewhat higher DOC content are dominated by hyaline conspecifics (Hebert and Emery, 1990; Hessen, 1996). Melanic morphs or clones seem to be competitively inferior to hyaline forms, probably owing to the need for energy allocation to melanin resynthesis after moulting (Hessen, 1996), yet they are dominant in most high Arctic localities, suggesting an extensive need for photoproduction in these habitats. Most clones seem to shut down the melanin synthesis immediately in the absence of ambient short-wave radiation. Tests with a melanic clone of Arctic *D. tenebrosa* demonstrated that animals became hyaline after one or two moults in the absence of short-wave radiation, and UV susceptibility increased radically in non-melanic individuals (Hessen *et al.*, 1999). Most likely the major role of the carapace melanization is simply light screening, but although the absorption spectrum of synthetic melanin *in vitro* in well known, there are no data on the spectral absorption properties of the coloured carapaces of *Daphnia*. Neither is there any information on the spectral properties triggering melanin synthesis, and short-term adaptations to different light regimes in nature.

A second line of UV-R defence is the suite of quenching agents and anti-oxidant enzymes that neutralize reactive oxygen species (ROS). Previous studies on different species and populations of *Daphnia* revealed no major diference between melanized and hyaline populations with regard to tissue concentrations of bulk carotenoids (CAR), superoxide dismutase (SOD), catalase (CAT) and glutathione transferase (GST) (Borgeraas and Hessen, 2002a). Studies on diurnal variations indicated midday maxima in concentrations of CAT and SOD in highly light-exposed populations of Alpine *Daphnia*, while a midday minimum in GST (Borgeraas and Hessen, 2002b). There are no data on how, or if, zooplankton may adjust their anti-oxidant expression to changes in ambient DOC and UV-R however. While increased DOC increases UV attenuation rapidly and thus offers a protection for direct damage, it also provides a basis for increased concentrations of photo-products in surface waters, of which increased levels of hydrogen peroxide ($H_2O_2$) may be prominent (Scully *et al.*, 1996a,b). CAT primarily aims at detoxifying $H_2O_2$ to oxygen and water. Increased ambient concentrations of free radicals and $H_2O_2$ could thus affect CAT activity, provided that ambient $H_2O_2$ may affect intracellular processes. In contrast, GST which neutralizes peroxidized macromolecules and detoxifies breakdown products after lipid peroxidations is assumed to work mostly at the intracellular level.

In this study, we wanted to test how a gradient of DOC would affect these basic photo-protective properties of Arctic *Daphnia*. This was done by creating an artificial gradient of DOC by means of freeze-dried humus in large enclosures.

## METHOD

The study was performed in the small lake Brandallaguna (max. depth: 2.5 m, surface area: 125 160 $m^2$, location: 78°57′N, 11°52′E) at Ny-Ålesund, Spitsbergen. Six plastic enclosures fixed to a wooden framework were anchored in the middle of the lake. The enclosures had a volume of ~2 $m^3$ (1 m deep, 1.6 m diameter) and were constructed of a UV-B transparent plastic (Figure 1). A gradient of DOC was made by adding freeze-dried humus, isolated from a humic lake. Water from Lake Skjervatjern, western Norway was subjected to a reverse osmosis procedure [cf. (Gjessing *et al.*, 1998; Hessen and Færøvig, 2001)]. The lake water was pumped through a pre-filter and feed water was passed through an in-line cation exchanger for the exchange of polyvalent and monovalent cations with $Na^+$. The feed water was then passed through a high-pressure pump that boosted the pressure to almost 250 psi and through the reverse osmosis membranes that separated the permeate from the retentate. After repeated filtration, a total of concentrated humus in 5 l was freeze-dried to yield ~75 g solid sample. The elemental composition of this solid matter was 37.8% ash, 50.2% carbon, 4.8% hydrogen, 1.0% nitrogen and 1.6% sulphur. This substance was highly water soluble, and was used to create a DOC gradient. Bags 1 and 2 were kept as controls, while freeze-dried humus was added to bags 3 to 6 to give final, nominal concentrations of 1, 2.5, 5 and 10 mg C $l^{-1}$ respectively. Bag 1 was covered by a mylar-filter on top, to screen off UV-B, thus serving as a control for the UV-B absorbing effects of humus.

The experiments were performed during 3 weeks from mid-July, just after disappearance of the ice, in 1997. A radiation station was mounted on the floating platform with measurements of incoming global radiation, UV-B radiation and photosynthetically active radiation (PAR), as well as surface and water temperatures. Recordings were made with minute resolution, incoming global radiation was measured with a Kipp and Zonen meteorological grade pyranometer type CM11, surface UV-B radiation was measured with a meteorological grade Solar Light Company CIE-weighting UV-Biometer type SL501 as well as a Biospherical Instruments filter-radiometer type GUV511 with filter channels at 308, 320, 340 and 380 nm and PAR. The vertical attenuation profiles in all bags were obtained with a Licor 1800UW UV-spectrometer scanning from 300 to 800 nm with 1 nm steps, as well as a Biospherical Instruments type PUV filter radiometer with similar channels as the GUV. Measurements were performed in all bags 2 days after addition of DOC. The bags

were filled with surface water and zooplankton was added from a vertical net-haul (2–0 m, 20 cm diameter net, 45 μm mesh size). Zooplankton (almost exclusively *Daphnia*) were collected every third day from a mixed 15 l sample from three depths taken with a 5 l Schindler trap. Individual length was measured to the nearest 0.1 mm, and carapace melanization was visually scored on a scale from 0 to 4, where 0 is totally non-melanic animals devoid of carapace pigmentation; 1 is animals with a hyaline appearance, but with a weak pigmentation on the inner segments of the antenna and/or a weak dorsal shading of the carapace; 2 represents individuals that are clearly melanic, yet not very dark, and 3 are heavily melanized individuals. Hyaline individuals did occur, and some of these may have been recently molted individuals. Occasionally, egg-carrying hyaline individuals were recorded, however, suggesting the existence of a (rare) non-melanic morph or clone. Taxonomic affinities were scored by sequencing of the 12S rRNA on both hyaline and melanic individuals. Both were assigned to *D. tenebrosa* (Hessen *et al.*, 1999). The genetic screening was insufficient for determination of clonal determination, however, and given the high frequency of multiclonal population in Arctic *Daphnia* (Colbourne *et al.*, 1998, Weider *et al.*, 1999), we assume that these colour-morphs in fact represented different clonal lineages.

To test for the actual spectral absorption properties of the carapaces of *Daphnia* with different levels of pigmentation, several scans were performed on exuvia that were dissected off intact animals with different levels of melanization. These were placed fresh on a 1 × 1 mm fibre-optic sensor with a 1 nm resolution from 300 to 700 nm. A full spectral range from 300 to 700 nm was provided by a DH200 (Ocean Optics) deuterium and halogen light source unit through a 400 μm diameter fibre-optic cable. Spectral transmission was analysed by a SD2000 Ocean Optics spectrometer fitted with a fibre-optic cable [cf. (Gauslaa and Solhaug, 2001)]. This allowed the absorptive properties of intact carapaces with different levels of melanization to be compared.

Activities of the major anti-oxidants CAT and GST were assessed at 6 days with highly different irradiation in the control bag (no DOC-addition) and the bag with nominal concentration of 10 mg DOC $l^{-1}$. Animals were sampled by vertical thawing of a net during midday, and immediately frozen on liquid N. After transport to the laboratory, they were transferred to an ultra-freezer (–70°C), and stored until analysis. At least 15 animals were homogenized using a glass-Teflon Potter-Elvehjem homogenizer in 1 ml of ice-cold 50 mM potassium phosphate buffer pH 7.7 containing 1 mM EDTA and 0.1% Triton X-100. Supernatants (1000 ***g*** for 20 min at 4°C) of homogenates were used directly as the enzyme source. Measurements of enzymatic activities were carried out with a Beckman DU 62-spectrophotometer at a constant temperature of 30°C. CAT activity was determined as described by Claiborne (Claiborne, 1985) using 20 mM $H_2O_2$ as substrate. One unit (U) is defined as μmol $H_2O_2$ decomposed per min, at pH 7.0. The activity is completely inhibited by 0.12 mM sodium azide. GST activity toward 1-chloro-2,4-dinitrobenzene (CDNB) was measured as described by Habig *et al.* at pH 6.5 (Habig *et al.*, 1974). One unit is defined as nmol CDNB conjugated per min. Protein concentrations were determined according to Bradford using dye reagent from Bio-Rad and with bovine serum albumin as standard (Bradford, 1976).

## RESULTS

A test on spectral properties of the plastic used for the enclosures revealed an almost complete transmission of UV-A and UV-B, as well as longer wavelengths (Figure 1). Thus both direct and indirect UV-R in the bags should be fairly representative for the natural solar exposure in the locality, although some shading was anticipated from the wooden platform to which the enclosures were fixed. Measurements of the attenuation profiles within the control bag and in the free lake indicate a bias in attenuation coefficients of 0.2–0.3 $m^{-1}$. This error only applies of course when comparing the bag experiments to the situation in the undisturbed lake. Here, all experiments refer to the control bag, all bags experienced the same incident light environment and the shading effect is regarded as small.

During the experimental period from July 15 to August 6 the weather and radiation conditions varied greatly, with periods of complete cloud cover following clear sky conditions and vice versa (Figure 2). The lower panel of Figure

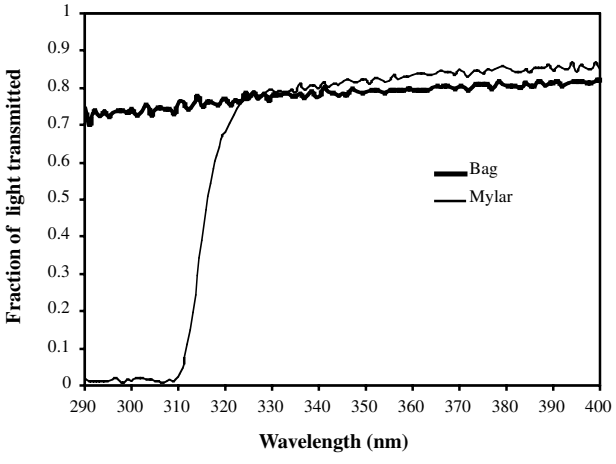


**Fig. 1.** Transparency of the plastic used in the enclosures, as well as the mylar sheets used for screening off direct UV-B in enclosure 1.

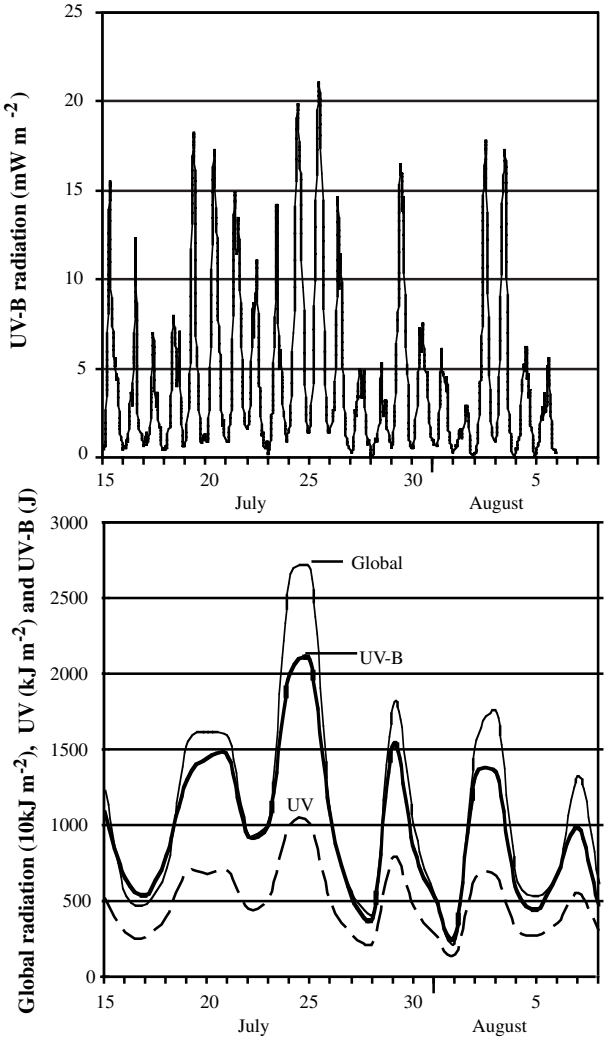


**Fig. 2.** Ambient, solar UV-B irradiation over the experimental period expressed as hourly UV-B (upper panel) and daily integrated incoming global radiation, and CIE-weighed total UV and UV-B (see text for data on instruments and resolution).

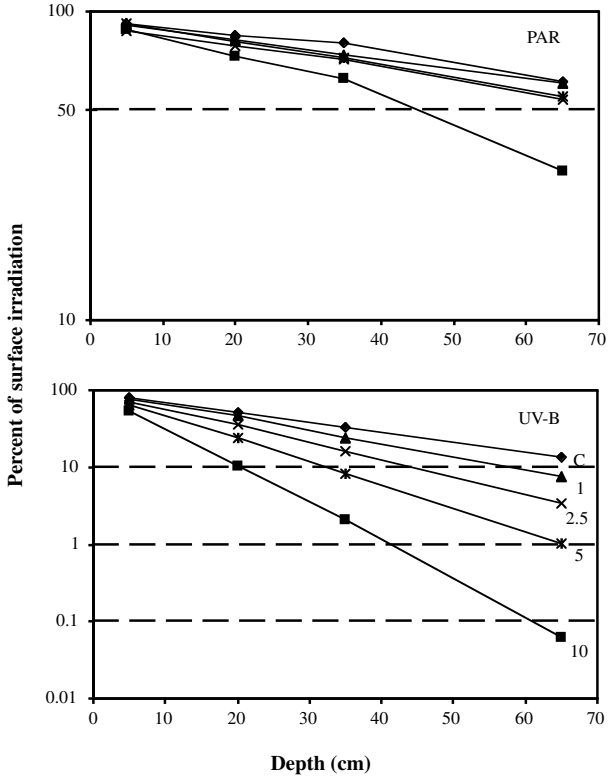


**Fig. 3.** Attenuation of integrated photosynthetically active radiation (PAR) (upper panel) and integrated UV-B expressed as remaining per cent of surface radiation at different depths. DOC concentrations given as mg C $l^{-1}$.

2 shows that the total daily CIE-weighted (International commission on illumination) doses of UV-B radiation varied from 250 J $m^{-2}$ on days of complete cloud cover to more than 2000 J $m^{-2}$ on the bright days. The upper half of the figure shows that the corresponding peak UV-B radiation at noon varied from less than 3 mW $m^{-2}$ to more than 20 mW $m^{-2}$. The addition of freeze-dried DOC yielded a strong gradient in UV-B and PAR attenuation, but less pronounced for PAR (Figure 3). The depth at which 10% of surface UV-B remained decreased from 0.75 m in the control to 20 cm in the 10 mg DOC treatment. The corresponding 1% depth was reached at 40 cm depth for the 10 mg C $l^{-1}$ treatment but exceeded maximum depth in the control bag (> 1 m). The attenuation coefficients as measured with spectral resolution increased at 320 nm from 3 $m^{-1}$ in the control to approximately 11 $m^{-1}$ at 10 mg DOC $l^{-1}$ (Figure 4).

While the apparent level of carapace melanization differed somewhat over the experimental period, there

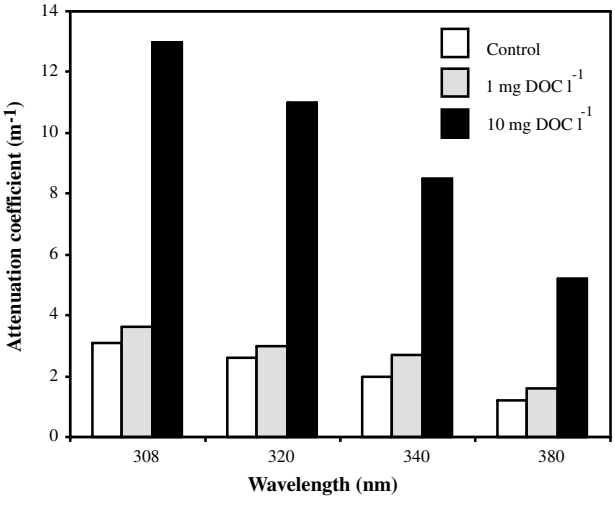


**Fig. 4.** Attenuation coefficients for three selected wavelengths in the control (~0.6 mg DOC $l^{-1}$) enclosure and in the enclosures with 1 and 10 mg DOC $l^{-1}$ added.

were no major differences between bags that could be accredited with the different levels of DOC (Figure 5). On July 19, following a period with cloudy weather, the majority of individuals in all bags had pigmentation scores of 2, i.e. moderately melanized. On July 27, a cloudy day but succeeding 3 days with bright sky and high irradiation, the majority in all bags were heavily melanized. On the last day, August 6, again following 2 days with low irradiation, a major fraction in all bags were only weakly pigmented. These shifts in pigmentation score also coincided with egg-production and probably also the moulting cycle, however. While no individuals with eggs were recorded during the first days of the experiment, and only a few (<10% of total population) on July 27, a large fraction (30–40%) were carrying eggs in early August, and there was an increasing number of neonates. This would normally imply that a rather synchronous moulting would take place during hatching.

Most of the *Daphnia* population were melanized individuals, although the degree of melanization displayed both individual variability and variability with time. Some hyaline animals were always recorded in the samples and some of these had eggs, suggesting that these individuals had not moulted recently and thus indeed were truly hyaline individuals. Melanized carapaces acted as a highly efficient UV screens (Figure 6), yet light was absorbed with high efficiency over a wide spectral range. The most pronounced pigmentation was at the dorsal side, with a gradual decrease in pigmentation towards the ventral side of the animals. While there were marked differences in absorbance of the melanic carapaces, they all had far stronger absorbance at all wavelengths than their hyaline counterparts.

CAT and GST was analysed from the control bag and the 10 mg DOC treatment, and the similarity between bags for both enzymes suggested no major effects of DOC (Figure 7). However, on July 24, the day with highest irradiation, there was lower CAT activity in the control bag than in the 10 mg DOC treatment (Student's *t*-test, $t = 2.558$, $P = 0.038$). There were marked day-to-day fluctuations of both CAT and GST, however, and the two enzymes yielded somewhat contrasting responses. During the experimental run, from July 15 to August 8, the weather conditions varied from heavy cloud cover to bright sky. While levels of CAT were negatively correlated with daily integrated UV-B, no such trend was found for GST (Figure 8). The CAT activity was directly related to all of the radiation measurements, but correlated best with the UV-B portion ($r^2 > 0.87$).

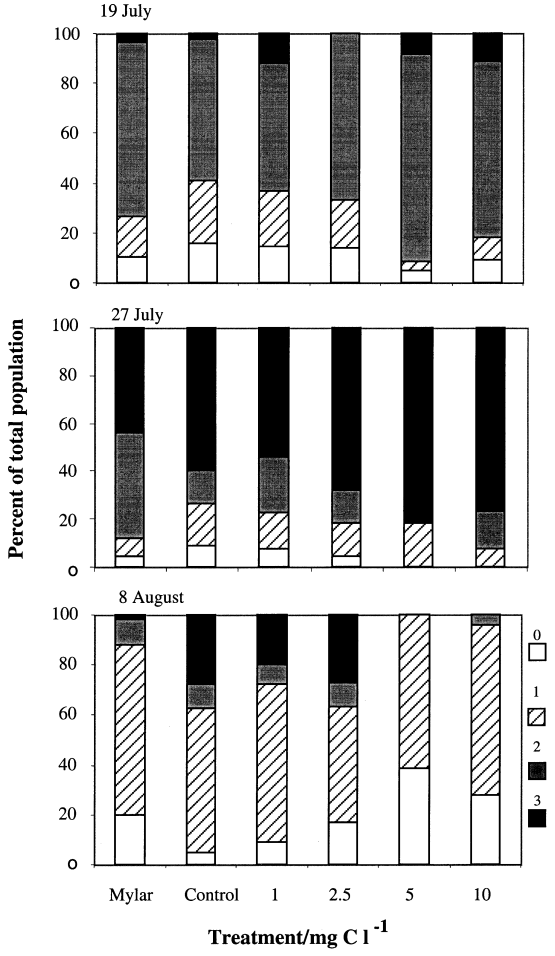


**Fig. 5.** Fraction of pigmentation scores in *D. tenebrosa* at three selected dates at different DOC concentrations. Pigmentation scores from 4 (dark: heavily melanized) to hyaline (white: no visual carapace melanization). See text for further details.

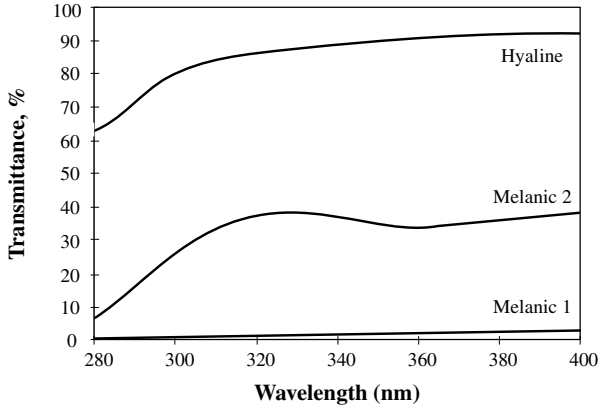


**Fig. 6.** Transmittance of irradiation of carapaces with different level of melanization. Melanic 1, dorsal part of heavily pigmented animal (pigmentation score 4); Melanic 2, dorsal part of less pigmented animals (pigmentation score 2); Hyaline, dorsal part of hyaline carapace (pigmentation score 0).

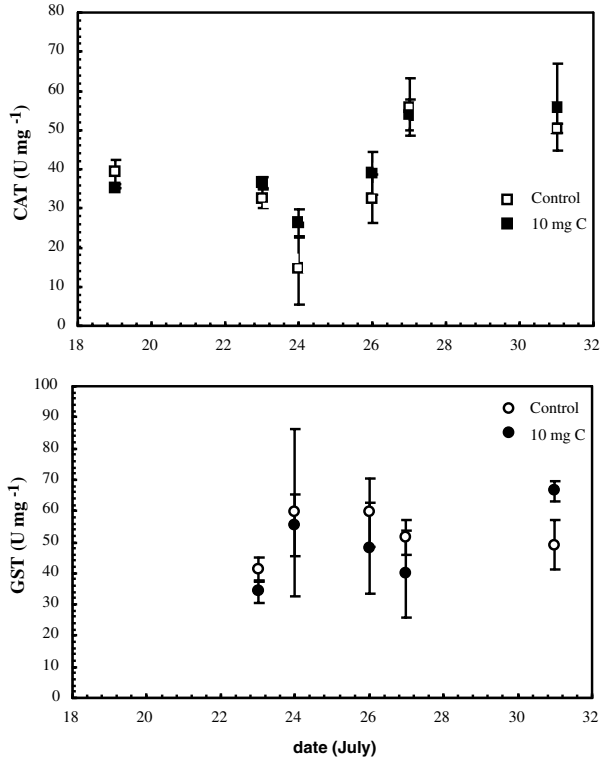


**Fig. 7.** Daily variation in specific activities of catalase (upper panel) and glutathione transferase in *D. tenebrosa* collected from the control (~0.6 mg DOC $l^{-1}$) enclosure and the 10 mg DOC $l^{-1}$ enclosure.

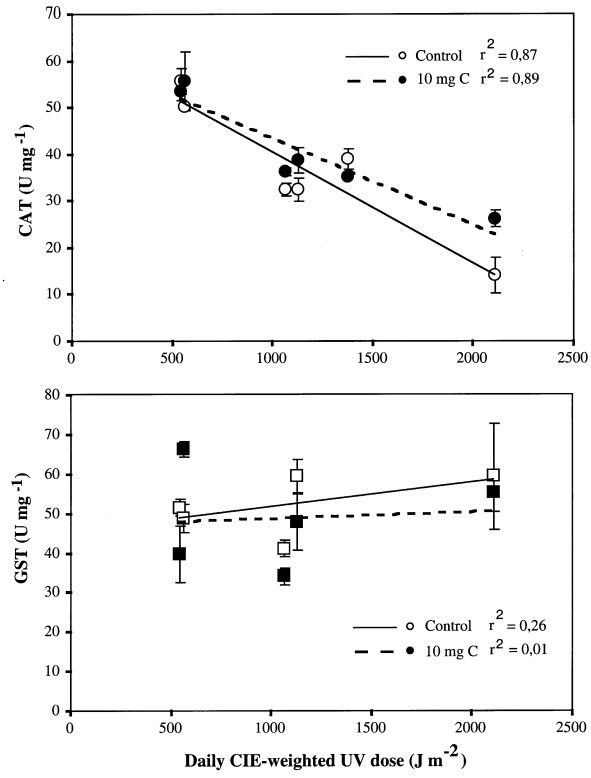


**Fig. 8.** Correlation between daily CIE-weighted dose at water surface during the study period of July 19–31 and levels of catalase (upper panel) and glutathione transferase in *D. tenebrosa* collected from the control (~0.6 mg DOC $l^{-1}$) enclosure and the 10 mg DOC $l^{-1}$ enclosure.

## DISCUSSION

The major role played by humus DOC in the absorption of short-wave radiation was clearly seen from the attenuation profiles in the various bags. Basically, these findings support the previous study by Scully and Lean (Scully and Lean, 1994) who found a UV-B-integrated attenuation coefficient largely related to DOC, and that by Morris *et al.* (Morris *et al.*, 1995), who found that among-lake variation in UV-B attenuation coefficients was mainly explained (87–96%) by differences in DOC concentrations. The effect of the DOC gradient also affects the attenuation of PAR (400–700 nm). The effect on PAR was far less than for UV-B, and most pronounced for the highest DOC concentration. This means that there will also be major shifts in the PAR : UV ratio with increasing DOC. While there is strong evidence that DOC does indeed offer an ecologically important UV-R protection for zooplankton (Williamson *et al.*, 1994; Zagarese *et al.*, 1994; Zellmer, 1995) there are still inherent problems with comparisons among lakes of different DOC, because not only may humus quality differ among lakes, but also physico-chemical parameters, adaptations at the species or clonal level, as well as food web structure will make predictions of the net effect of DOC difficult. The use of a gradient of DOC created by a standardized freeze-dried isolate circumvents these problems and allows for direct comparisons among treatments.

Humic matter plays a dual role with regard to the light effects. The strong absorption of PAR implies a potential for lowered photosynthesis. On the other hand, aquatic humus effectively blocks harmful UV-A and UV-B. Also, the suite of low molecular weight compounds that originate from the degradation of aquatic humus by sunlight has attracted considerable interest, owing to the potential effects on aquatic secondary production (Lindell *et al.*, 1995; Wetzel *et al.*, 1995; Moran and Zepp, 1997; Herndl *et al.*, 1997). However, the pronounced absorption of photons in the surface also generates a complex photochemistry, involving a set of highly reactive, strong oxidants that may interact with the biota (Cooper *et al.*, 1989, 1994; Miller and Moran, 1997; Lean, 1998; Miller, 1998). Thus the net effect of humus DOC on the aquatic biota may not be easily predicted, but a set of laboratory UV-R exposure experiments along a gradient of the same humus

quality demonstrated a strong photoprotective effect of humus for *Daphnia magna*, but negligible negative effects from ambient photoproducts (Hessen and Færøvig, 2001).

These *in situ* experiments did not suggest any major effects of DOC concentrations on *Daphnia* performance over a 3 week period, however. The shifts in pigmentation over time did not differ among treatments, and are most probably a result of moulting because melanization has to be reconstituted after each moult. These animals hatch during ice-melt early in July, and thus there is a slow and almost synchronous moulting in the population. Previous tests (Hessen *et al.*, 1999) have clearly verified the ability to cease melanin synthesis in the absence of short-wave light in these arctic *Daphnia*, yet the spectral cue for melanin synthesis is not settled. The test on spectral properties of the carapace showed a major absorbance in the UV region, but also of longer wavelengths, and judging from the similarity among the different bags, this light gradient was insufficient to create a melanization response. A simple test on development of carapace melanization under different light regimes was performed with melanic *Daphnia* from the lagoon incubated *in situ* at a depth of 10 cm for 6 days. Twenty individuals were added in triplicate to bottles with different optical properties, either quartz (UV + PAR), glass (PAR) or dark bottles, but these preliminary experiments (Hessen *et al.*, 1999) did not reveal any capacity for rapid shifts in carapace melanization. Although tests with a finer spectral solution, or preferably monochromatic light, should be performed to settle the real spectral cues for melanin synthesis, the present experiments suggest that also PAR may induce melanin synthesis, explaining the similarity in pigmentation score among different enclosures.

These findings do not suggest that light regimes are irrelevant for pigmentation however. Melanin synthesis is an energetically costly process, and there is an apparent trade-off between the need for photo-protection and the costs associated with repeated melanin synthesis (Weider, 1987; Hessen, 1996). This is normally reflected as a strong association between high water transparency (low DOC) and melanization (Hebert and Emery, 1990). Under the prevailing low temperatures (4–6°C), development and moulting rates are too slow to allow for rapid responses, and one season is probably not only insufficient time for competitive take-over by the hyaline morphs or clones, but also insufficient time for major shifts in pigmentation.

Both invertebrate and mammalian studies involving the effects of UV-B radiation have observed changes in the activity of the antioxidant system. Vega and Pizarro found the CAT activity to be elevated by UV-B in *D. longispina* (Vega and Pizarro, 2000). GST is known to be induced by chronic UV-B irradiation in mammalian skin (Hiratsuka *et al.*, 1999). In addition, studies on mammalian cell lines have indicated a reduction of antioxidant enzyme activity after acute exposure to UV-R (Fuchs *et al.*, 1989; Shindo *et al.*, 1994). A large difference in ambient UV-B exposure exists between the control and the 10 mg DOC enclosure because of the effect of DOC on UV-B attenuation. Thus, we expected differences between DOC treatments in *Daphnia* antioxidant activity, especially on days with high irradiance.

The activity of GST was somewhat lower at the 10 mg DOC treatment relative to the control (~0.6 mg DOC $l^{-1}$), but the difference was not significant and the activity was not related to the intensity of surface UV-B radiation (Figure 8). However, on the day with highest irradiance (July 24) lower activity of CAT was found in the control bag and the activity was negatively correlated with the surface UV-B dose. This may indicate that *Daphnia* CAT is inhibited or inactivated by UV-B. However, the same trend was also found in the 10 mg DOC bag, which indicates that it is not the direct absorbance of UV-B radiation that is involved in the inactivation. However, UV-A and visible light, which are less attenuated by DOC, are also able to inactivate CAT (Cheng and Packer, 1979; Fuchs *et al.*, 1989).

The causality is complicated by the fact that UV-R is known to induce formation of $H_2O_2$ and other ROS in surface waters, and there is a strong relationship between formation rates and DOC concentration (Scully *et al.*, 1996). Studies on freshwater invertebrates are lacking, but in marine worms, CAT activity is increased by exogenous exposure of $H_2O_2$ (Buchner *et al.*, 1996; Abele *et al.*, 1998). Among alpine populations of *D. longispina* we have found a significant positive correlation between absorbance (300 nm) of the pond water and CAT activity, which could be related to ambient levels of photoinduced $H_2O_2$ production (Borgeraas and Hessen, 2002b). Accordingly, higher levels of CAT are expected in the DOC treatment. However, the negative correlation between CAT and surface UV-B in the high DOC treatment suggests that UV-induced ROS in the water column is also involved in the inactivation. Other photoproducts, which may have the potential to affect biological processes, such as singlet oxygen and superoxide are also formed in natural waters through a sensitizer such as DOC (Cooper *et al.*, 1994).

The chromophores responsible for $H_2O_2$ formation are probably the same as those responsible for the attenuation of UV radiation (Scully *et al.*, 1996a,b). Consequently, in the 10 mg C enclosure the production is limited to the upper few cm, whereas in the control $H_2O_2$ production may be significant also at the bottom of the enclosures. Scully *et al.* found the daily area-specific cumulative production of $H_2O_2$ to be independent of the DOC concentration of lake waters (Scully *et al.*, 1996a,b).

For the Arctic ecosystems, increased temperature and subsequent permafrost thawing could be a major determinant of the light climate. Climate models forecast a significant warming and increased precipitation for the Arctic (Hanssen-Bauer and Førland, 1998). A number of studies show a general observed annual increase in precipitation (Dai *et al.*, 1997) and permafrost thawing (Majorowicz, 1996) at high latitudes. This would most probably increase input of allochthonous DOC to Arctic ponds that would offer a protection from direct UV-R that could offset the effect of increased ozone losses. On the other hand, increased levels of DOC in these shallow ecosystems could pose an increased stress from ambient photoproducts like $H_2O_2$.

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