

- Murata [eds.], Aerobic photosynthetic bacteria. Japan Scientific Society.
- IMHOFF, J. F. 1992. Taxonomy, phylogeny, and general ecology of anoxygenic phototrophic bacteria, p. 53–92. *In* N. H. Mann and N. G. Carr [eds.], Photosynthetic prokaryotes. Plenum.
- KOLBER, Z. S., AND OTHERS. 2001. Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. *Science* **292**: 2492–2495.
- , C. L. VAN DOVER, R. A. NIEDERMAN, AND P. G. FALKOWSKI. 2000. Bacterial photosynthesis in surface waters of the open ocean. *Nature* **407**: 177–179.
- MANTYLA, A. W., E. L. VENRICK, AND T. L. HAYWARD. 1995. Primary production and chlorophyll relationships, determined from ten years of CalCOFI Measurements. *CalCOFI Rep.* **36**: 159–166.
- MAUSERALL, D. 1978. Bacteriochlorophyll and photosynthetic evolution, p. 223–231. *In* R. K. Clayton and W. R. Sistrom [eds.], The photosynthetic bacteria. Plenum.
- MULLINS, T. D., T. B. BRITSCHGI, R. L. KREST, AND S. J. GIOVANNONI. 1995. Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. *Limnol. Oceanogr.* **40**: 148–158.
- PROCTOR, L. M. 1997. Nitrogen-fixing, photosynthetic, anaerobic bacteria associated with pelagic copepods. *Aquat. Microb. Ecol.* **12**: 105–113.
- SCHEER, H. 1991. Structure and occurrence of chlorophylls, p. 3–30. *In* H. Scheer [ed.], Chlorophylls. CRC.
- SHIBA, T., U. SIMIDU, AND N. TAGA. 1979. Distribution of aerobic bacteria which contain bacteriochlorophyll *a*. *Appl. Environ. Microbiol.* **38**: 43–45.
- YURKOV, V. V., AND T. BEATTY. 1998. Aerobic anoxygenic phototrophic bacteria. *Microbiol. Mol. Biol. Rev.* **62**: 695–724.

Received: 12 June 2001

Accepted: 31 July 2001

Amended: 6 September 2001

Limnol. Oceanogr., 47(1), 2002, 295–299
© 2002, by the American Society of Limnology and Oceanography, Inc.

UV-induced pigmentation in subarctic *Daphnia*

Abstract—The distribution of the planktonic crustacean *Daphnia* and the occurrence of ultraviolet (UV)-protective melanin pigmentation in its body wall were studied in relation to the UV transparency in subarctic ponds and lakes in Finnish Lapland. In shallow ponds, *Daphnia* only occurred in those with sufficient amounts of UV-screening dissolved organic matter. The highest pigment concentration was found in these *Daphnia* and in populations that occurred in lakes with low organic carbon content (<2 mg L⁻¹). Pigment synthesis was extremely low during the long winter and peaked immediately after the ice break-up at the time of the maximum underwater UV intensity. We propose that the predicted increase in UV irradiance from ozone depletion in the Northern Hemisphere spring, along with the earlier ice break-up associated with Arctic warming may favor the survival of those organisms with the ability to produce UV photoprotective pigments.

The most conspicuous feature of polar regions is the large seasonal variation in incoming solar radiation, from zero in winter months to 24 h of continuous sunlight in the summer. The poles therefore receive more solar radiation in summer than equatorial locations, yet the momentary radiation intensity never exceeds equatorial values. Ozone depletion in northern latitudes and the resultant changes in incident ultraviolet radiation have increased markedly during past decades, with some sectors of the Arctic experiencing upwards of 20% reductions in ozone and more than a 40% increase in ultraviolet (UV) radiation, the trend being most pronounced during spring months (WMO 1998). Although solar UV accounts for less than 5% of the total radiation reaching the surface of the earth, it contains the most energetic and biologically harmful wavelengths. These wavelengths are known to have broad effects on aquatic ecosystems, including the photoproduction of toxic compounds, mutagenesis, and physiological stress (Vincent and Neale 2000).

The penetration of UV radiation in lakes is known to be largely a function of the concentration of dissolved organic carbon (DOC; Morris et al. 1995). Because of poorly developed soils, sparse terrestrial vegetation, and low phytoplankton production, most of the waters in northern Fennoscandia situated above the tree line are poor in both allochthonous (watershed-derived) and autochthonous (originating within the lake) DOC, with values usually less than 5 mg L⁻¹. Northern Fennoscandian waters are also shallow, the mean depth being less than 5 m (Blom et al. 1998). This means that in many cases high levels of UV radiation can penetrate to the bottom of the water body. The open-water fauna of high-latitude lakes is therefore experiencing high enough UV intensities to cause increased mortality (Zellmer 1998).

To address the potential effect of increased UV radiation, we studied one of the most common and abundant groups of the subarctic open-water fauna, *Daphnia*, in relation to underwater UV conditions. First, we determined the occurrence of *Daphnia* in water bodies with different optical characteristics. Second, we measured the melanin pigment concentration of the body wall of *Daphnia*, which is suggested to depend on exposure to UV radiation (Hebert and Emery 1990; Hessen and Sørensen 1990). The growth of a crustacean takes place through the moulting of the shell and the formation of a new larger carapace. Melanin synthesis has to be repeated after each moult and has been considered to be energetically costly (Hebert and McWalter 1983; Hessen 1996). In laboratory experiments, the melanic morphs have been shown to have lower growth rates than the nonmelanic ones, thus being competitively inferior to the unpigmented forms (Hessen 1996). If pigment synthesis requires energy, the few resources available are not only allocated to growth and reproduction, but also to UV protection. In addition,

pigmented morphs are more vulnerable to visually hunting predators than nonpigmented ones (Hairston 1979). Thus, there occurs a considerable trade-off between the costs of pigmentation and the threats from UV radiation and predation (Hansson 2000). Hence, a plausible hypothesis would be that pigment synthesis is constrained to periods when it is really needed.

Methods—A survey of ponds and lakes with varying optical properties was conducted in Finnish Lapland in summers 1998 and 1999. We sampled 27 fishless ponds with different DOC concentrations in July 1998 and in July–August 1999 for the occurrence of *Daphnia*. The numerous ponds in northern Finland are shallow, seldom exceeding 1.0 m in depth. The clearest localities are exposed to high levels of UV radiation, whereas some contain high amounts of UV-absorbing DOC from wetlands in their watersheds. Deeper water bodies offer some refugia for migrating plankton to avoid exposure to harmful levels of radiation. To analyze the effects of such depth refugia on *Daphnia* pigmentation, we also studied 16 larger and deeper lakes ranging in latitude from 68°01'N to 69°26'N in an area that spans the biogeographical limits of the Norwegian spruce, Scots pine, and mountain birch (Fig. 1). The lakes were sampled with a helicopter during 20–22 July 1998. The maximum depth of the lakes averaged 8.4 m (range 2–24 m). The majority of all the lakes were inhabited by planktivorous white fish (*Coregonus* sp.) or arctic char (*Salvelinus alpinus*). We also followed seasonal patterns in melanin concentrations in a lake population of *Daphnia umbra* for a 1-yr period to test the hypothesis that the occurrence of pigmented organisms is restricted to times that pigmentation is really required (i.e., open-water period). The shift in the pigmentation pattern can occur either by physiological adaptation of individuals or by temporal changes in the clonal structure of a population (Hessen et al. 1999).

Pigment extraction followed the method by Hebert and Emery (1990) and Hobæk and Wolf (1991). Live *Daphnia* were separated from plankton samples that were taken with a 200- μ m net from the whole water column. Adult females (30–100) from each sample were preserved in 4% formalin solution to prevent melanin dilution (Hobæk and Wolf 1991) and frozen. Because of low density of *Daphnia* in the samples, three replicate net hauls were pooled in order to get enough individuals for pigment analysis; thus, only one pigment measurement is available for each sampling location. Pigment analysis took place 3 months after sampling. The formalin was washed off and individuals were measured. Some of the individuals were reserved for carbon analysis. The remaining *Daphnia* from each site were placed in a test tube in 5 ml 5 M NaOH and homogenized with an ultrasonic rod (Branson Sonifier Cell Disruptor B15) for 4 min. The tubes were heated to approximately 65°C once a day for 5 d by placing them in a jar with hot tap water and were then left to cool. Finally, the samples were filtered (Whatman GF/F 0.2- μ m-membrane filters), and absorbance of the filtrate was measured spectrophotometrically (Shimadzu UV-2100) through a 1-cm cuvette at 350 nm. Absorbance was related to concentration by means of a reference curve calculated from synthetic melanin (Sigma No. M8631), and the rela-

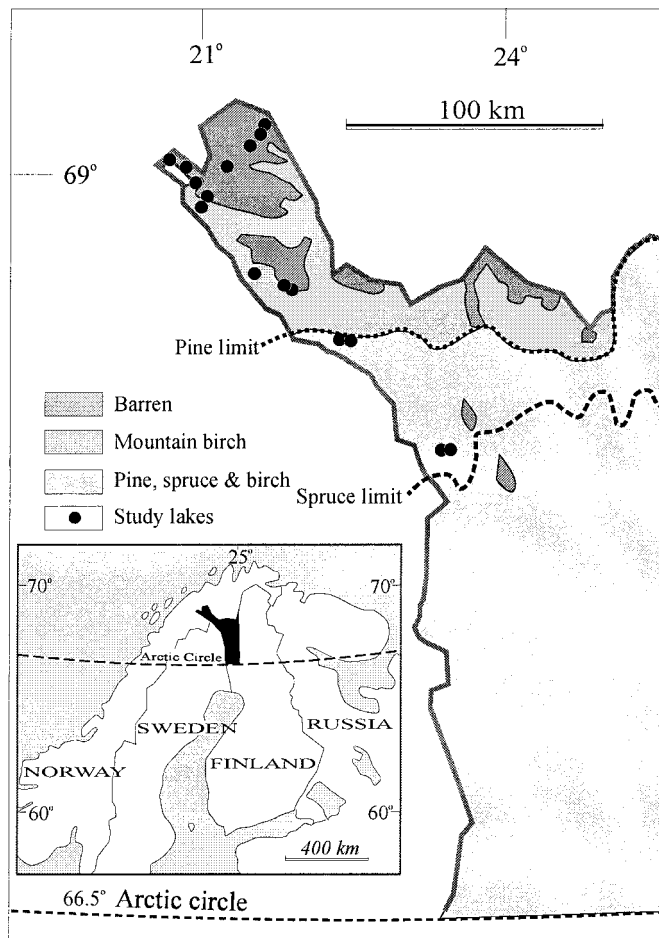


Fig. 1. Locations of the study lakes in Finnish Lapland. Studied ponds (not shown in the figure) were all located above 69°N. Altitude for the study sites ranged between 249 and 897 m above sea level (a.s.l.).

tionship between carbon content and body length was determined using regression analysis in the expression of cuticular melanin (μ g melanin per μ g carbon). The carbon content of individual *Daphnia* of different body sizes was analyzed with the Universal Carbon Analyzer using the carbon combustion method of Salonen (1979). The same method was used for measuring DOC from water. Samples for DOC were taken from 1 m deep in the lakes and from the surface in the studied ponds. Solar radiation measurements in 1998 were made every 30 min at a height of about 3.5 m above the surface of Lake Saanajärvi using a Vaisala Mii-los 500 automatic weather station located about 15 m from the southeastern lake shore.

Results and discussion—Clear ponds with DOC less than 5 mg L⁻¹ lacked *Daphnia*, whereas all but one of the more humic sites were inhabited by *Daphnia* (Fig. 2A). All recorded pond populations were melanic, with the level of pigmentation being high but variable between samples (Fig. 2B). We propose that the shallow depth (<0.5 m) and resultant lack of depth refugia explain the high melanin concentration despite high DOC. The high variation in the mel-

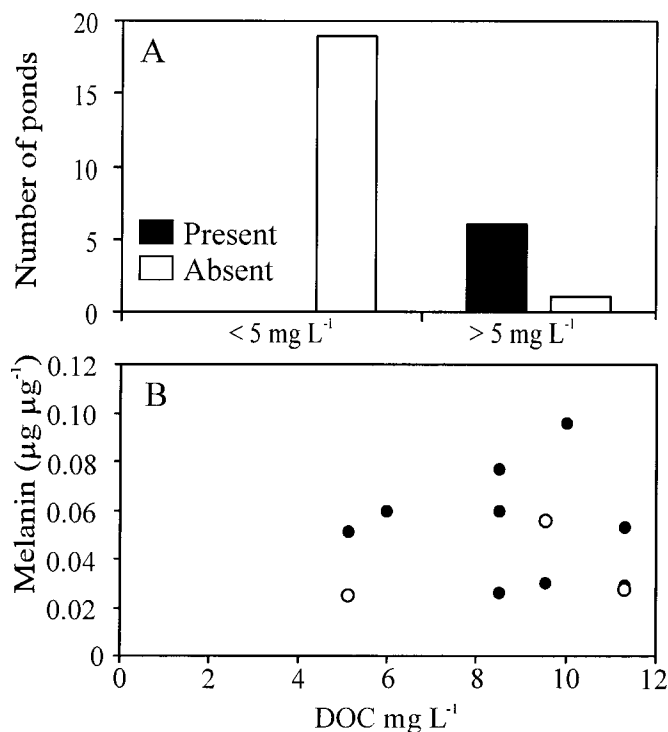


Fig. 2. Occurrence and pigmentation of *Daphnia* in ponds with different DOC concentrations. (A) White bars indicate the number of ponds where *Daphnia* is absent, and the black bar the number of ponds with *Daphnia*. DOC (5 mg L⁻¹) defines the transition from oligohumic to mesohumic waters. (B) The relationship between DOC and melanin in the six pond populations of *Daphnia* in 1999 (solid circles). For some populations, the melanin was measured additionally in 1998 (open circles). In 1999, the samples were taken once a week in July–August. The melanin concentration of *Daphnia* is related to carbon biomass.

anin concentration between the samples could have resulted from the different molting phases of the individuals during different sampling periods, which affect the total pigment concentration of the population. The lack of *Daphnia* from the clear-water ponds may also be due to other reasons apart from UV. Humic substances such as DOC are known to serve as food source for *Daphnia* (Salonen and Hammar 1986), in addition to providing a UV screen, therefore making humic ponds more favorable for *Daphnia* than clear ponds. Humic ponds were also generally shallower than clear ponds ($Z_{\text{mean}} = 0.4$ and 1.3 m, respectively) and were inhabited by phantom midge larvae (*Chaoborus* sp.), which prey upon *Daphnia*. However, these features (i.e., poorer depth refugia and greater risk for predation) rather make humic ponds less attractive to *Daphnia* than clear ponds. Hence the absence of *Daphnia* from clear ponds is likely a result from UV radiation or interaction of UV radiation with other stressors such as an unfavorable food regime.

In studied lakes, a steep gradient of DOC concentrations was observed, ranging from <1 to almost 10 mg L⁻¹, with minimum DOC values in lakes above the timberline (Fig. 3A). The UV absorbance of lake water was found to be closely related to DOC (Fig. 3B), which gave us an estimate of the underwater UV radiation and suggests that most

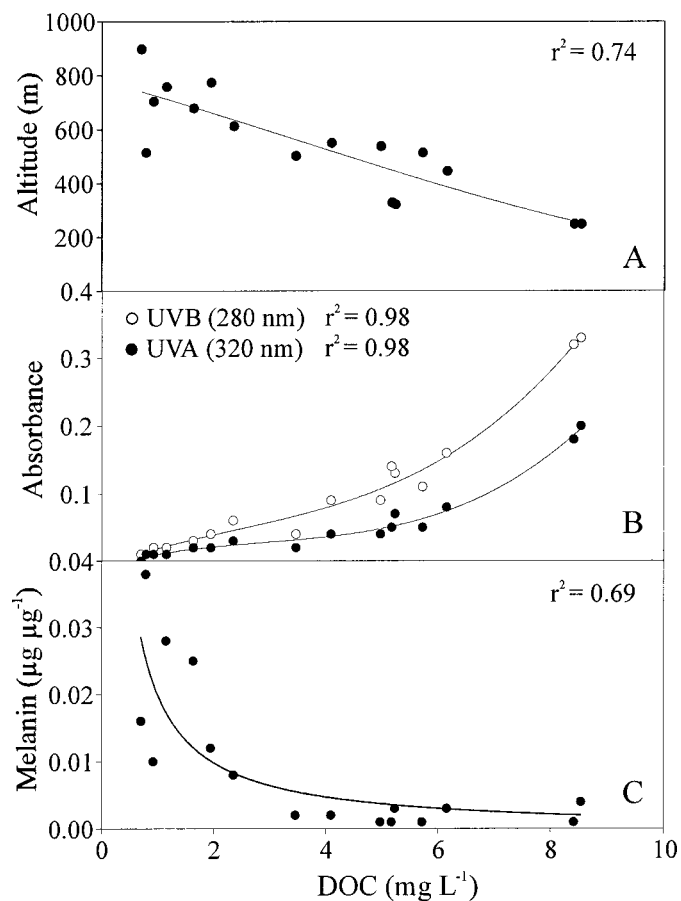


Fig. 3. The relationship ($P < 0.001$) between measured DOC concentration and (A) altitude above sea level, (B) UV absorbance, and (C) cuticular melanin content in *Daphnia* in the 16 study lakes. Melanin concentration is related to *Daphnia* carbon biomass.

DOC-poor lakes are exposed to UV radiation to a depth of several meters. The level of pigmentation in *Daphnia* varied between lakes. Melanin pigments were most abundant in *Daphnia* that inhabited lakes with low concentrations of DOC (<2 mg L⁻¹; Fig 3C). Similarly with the studied ponds, the molting phase of *Daphnia* may have influenced the variability in melanin concentration in the pigmented populations, as can be seen by the scattering of points around the regression curve in low-DOC values (Fig. 3C). However, the major pattern (i.e., the increase in melanin concentration with low-DOC values) is clear despite the variability in finer scale. The rise in the melanin concentration in low-DOC values relates to the biooptics of water. At DOC concentrations <4 mg L⁻¹, there is a documented change in spectral irradiance across the UV and PAR (photosynthetically available radiation) range, indicating a higher attenuation of PAR relative to UV (Laurion et al. 1997). Such change is of biological importance because certain damage–repair effects are responsive to wavelength ratios in the incident-light field (Vincent and Roy 1993).

The total amount of radiation reaching ground level is highest in mid-June in Lapland (Fig. 4). At this time, however, lakes above tree line are still covered by ice and snow, which effectively absorbs and reflects the incoming radi-

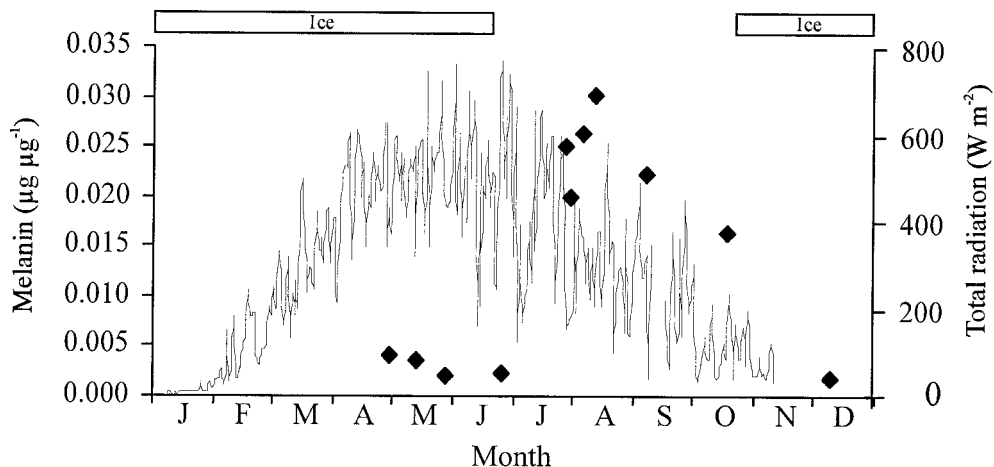


Fig. 4. Changes over time in the ground level total radiation and the *Daphnia umbra* melanin concentration in Lake Saanajärvi (69°05'N, 20°87'E; maximum depth = 24 m, altitude = 679 m a.s.l., DOC = 1.6 mg L⁻¹), showing the effects of increased underwater light intensity on pigment synthesis. The thickness of the ice exceeds 1 m in late spring.

tion. Under the ice, the cuticular melanin concentration of *Daphnia umbra* was negligible but increased immediately following ice cover break-up (Fig. 4). Toward fall, the melanin concentration decreased with the declining radiation. Our results thus suggest that despite being costly in terms of resource requirement and increasing vulnerability to fish predation (Hessen 1996; Hansson 2000), pigments are synthesized to increase survival of organisms under harmful UV radiation. Carapace melanization in cladocerans can be either a short-term response to high levels of UV or a genetic trait. Documented observations that pigmented individuals become transparent after 1–2 moults in the absence of short-wave radiation (Hessen et al. 1999) suggests that carapace melanization may result from phenotypic plasticity. However, Carvalho and Crisp (1987) and Geedey et al. (1996) have shown that, in an environment with regularly fluctuating conditions, some clones may become specialized to high and others to low levels of a given environmental factor. Therefore the dominance of nonmelanic individuals under the ice and melanic individuals during the open-water period in our study could also result from a selection of seasonal clones differing in their physiological flexibility to UV radiation. The population of *Daphnia umbra* studied in the present survey reproduced parthenogenetically throughout the year, and no males or ephippia were found in any of the samples, nor are they found in the sediment. The absence of sexual reproduction can act to maintain clonal variability on longer time scales because stable, long-term ecological differences among clones are thought to persist most likely where recombination is rare (Carvalho and Crisp 1987). Defining whether the variation in the melanin concentration was caused by phenotypic plasticity, clonal succession or a combination by both will require more work and is one of the objectives for our future investigations.

The findings reported here have direct implications for predicted future climate warming. One of the most obvious effects of higher mean air temperatures for alpine and arctic lakes is a shorter period of ice cover (Rouse et al. 1997).

Simulation studies assuming a doubling of carbon dioxide relative to present day values suggest that ice cover in Finnish lakes will melt 1–2 months earlier than in the present climate (Huttula et al. 1992), and a recent analysis of long time series of freeze and break-up dates provides evidence that the shortening of the duration of ice cover in lakes and rivers in the Northern Hemisphere is already in action (Magnuson et al. 2000).

Shorter ice cover duration leads to substantially improved light conditions and increases the exposure of northern lakes to the most intensive period of UV radiation. Numerical simulations, together with observations of lower stratospheric temperatures, suggest that the interannual variability of ozone depletion in the Northern hemisphere spring will increase considerably in the future. The doubling of atmospheric CO₂ concentration may lead to an Arctic ozone hole and nearly total local depletion of lower stratospheric ozone (Austin et al. 1992). We propose that an earlier disappearance of lake ice cover, especially at times of the highest UV-B flux in late spring/early summer, may impose strong stresses to aquatic organisms. Increased UV irradiance resulting from a combination of climatic warming and ozone depletion is likely to be lethal for those organisms that are not able to adjust their protection against harmful UV radiation. This scenario is further exacerbated by the interaction between photochemical and microbial processes that enhance the decomposition of DOC, the main protector of biota from the reactive components of UV radiation in lakes. According to recently published research, an increase in UV-B radiation would significantly increase photochemical mineralization of DOC in high-latitude waters (Vähätalo et al. 2000). Fluctuations in tree line position and associated catchment vegetation changes, which are caused by climate change and accompanied by variations in the export of DOC from the watershed, also influence the underwater UV radiation of waters (Pienitz and Vincent 2000). A combination of all these factors may ultimately result in changes in species composition, dominance patterns, and food web structure.

Moreover, because melanin synthesis is thought to be energetically costly and because pigmentation may cause increased vulnerability of organisms to predation, it is likely that even the pigmented clones could not tolerate the increased UV-B over sustained periods. This is particularly the case in high-latitude systems where planktonic communities, even in the absence of the harmful UV radiation, are facing considerable environmental stress during periods of snow and ice melt because of high dilution, pH decline, and increasing sunshine hours.

Milla Rautio and Atte Korhola

Department of Ecology and Systematics
Division of Hydrobiology
University of Helsinki
P.O. BOX 17
Helsinki FIN-00014
Finland

References

- AUSTIN, J., N. BUTCHART, AND K. P. SHINE. 1992. Possibility of an Arctic ozone hole in doubled-CO₂ climate. *Nature* **360**: 221–225.
- BLOM, T., A. KORHOLA, AND J. WECKSTRÖM. 1998. Physical and chemical characterisation of small subarctic lakes in Finnish Lapland with special reference to climate change scenarios, p. 576–587. *In* R. Lemmelä and N. Helenius [eds.], *Proceedings of the Second International Conference on Climate and Water*, Espoo, Finland.
- CARVALHO, G. R., AND D. J. CRISP. 1987. The clonal ecology of *Daphnia magna* (Crustacea: Cladocera) I. Temporal changes in the clonal structure of a natural population. *J. Anim. Ecol.* **56**: 453–468.
- GEEDEY, C. K., A. J. TESSIER, AND K. MACHLEDT. 1996. Habitat heterogeneity, environmental change, and the clonal structure of *Daphnia* populations. *Funct. Ecol.* **10**: 613–621.
- HAIRSTON, N. G., JR. 1979. The adaptive significance of color polymorphism in two species of *Diaptomus* Copepoda. *Limnol. Oceanogr.* **24**: 15–37.
- HANSSON, L.-A. 2000. Induced pigmentation in zooplankton: A trade-off between threats from predation and ultraviolet radiation. *Proc. R. Soc. Lond. B* **267**: 2327–2331.
- HEBERT, P. D. N., AND C. J. EMERY. 1990. The adaptive significance of cuticular pigmentation in *Daphnia*. *Funct. Ecol.* **4**: 703–710.
- , AND D. B. MCWALTER. 1983. Cuticular pigmentation in arctic *Daphnia*: Adaptive diversification of asexual lineages? *Am. Nat.* **122**: 286–291.
- HESSEN, D. O. 1996. Competitive trade-off strategies in Arctic *Daphnia* linked to melanism and UV-B stress. *Polar Biol.* **16**: 573–579.
- , AND K. SØRENSEN. 1990. Photoprotective pigmentation in alpine zooplankton populations. *Aqua Fenn.* **20**: 165–170.
- , J. BORGERAAS, K. KESSLER, AND U. H. REFSETH. 1999. UV-B susceptibility and photoprotection of Arctic *Daphnia* morphotypes. *Polar Res.* **18**: 345–352.
- HOBÆK, A., AND H. G. WOLF. 1991. Ecological genetics of Norwegian *Daphnia*. II. Distribution of *Daphnia longispina* genotypes in relation to short-wave radiation and water colour. *Hydrobiologia* **225**: 229–243.
- HUTTULA, T., A. PELTONEN, Å. BILALET DIN, AND M. SAURA. 1992. The effects of climatic change on lake ice and water temperature. *Aqua Fenn.* **22**: 129–142.
- LAURION, I., W. F. VINCENT, AND D. R. S. LEAN. 1997. Underwater ultraviolet radiation: Development of spectral models for northern high latitude lakes. *Photochem. Photobiol.* **65**: 107–114.
- MAGNUSON, J. J., AND OTHERS. 2000. Historical trends in lake and river ice cover in the Northern Hemisphere. *Science* **289**: 1743–1746.
- MORRIS, D. P., AND OTHERS. 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnol. Oceanogr.* **40**: 1381–1391.
- PIENITZ, R., AND W. F. VINCENT. 2000. Effect of climate change relative to ozone depletion on UV exposure in subarctic lakes. *Nature* **404**: 484–487.
- ROUSE, W. R., AND OTHERS. 1997. Effects of climate change on the freshwaters of arctic and subarctic North America, p. 55–84. *In* C. Cushing [ed.], *Freshwater ecosystems and climate change in North America*. Wiley.
- SALONEN, K. 1979. A versatile method for the rapid and accurate determination of carbon by high temperature combustion. *Limnol. Oceanogr.* **24**: 177–183.
- , AND T. HAMMAR. 1986. On the importance of dissolved organic matter in the nutrition of zooplankton in some lake waters. *Oecologia* (Berlin) **68**: 246–253.
- VÄHÄTALO, A. V., M. SALKINOJA-SALONEN, P. TAALAS, AND K. SALONEN. 2000. Spectrum of the quantum yield for photochemical mineralization of dissolved organic carbon in a humic lake. *Limnol. Oceanogr.* **45**: 664–676.
- VINCENT, W. F., AND P. J. NEALE. 2000. Mechanisms of UV damage to aquatic organisms, p. 149–176. *In* S. de Mora, S. Demers, and M. Vernet [eds.], *The effects of UV radiation in the marine environment*. Cambridge University Press.
- , AND S. ROY. 1993. Solar ultraviolet-B radiation and aquatic primary production: Damage, protection, and recovery. *Environ. Rev.* **1**: 1–12.
- [WMO] WORLD METEOROLOGICAL ORGANIZATION. 1998. Scientific assessment of ozone depletion: 1998. WMO global ozone research and monitoring project. Report 44, Geneva. World Meteorological Organization.
- ZELLMER, I. D. 1998. The effects of natural UVA and UVB on subarctic *Daphnia pulex* in its natural habitat. *Hydrobiologia* **379**: 55–62.

¹ Corresponding author (milla.rautio@helsinki.fi).

Acknowledgments

We thank S. Sorvari and J. Weckström for assistance in the field, P. Junttila for identifying *Daphnia umbra*, and the Kilpisjärvi Biological Station and the Finnish Forest Research Institute, Kilpisjärvi Branch, for logistic help. Discussions and constructive comments by M. Järvinen, R. Psenner, J. Catalan, W. F. Vincent, and two anonymous reviewers are highly appreciated. This work was supported by the Academy of Finland.

Received: 22 January 2001
Accepted: 5 October 2001
Amended: 16 October 2001