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Photoprotective Pigments in a Pond Morph of *Daphnia middendorffiana*

CHRIS LUECKE[1,2] and W. JOHN O'BRIEN[1]

ABSTRACT. Two morphs of *Daphnia middendorffiana*, a pigmented form with a dorsal black patch found commonly throughout Alaska in ponds and a nonpigmented form found in some lakes in the same vicinity, were exposed to natural sunlight conditions. The nonpigmented morph suffered higher mortality in sunlight than did the dark morph, and the black patch was lost when animals were protecte from exposure to light. The pigmentation appears to protect *Daphnia middendorffiana* from the harmful effects of natural radiation. This pigmentation pattern is an adaptation to living in shallow ponds exposed to high light intensities and few visual-feeding predators.

Key words: light toxicity, *Daphnia middendorffiana*, pigmented pond morph, Alaska, arctic ponds

RÉSUMÉ. Deux morphes de la *Daphnia middendorffiana*, l'une, une forme pigmentée avec une tache dorsale noire, souvent trouvée partout en Alaska dans les mares, et l'autre, une forme non pigmentée trouvée dans certains lacs dans la même région, ont été exposés à des conditions naturelles de lumière solaire. Le morphe non pigmenté a souffert dans la lumière solaire un taux de mortalité plus élevé que celui du morphe foncé, et la tache noire disparaissait lorsque les animaux étaient protégés contre l'exposition à cette lumière. La pigmentation semble protéger la *Daphnia middendorffiana* des effets néfastes de la radiation naturelle. Cette pigmentation figure comme une adaptation à la vie dans les mares peu profondes exposées à une lumière à forte intensité et où vivent peu de prédateurs à alimentation visuelle.

Mots clés: toxicité de la lumière, *Daphnia middendorffiana*, forme pigmentée, Alaska, mares arctiques

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INTRODUCTION

Zooplankton pigmentation has often been viewed solely as a detrimental characteristic contributing to increased vulnerability to visual-feeding planktivores (Zaret, 1980; O'Brien *et al.*, 1979). However, in situations where visual-feeding predators are sparse or absent, zooplankton are often intensely pigmented (Nilsson and Pejler, 1973; Byron, 1982). One of the advantages of pigmentation is photoprotection. The carotenoid pigments present in at least two species of calanoid copepods protect the organism from the damaging effects of sunlight (Hairston, 1976, 1979; Luecke and O'Brien, 1981). In all three of these studies a relatively unpigmented morph of the same species was present in nearby lakes that contained visual-feeding predators. In lakes without the predators the carotenoid pigmentation allowed the copepods access to the upper waters of the lake during the day, resulting in increased ability to feed, grow, and reproduce (Hairston, 1980; but see Byron 1982 for an alternate interpretation). In the only study done to date on cladocerans, Siebeck (1978) reports that darkly pigmented *Daphnia pulex* survive the effects of ultraviolet radiation better than non-pigmented individuals.

In the lakes and ponds north of the Brooks Mountain Range in central Alaska, we have found two color morphs of *Daphnia middendorffiana*. In deep lakes inhabited by planktivorous arctic grayling, *D. middendorffiana* are relatively unpigmented, whereas in shallow ponds which have no visual-feeding fish, *D. middendorffiana* has a pale brown carapace and a large conspicuous black area on the dorsal side of the head and anterior body. When the organism swims, the black patch is located uppermost. Siebeck (1978) reported a similar pigment patch in mountain pool populations of *Scapholeberis mucronata* and speculated that such pigmentation may provide protection from ultraviolet radiation.

In the Arctic, the abundance of shallow ponds and the constant summer light conditions suggest that photoprotective pigments may be especially important to zooplankton. In fact, we earlier found that *Heterocope septentrionalis*, a large predaceous calanoid copepod occurring in the same ponds as the dark *D. middendorffiana* morph, contains a carotenoid pigment that protects these individuals from the damaging effects of natural light intensities (Luecke and O'Brien, 1981).

We conducted experiments to determine if the unusual pigment pattern of the dark morph of *D. middendorffiana* also affords photoprotection for the organism.

MATERIALS AND METHODS

The experiments were conducted in the summer of 1979 at the Toolik Lake limnological station located 200 km south of Prudhoe Bay, Alaska, along the Trans Alaska Oil Pipeline. Unpigmented morphs of *Daphnia middendorffiana* were collected from Toolik Lake, a deep (20 m maximum depth), oligotrophic lake containing plantivorous arctic grayling. Pigmented individuals were collected from Camp Pond, a small, shallow (1.5 m maximum depth) pond located within 100 m of Toolik Lake, but lacking fish.

Individual *D. middendorffiana* were placed in open-ended plexiglass cylinders (9 cm diameter, 5 cm deep). Eighty of the cylinders were fixed to a sheet of plexiglass and partially submerged in a small wading pool. A small hole (6 mm) drilled in the bottom of each cylinder was covered by 350-μm plankton netting so that water could circulate into each cylinder from the pool. Once a day water was flushed in and out of the small hole to make certain each cylinder was replenished with fresh water and lake algae. A control was established by setting up cylinders in a second pool in the same manner, and shading this pool with a space blanket suspended 20 cm above the pool. Toolik Lake water was continuously pumped into each of the pools to minimize differential heating and provide fresh water and phytoplankton food for the *Daphnia*. Temperatures in the two pools were measured daily

[1]Department of Systematics and Ecology, University of Kansas, Lawrence, Kansas 66045, U.S.A.
[2]Present address: Department of Zoology, University of Washington, Seattle, Washington 98195, U.S.A.

and never varied more than 1.5°C. Mortality of individual *Daphnia* was checked daily. A quantum radiometer recorded the total incident radiation received each day at the experiment site.

In a second experiment, populations of pigmented *Daphnia* were maintained throughout the summer under three light intensities. One 40-L aquarium was enclosed in a light-tight container. A moderate light-intensity treatment was achieved by surrounding another 40-L aquarium in four layers of window screening, thus allowing transmission of approximately 1/16 of the incident light. A high light-intensity treatment was achieved by exposing a third 40-L aquarium to direct sunlight. In all three aquaria the water level was maintained at a depth of 24 cm. About 10 mL of a rich algal culture was added to each aquarium every third day. Every four days the degree of pigmentation of representative individuals from each treatment was qualitatively compared with the aid of a dissecting microscope.

RESULTS

In the first experiment, when *D. middendorffiana* were exposed to sunlight in plastic cylinders, we found the unpigmented *Daphnia* to suffer higher mortality rates than the pigmented morph (Fig. 1). In the first trial 89% of the lake *Daphnia* (unpigmented) died after being exposed to the sun for four days, whereas only 25% of the pond *Daphnia* (pigmented) died under the same conditions (Fig. 1A). A Kolmogorov-Smirnoff test (Sokal and Rohlf, 1969) showed significant differences in mortality betweenunpigmented and pigmented individuals exposed to the sun (D_{max} = .63). In the control populations, which were shaded from direct sunlight, 11% of the pigmented morph and 21% of the unpigmented morph died during the experiment. Both unpigmented (lake) and pigmented (pond) forms in the sun suffered higher mortalities than their respective controls (D_{max} lake = .68, D_{max} pond = .36). The two control populations were not significantly different (D_{max} = .15).

In contrast to the first trial, when initial days were clear and sunny, periods of overcast skies characterized the first few days of the second trial. Because a physiological delay exists between the photo-oxidation of proteins and the death of an organism (Krinsky, 1971), it is difficult to correlate daily radiation loads and mortality rates of *Daphnia* in these experiments. The total accumulated solar radiation loads presented in Table 1 allow a direct comparison of trials conducted over periods of differing light conditions (Luecke and O'Brien, 1981). During the second trial, the survival of both morphs was quite high for the first four days; however, by the eighth day 79% of the unpigmented *Daphnia* died (Fig. 1B). compared to only 16% in the controls (D_{max} = 0.68). Interestingly, there was no statistical difference in survival rates of the pigmented morph between the two conditions (D_{max} = 0.26), perhaps because of the lowered light intensities during the second trial.

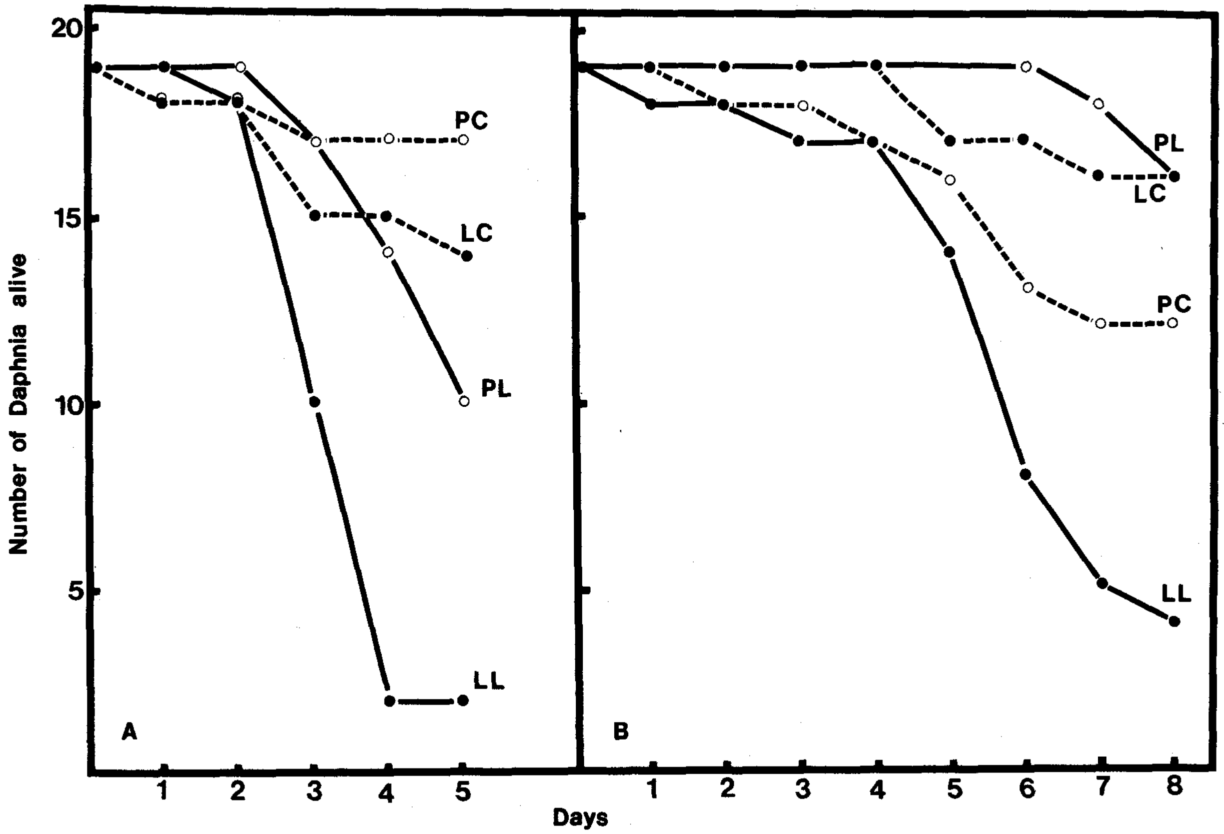

FIG. 1. A. The survivorship of *Daphnia middendorffiana* exposed to the sun (PL = pond individuals in the light, LL = lake individuals in the light) and of the control (PC = pond individuals kept in shade, LC = lake individuals kept in shade). B. Second trial, legend same as in A.

TABLE 1. Cumulative solar radiation (cal·cm^{-2}) received during both trials of the light-toxicity experiment

Day	Trial 1	Trial 2
1	495	262
2	960	584
3	1147	989
4	1462	1139
5	1837	1304
6	—	1506
7	—	1840
8	—	2155

The longer-term experiment, where pigmented *Daphnia* cultures were exposed to continuous conditions of total darkness, moderate natural light intensities, and high natural light intensities, showed that *Daphnia* reared in constant darkness lost any perceptible black pigment patch by the twelfth day. *Daphnia* reared in moderate light intensities (7% of ambient) also lost the black patch, but under these conditions it took 26 days. *Daphnia* reared in high light intensities remained pigmented throughout the experiment.

DISCUSSION

The results of these experiments suggest that the pigmentation of the dark morph of *Daphnia middendorffiana* provides protection from sunlight, and that exposure to light is necessary for the maintenance of such pigmentation.

Ringelberg (1980) offers an alternative hypothesis to explain greater mortality of nonpigmented compared to pigmented *Diaptomus nevadensis* when both are exposed to light (Hairston, 1976). Since the *Diaptomus* were not fed during the experiments, individuals containing the lipid-rich carotenoid pigments may have survived longer because of their superior nutritional condition. This cannot be the case for *Daphnia middendorffiana.* Food was available to both morphs in the form of algae in the circulating lake water. Control animals of both morphs appeared in good physiological condition throughout the experiments. In fact, unpigmented *Daphnia* should have been better adapted to experimental conditions since they were collected from the same water in which the experiments were run. Ringelberg also cautions researchers to separate the effects of light and heat because higher metabolic rates may also favor deeply pigmented zooplankton forms. Because the water temperature between the pools in our experiment never varied more than 1.5° C, the differences in metabolic rates were slight.

It appears that some characteristic of the pigmented morph of *D. middendorffiana* serves to protect the organisms from the harmful effects of sunlight. The most obvious difference between the two morphs is the pigmentation pattern. Although melanic pigments are known to act as strengthening agents, Dodson (in press) has concluded that the pigmentation differences of *D. middendorffiana* and *D. pulex* are not related to differences in carapace strength. The fact that sunlight is necessary for maintenance of the pigmentation provides strong circumstantial evidence that the pigmentation pattern is responsible for the differences in the mortality rate of the two morphs when exposed to natural light conditions. Because *D. middendorffiana* commonly exist in ponds as shallow as 0.5 m, they must be able to survive periods of high light intensity. The unpigmented lake form, on the other hand, can escape intense sunlight by migrating away from the surface.

The production of a black pigment patch in pond *Daphnia* is at least partially controlled by environmental stimuli, in that high natural light intensities are necessary for it to be maintained. Unfortunately, we could not investigate the induction of pigmentation in the unpigmented morph because we were not able to keep the unpigmented lake *Daphnia* alive at high light intensities. By rearing lake *Daphnia* under different light intensities, we would have gained valuable insights into the interaction between environmental and genetic factors in *Daphnia* pigmentation.

The position of the black patch in *Daphnia middendorffiana* suggests that it provides shade from the sun. Assays to determine what type of pigment or pigments are contained in this black patch were not possible under lab conditions at Toolik Lake. We do not know whether the pigment works similarly to carotenoids, which bind up the free oxygen radicals produced by photo-oxidation (Krinsky, 1971), acts simply as a light screen, or functions by some other mechanism. It is evident, however, that exposure to sunlight is necessary for the maintenance of the black pigment in the pond morph of *Daphnia middendorffiana*, and that the presence of this dark pigmentation reduces mortality in conditions of natural light.

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