Photoprotection by carotenoid pigments in the copepod *Diaptomus nevadensis*

(vertical migration/zooplankton)

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ABSTRACT   Individuals of the copepod *Diaptomus nevadensis* that contain high concentrations of carotenoids survive significantly better in natural intensities of visible light than less pigmented copepods. Vertical migration and behavior in light of different wave lengths are related to the degree of pigmentation.

Many investigators of aquatic communities have shown that the more visible zooplankters are the preferred prey of fish and other predators that hunt by sight (1–3). Strikingly visible, bright red copepods, however, are common in many lakes and ponds. While Nilsson and Pejler (4) showed that large red copepods occur in Swedish lakes only when the planktivorous whitefish (*Coregonus*) are absent or rare, the question of why any should be red remained unanswered.

Diaptomus nevadensis Light, a calanoid copepod, is found in two lakes in central Washington. Those living in Lake Lenore are pale blue, clear, or only slightly red, while those in Soap Lake are deep red in color. Soap Lake is quite saline (17 g/liter) and contains no vertebrates, while a population of predatory salamanders (*Ambystoma tigrinum*, Green) is found in Lake Lenore (salinity 1.7 g/liter) as well as greater numbers of aquatic insects. In the laboratory, salamanders from Lake Lenore consume red copepods in preference to pale ones. Studies on stomach contents of animals from the lake are in progress.

Species of *Diaptomus* have, in several cases, been shown to contain carotenoid pigments which give them their red color (5–7). While many hypotheses have been forwarded to explain this phenomenon, none had up to the present time been proven (8, 9). One of the more plausible of these explanations first proposed by Griffiths *et al.* (10) suggests that the carotenoids protect the animals from potentially damaging, visible wave lengths of light. These pigments have been shown by many investigators to enhance survival of a wide variety of bacteria and plants, probably by preventing the photooxidation of sensitive molecules (10–12). To the present, examples of this function in animals appear to be limited to trout eggs, mice, and humans (12).

Reports of the damaging effect of visible light on copepods are common (13), and J. M. Harvey in 1930 showed that the cause could be attributed much more to blue light than to green or red (14). This agrees with the observation that porphyrins and flavins, likely candidates for the sensitizers in photodynamic action (11, 15), show peak absorbance in blue light. Proceeding along the same line of reasoning, Mathews and Sistrom (16) showed that carotenoidless mutants of nonphotosynthetic bacteria were killed by sunlight but not by light from fluorescent or tungsten lamps because the sun produces relatively much more light of wave lengths shorter than 500 nm. Strains of the bacteria with carotenoids survived normally in sunlight.

F. E. Smith and E. R. Baylor (17) found that the reaction of planktonic cladocerans to blue light is different from their reaction to red light. At wave lengths longer than 500 nm, the animals were attracted to a light source shone from above and swam placidly. With wave lengths shorter than 500 nm, they sank from the source and wandered erratically about the aquarium. This behavior, originally reported in 1913 by K. von Frisch and H. Kupelwieser (18), has recently been elaborated by S. Stearns (19). Smith and Baylor suggested that the observed difference in behavior in red and blue light was an adaptation for locating concentrations of algae, but a more likely explanation might be that the animals were avoiding photooxidizing wave lengths of light. It may then be predicted that animals possessing carotenoid protection from this light should react differently to a change in light color than animals without protection.

To test the importance of these considerations in *Diaptomus nevadensis*, experiments were made comparing the survival of dark red copepods with that of pale copepods in natural intensities of light, and comparing their behavior in different colors of light.

METHODS

Diaptomus nevadensis were collected by plankton net and stored in the laboratory at 10°. The animals were used within 5 days of capture to ensure that they were in good condition. Field measurements of light were made with a pyrheliometer and a submersible photocell with glass cut-off filters.

Photodamage Experiments. Two experiments were run to test the effect of continuous visible light on the survival of *D. nevadensis* with large and small amounts of carotenoid pigment. Eight 250-ml plastic tissue culture flasks each containing 200 ml of filtered lake water were placed upright and exposed from the side to light from 25 W blue fluorescent lamps. These lamps produce a peak intensity at 450 nm, with a half-width of 100 nm. The effects of ultraviolet radiation are not considered since the energy produced by the lamps is negligible below 350 nm. Four of the flasks each contained 20 pale copepods from Lake Lenore, and each of the other four contained 20 red copepods from Soap Lake. Reddest animals were chosen to ensure high carotenoid content. Similarly, eight flasks were placed in a dark box as controls. The experiments were run at 15° ± 2°.

In the first experiment four lamps were used to produce an intensity of 1.6 mW/cm^2 (measured between the lamps and the flasks), approximately the intensity of blue light (400–500 nm) reaching the lake surface in midsummer. In the second experiment only one lamp was used (0.38 mW/cm^2), giving about the amount of light of wave lengths 400 nm to 500 nm reaching 3.0 m in Soap Lake and 1.5 m in Lake Lenore in midsummer or the amount of blue light reaching the lake surface on a sunny winter day.

Table 1. Peak transmittances of interference filters, filter half-widths, and energies used in copepod behavior experiment

Wave length (nm)	420	460	520	560	620	660
Half-width (nm)	6.0	5.7	5.6	4.8	5.2	5.2
μW/cm^2	57	52	46	43	39	37

Energies were adjusted to give approximately $1.2 \times 10^{14} \pm 0.1 \times 10^{14}$ quanta/cm^2 per sec at each wave length. The filters used have side band blocking to 10^{-4} in the range x-ray to infrared.

Dead animals were counted and removed once each day, except that in the experiment at lower intensity after the 10th day animals were counted and removed once every 3 days.

Behavior Experiments. An experiment to test behavior of differently pigmented copepods in light of different colors was run using basically the set up described by Stearns (19). Animals were placed one at a time in an experimental chamber made of Plexiglas 1 cm deep, 10 cm high, and 10 cm wide, restricting their movement to a vertical plane. Light was produced by a Xenon arc lamp (Oriel model 6137), and light of six wave lengths was produced using Oriel interference filters (Table 1). The light was projected from above through one or more pieces of Parafilm to diffuse the light and help adjust intensity. The energy of the light was measured with a Hewlett–Packard 8330A radiant flux meter and adjusted so that, as nearly as possible, equal numbers of quanta were produced at each wave length. The energies used are quite low relative to what the animals can receive in the lakes.

Fourteen copepods were tested from each lake. In a darkened room at 20° ± 2° each copepod was placed in the chamber and presented with the six wave lengths in random order. The face of the chamber was marked off in a grid of 1-cm squares, and movement of the animals was measured by recording the number of vertical and horizontal lines crossed by an animal in 3 min time. The distance moved was then calculated by multiplying this number by $\pi/4$ as described by Olson (20).

Additionally, two extra intensities at 460 nm and 620 nm were used with six animals from each lake. These were one-half the intensity of the main experiment and double that intensity, respectively. The purpose was to ensure that the experiment was performed at an intensity to which the copepods were sensitive.

Extraction and Identification of the Carotenoid Pigments. To identify the pigment, carotenoids of animals from Soap Lake were extracted in acetone. One-half of the extract was taken into ether and saponified in a 10% KOH/methanol solution. When this solution was placed in a separatory funnel with ether and water was added, a precipitate characteristic of astacene formed at the interface. The saponified extract was then taken completely into ether. Muscle from
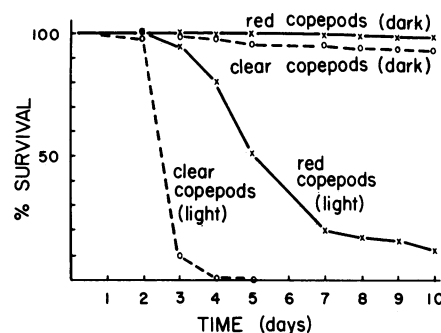

FIG. 1. Survivorship of *Diaptomus nevadensis* with large (red copepods) and small (clear copepods) amounts of carotenoid pigment when exposed to visible blue light (450 nm) at peak summer intensity (1.6 mW/cm^2).

coho salmon (*Oncorhynchus kisutch*, Walbaum), a known source of astaxanthin (21), was treated in the same manner and used as a standard for comparison.

Absorption spectra of copepod and salmon pigments were identical and showed single broad peaks with maxima at 474 nm in ether and hexane and at 505 nm in carbon disulfide. Cochromatography of unsaponified extracts of salmon and *D. nevadensis* carotenoid on three adsorbents showed only astaxanthin in salmon, while the copepods showed two pigments. One of these migrated with the salmon astaxanthin and the other migrated slightly faster and proved to be an ester of astaxanthin. Saponified extracts of salmon and copepod carotenoid showed only the single pigment astacene in cochromatography on three adsorbents.

Pigment was extracted from copepods from Lake Lenore. Cochromatography with pigment of Soap Lake copepods showed that animals from both lakes contained the same two pigments.

Throughout the year 1973 to 1974, the amount of pigment in animals from the two lakes was measured quantitatively. Determinations were made bimonthly by extraction from known numbers of animals in ethanol and reading peak absorption on a Bausch and Lomb Spectronic 505 spectrophotometer. Dry weights were obtained by drying 10 males and 10 females at 60° and weighing them on a microbalance. The amount of pigmentation proved to vary seasonally, with a maximum in winter and a minimum in summer (Table 2). Quantitative measurements of pigment were also made on animals taken from those used in the experiments. In every case, animals from Soap Lake contained more carotenoid than those from Lake Lenore.

RESULTS

In the photodamage experiments, at high intensity, the copepods with less carotenoid were all dead within 5 days, whereas those with more pigment survived much longer (Fig. 1). The average time to death for the pale copepods from Lake Lenore was 3.06 days and for red Soap Lake co-

Table 2. Concentration of carotenoid pigment (absorbance at 474 nm/mg of dry weight) in *D. nevadensis* from two lakes, showing seasonal maxima and minima and values at the start of the three experiments described in the *text*

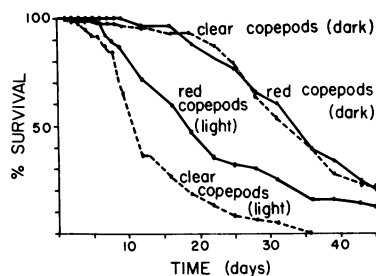|	Maximum (winter)	Minimum (summer)	Photodamage experiments		Behavior experiment
			High light	Low light	
Soap Lake	1.114	0.146	0.708	0.860	0.139
Lake Lenore	0.256	0.020	0.044	0.026	0.014

FIG. 2.   Survivorship of *Diaptomus nevadensis* with large (red copepods) and small (clear copepods) amounts of carotenoid pigment when exposed to visible blue light (450 nm) at relatively low intensity (0.38 mW/cm^2).

pepods 6.54 days. This difference is highly significant (*t* test, $P < 0.001$). Survival in the dark controls was very high and was not significantly different for copepods from the two lakes.

At low light intensity, red copepods again survived significantly better than pale (*t* test, $P < 0.001$), while dark controls were quite similar for animals from the two lakes (difference not significant, Fig. 2). Death due to starvation became important after day 15, as shown by the dark controls. The average time to death for the pale copepods in the light was 12.9 days, while red copepods averaged 20.4 days.

In the behavior experiment, copepods from both lakes swam significantly faster in light of 420 nm than they did in light of 660 nm (*t* test, $P < 0.05$ Soap Lake, $P < 0.001$ Lake Lenore, Fig. 3). Swimming speed at 420 nm is not significantly different between copepods from Soap Lake and Lake Lenore, but copepods from Soap Lake swim faster than those from Lake Lenore at all other wave lengths. Differences become increasingly significant as wave length increases (*t* test, $P < 0.05$ 460 nm, $P < 0.01$ 520 and 560 nm, $P < 0.001$ 620 and 660 nm). A fit of straight lines to the data shows that the slopes of both lines are significantly different from zero (*t* test, $P < 0.02$ Soap Lake, $P < 0.001$ Lake Lenore) and that these slopes are significantly different from each other (*F* test, $P < 0.05$).

When the intensity of light at 460 nm was varied from one-half the experimental intensity to double it, a significant increase in activity occurred in animals from both lakes (*t* test, $P < 0.05$), but in red light (620 nm) a change in intensity produced no change in swimming speed. Thus it seems that the swimming speed observed at 620 nm and 660 nm is the animals' basal rate.

DISCUSSION

Diaptomus nevadensis from Soap Lake and Lake Lenore are pigmented by the carotenoids astaxanthin and astaxanthin ester, and those from Soap Lake contain much more of this pigment than those from Lake Lenore. The results of the photodamage experiments show that copepods with large amounts of these pigments are less subject to the lethal effects of exposure to visible light than copepods that are only slightly pigmented. It remains to be shown why, in the face of this, animals from Lake Lenore have little pigment, but a likely explanation is that if they were red, they would suffer a high cost in visibility to predators. In summer, many of the Lake Lenore copepods turn pale blue as a result of the carotenoid binding to a protein (9). Since the photoprotective action of the carotenoid is a function of the number of conjugated double bonds in the molecule (12), protection is probably not eliminated by association with a protein. It is possible that becoming blue gives an animal some protection
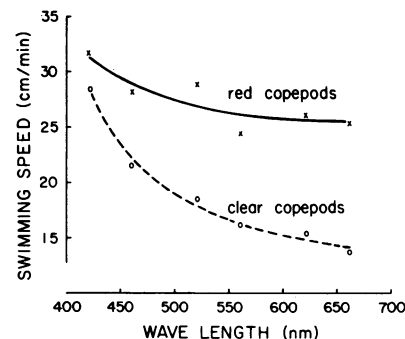

FIG. 3.   Response of *D. nevadensis* with large (red copepods) and small (clear copepods) amounts of carotenoid pigment to 1.2 × $10^{14} \pm 0.1 \times 10^{14}$ quanta/cm^2 per sec of light at six wave lengths. Swimming speeds in red light probably represent basal rates.

from sunlight while allowing it to remain relatively inconspicuous.

Many species of zooplankton, including *D. nevadensis* in spring and summer, migrate to deep water during the day and to the surface at sunset. Several hypotheses have been proposed to account for this diurnal cycle (23). One of the more likely hypotheses is that by remaining at low levels of light intensity the zooplankton avoid being seen and eaten by fish (13, 24, 25). It is generally accepted that they must return to the surface at some time to feed on the algae which are more abundant there.

Solar radiation has in the past been denied as an ultimate cause of vertical migration on the basis that ultraviolet light does not penetrate more than two meters into water (13). Effective intensities of blue light, however, may penetrate more than 20 meters in clear lakes (26). Copepods such as *D. nevadensis* that carry carotenoid pigments to protect them from this radiation would have to migrate only slightly to avoid peak day-time intensities. Since this results in a cost in vulnerability to predation (4, 22, 27), only animals with reduced carotenoid content can live where predators are present. Thus predators that hunt by sight can affect the vertical migration of zooplankton in two ways: first, as a direct selective force keeping the prey at day-time depths where the light is too low for them to be seen, and second, as a selective force for reduced prey pigmentation which in turn requires them to stay at lower light intensities than they otherwise would. The relative importance of these two forces remains to be investigated.

Finally, the swimming speed experiment shows that *D. nevadensis* behaves similarly to the cladocerans studied by Smith and Baylor (17), swimming faster in blue light than in red. The selective advantage to animals from Lake Lenore swimming more slowly on the average than those from Soap Lake may be that in so doing they remain less obvious to predators (22, 24). Less pigmented animals from Lake Lenore are much more responsive to a change of color than red animals from Soap Lake. It seems very likely that this difference is due to the greater sensitivity of Lake Lenore animals to blue light. Preliminary experiments in a graduated cylinder show that the copepods tend to be attracted to red light and swim away from blue light. By sinking and swimming faster, they may reach a place of reduced intensity. While Smith and Baylor suggest that the behavioral difference represents a method of finding concentrations of algae, it is interesting that they, in fact, observed that, "Prolonged exposure to this light [shorter than 500 nm] has literally driven populations to death."

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