The mortality effect of ultraviolet radiation in a translucent and in a red morph of *Acanthodiaptomus denticornis* (Crustacea, Copepoda) and its possible ecological relevance

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Abstract

Ultraviolet radiation induced a lower mortality in morphs of *Acanthodiaptomus denticornis,* coloured red by carotenoid pigments, than in translucent morphs. Calculations showed that ultraviolet penetration in a lake of low chlorophyll-a content may cause a hazardous dose in the surface layers. Experiments confirmed this.

Introduction

Many calanoid copepods inhabiting high altitude lakes are coloured red by carotenoid pigments. These pigments, mostly astaxanthin and hydroxyechinenone, are oxidative metabolic products of β-carotene derived from algae eaten by the animals (Paanakker & Hallegraeff, 1978). In some instances, two different morphs of the same species occur, inhabiting different lakes. For instance, *Diaptomus nevadensis* has a red morph living in Soap Lake, and a bluish transparant one in the neighbouring Lake Lenore (U.S.A.) (Hairston, 1979). Another example is *Acanthodiaptomus denticornis,* red in Lac Pavin, but bluish and translucent in Lac de Montcineyre (France) (Ringelberg, 1980). According to Hairston (1976), an important ecological function of this pigment is protection from damage caused by radiation in the visible part of the spectrum. Compared to animals kept in darkness, the mortality effect of blue light is more pronounced than that of red light. Siebeck (1978) emphasized the deleterious action of ultraviolet-B radiation. Pigmented cladocerans (*Daphnia pulex, D. longispina*) from shallow mountain ponds were able to tolerate a higher dose than less pigmented individuals from lowland areas. In both cases the mortality after radiation could be diminished by placing the animals in visible light. According to him (Siebeck, 1981), this photoreactivation effect, also present in U.V.-treated reef corals (Great Barrier Reef), is most effective in visible light in the blue-violet range. With regard to the activity of blue-violet light it seems that we are possibly dealing with counteracting physiological processes: on the one hand a deleterious, even deadly effect caused by oxidative damage, on the other hand recuperation of UV-damaged molecules by activation of the photoreactivating enzyme. Of course, both authors experimented with different species, calanoid copepods on the one hand, cladocerans and corals on the other. However, since the damaging effect of U.V.-B radiation and the photoreactivation of the shorter wavelengths in the visible spectrum are common phenomena in plants (Caldwell, 1971) and in such highly different animals as cladocerans and corals (Siebeck, 1978, 1981), it seems that these phenomena can be expected to be of a general nature. Following similar lines to those of Siebeck's experiments, we studied the mortality effect of U.V.-B radiation and subsequently the reactivating effect of visible light in a calanoid copepod comparable to the species used by Hairston.

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Methods

A red morph of *A. denticornis* was caught in Lac Pavin, France, by taking net hauls from about 30 m to the surface. This oligotrophic lake has a maximum depth of 90 m, but in daytime most animals concentrate at a depth between 10 and 20 meters. A translucent morph was caught in Lac de Montcineyre, France, at a distance of about 5 km from Lac Pavin. In most places, Lac de Montcineyre is shallower than 20 m and the animals were gathered by oblique net hauls. Both lakes are described extensively in Flik *et al* (1973) and Hallegraeff *et al* (1975–76). U.V.-B radiation was administered by placing the animals 30 cm beneath two parallel fluorescent tubes (Philips TL 40W/12, the type used by Siebeck, 1978). Irradiation intensity was calculated (by Dr. R. Lingeman) from the characteristics provided by the manufacturer. Also, intensity was measured using a radiometer calibrated in the U.V. and blue from 250–500 nm. At the centre of the tubes, both methods gave an irradiation of 6.4×10 W cm^{-2}. However, the radiometer measurements showed a decrease of 9%, 12% and 22% at respectively 20, 30 and 40 cm from the centre. Different doses of radiation were administered, depending on the period of exposure. For every dose, four small Petri dishes (without cover) were used, containing 3 ml lake water and five females. Most female morphs from Lac de Montcineyre carried eggs, those from Lac Pavin only occasionally. Before the radiation was administered, the animals had been in the laboratory for one night. It was thought that at least part of the effects of capture and transport would be lost in this way. Nine different doses of U.V.-B radiation were given. For comparison, with each experiment a set of animals was not irradiated. In a preliminary experiment, the translucent morph had been found to be much more sensitive than the red one. Therefore, the five lower doses were applied to the translucent morph and the five higher doses to the red morph.

After the radiation period, for each dose, two dishes were placed in darkness and two under continuous light from a white fluorescent lamp (Blanc Visseaux, 40 Watt). Since no radiation meter, calibrated in energy units, was available at the time, light intensities were measured with a Licor quantum meter. Afterwards, using a more or less comparable fluorescent lamp, values of this quantum meter were compared with those of a Licor radiometer. The measured intensity of 6.5 $\mu E\ m^{-2}\ s^{-1}$ proved to be comparable with about 0.4 W m^{-2}. Temperature varied between 16° and 19 °C.

For five successive days, dead animals were counted and removed every morning at about 9 h and every evening at about 21 h. Each day, the water in the dishes was partly replaced by fresh lake water.

For each dose, the increase of mortality with time was approximated by a linear regression, combining the replicates. The first set of observations to enter the calculation was the one preceding the one where dead animals were observed for the first time. The significance of the regression was high, all correlation coefficients varying between $R = 0.80$ and $R = 0.95$.

Forty-eight hours after the U.V. radiation was terminated the mortality percentage was calculated from the regression equations. These percentages for each dose are presented in Fig. 1.

U.V. penetration in the lake was not measured. However, to obtain some impression of the ecological significance of the lethal doses found, U.V.-irradiance at different depths was calculated from data found in the literature. From Bener (1963) cited by Caldwell (1971), global U.V.-B irradiances were taken between 07 and 17 hours at wavelengths ranging from 295 to 320 nm measured at Davos, Switzerland. Latitude and altitude in Davos are 47 NB respectively 1 590 m, and comparable to Lac Pavin at 46 NB with an altitude of 1 190 m. At wavelengths shorter than 295 nm, irradiance becomes very low due to ozone filtering. Diffuse attenuation coefficients for 5 nm wavelength intervals were taken from Fig. 4 in Smith & Baker (1981). These authors give k-values at 10 nm intervals ranging from 200 to 800 nm for the clearest ocean water and, in a graphical form, k-values for 330 to 460 nm for ocean water containing different concentrations of chlorophyll-a. Attenuation coefficients valid for 296–320 nm and 1.9, 3 and 5 mg chlorophyll-a m^{-3} were found by extrapolation, drawing lines parallel to the one for clearest ocean water. For the range of 295–320 nm, at 5 nm intervals, irradiance was calculated at different depths. A surface reflectance of 5% was used. The biological effectiveness of this U.V.-B radiation was calculated for 5 nm intervals multiplying the absolute irradiation energy by the relative energy effectiveness as determined by the

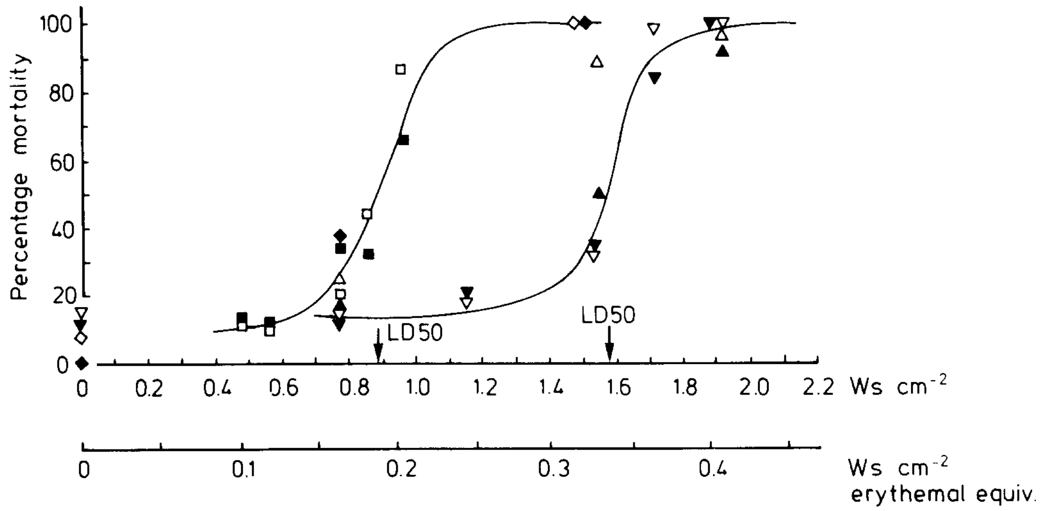

Fig. 1. Mortality percentage of *A. denticornis* 48 h after U.V.-B radiation treatment of different doses. Squares respectively triangles indicate results of two experiments with translucent morphs respectively red morphs. Black figures represent animals kept in darkness after treatment (see, however, text).

erythema (sunburning) action spectrum. This relative energy effectiveness was calculated by means of a formula presented by Green *et al* (1974) as cited by Damkaer *et al* (1980).

From the radiation spectrum of the fluorescent lamp as given by the manufacturer, relative energy was calculated at 5 nm intervals between 290 and 320 nm. These values multiplied by the total irradiation energy at the distance of 30 cm used in the experiments and multiplied by the relative erythemal effectiveness give the biological effectiveness of the U.V.-B used in the experiments. The mortality percentage presented in Fig. 1 is also made a function of this erythemal energy equivalent.

Results and discussion

In Fig. 1 the results of the ultraviolet radiation experiments are presented. From a zero-dose mortality of about 10–13% the radiation effect increases rather rapidly to a death rate of 100%. It is highly probable that mortality was caused by U.V. radiation. At the high doses, swelling of the body was observed, resulting in rupture of the integument and expulsion of body tissue, especially at the joints of the thorax segments. The red morph from Lac Pavin can tolerate a higher dose than the translucent one from Lac de Montcineyre. Although no strict proof is given, it is thought that the carotenoid pigments protect the animals against U.V. radiation.

There seems to be no difference in mortality between animals placed in the light or in the dark after treatment. The slight differences that may be observed are not significant and are inconsistent. This holds for the translucent as well as for the red morph. Apparently, no photoreactivation occurred. This is not in accordance with the findings of Siebeck (1978, 1981). In his experiments with *Daphnia*, LD50 values differed by a factor of 20 for post-radiation treatment with light or darkness. Such a large difference cannot be overlooked and the absence of it in our experiments, therefore, is conspicuous. Since many animals show photoreactivation (Siebeck, pers. commun.) and since it is considered a universal phenomenon also in plants (Caldwell, 1971), an experimental error on our part is the most plausible explanation. The white fluorescent lamp we used for photoreactivation had a low intensity of 0.4×10^{-4} W cm^{-2} providing a dose of 6.9 Ws cm^{-2} after 48 hours. The doses given by Siebeck to reactivate *Daphnia longispina galeata* for 50%, ranged from 0.10 at 440 nm to 0.65 Ws cm^{-2} at 470 nm (unpubl. data provided by Siebeck). They are at least smaller by a factor of 10. Even considering that most wavelengths of the fluorescent white light are rather ineffective in photoreactivation, it does not seem plausible that our irradiation dose was too small. Since the animals had been exposed to the light for at least a quarter of an hour twice daily in order to check on mortality, it may be possible that all our animals were photoreactivated.

The lethal dose at which 50% of the treated females of *A. denticornis* had died after 48 h amounted to 0.89 Ws cm^{-2} for the translucent morph from Lac de Montcineyre and to 1.58 Ws cm^{-2} for the red one from Lac Pavin. If these values hold for photoreactivated animals, these morphs of *A. denticornis* are more sensitive to U.V. radiation than either *D. longispina* or *D. pulex* (LD50 = 0.49 respectively 0.77 for the individuals kept in darkness and 9.2 respectively 15.2 Ws cm^{-2} for photoreactivated individuals according to Siebeck, 1978). Since the same lamps were used, these results are comparable. For comparison with results obtained by Damkaer *et al* (1980), U.V.-B erythemal effective equivalents have to be calculated (see below). The LD50 doses found for marine pelagic decapod larvae and the euphausid *Thysanoessa raschii* ranged from 0.36 to 0.45 Ws cm^{-2} and compare well with the 0.30 Ws cm^{-2} erythemal equivalent found for the red morph in this study.

The question arises as to whether our experiments have any ecological significance for *A. denticornis* in Lac Pavin. The accumulated U.V.-B radiation which animals would be exposed to between 7 h in the morning and 17 h in the afternoon at different depths in the water column was calculated from data found in the literature. These calculations were undertaken to provide a basis on which to assess the ecological value of the dose levels. No claim to precision is made. Moreover, the intensity of U.V.-B radiation strongly depends on the atmospheric turbidity due to haze, dust and air pollution (Caldwell, 1971). The results of the calculations are presented in Fig. 2. Ultraviolet radiation

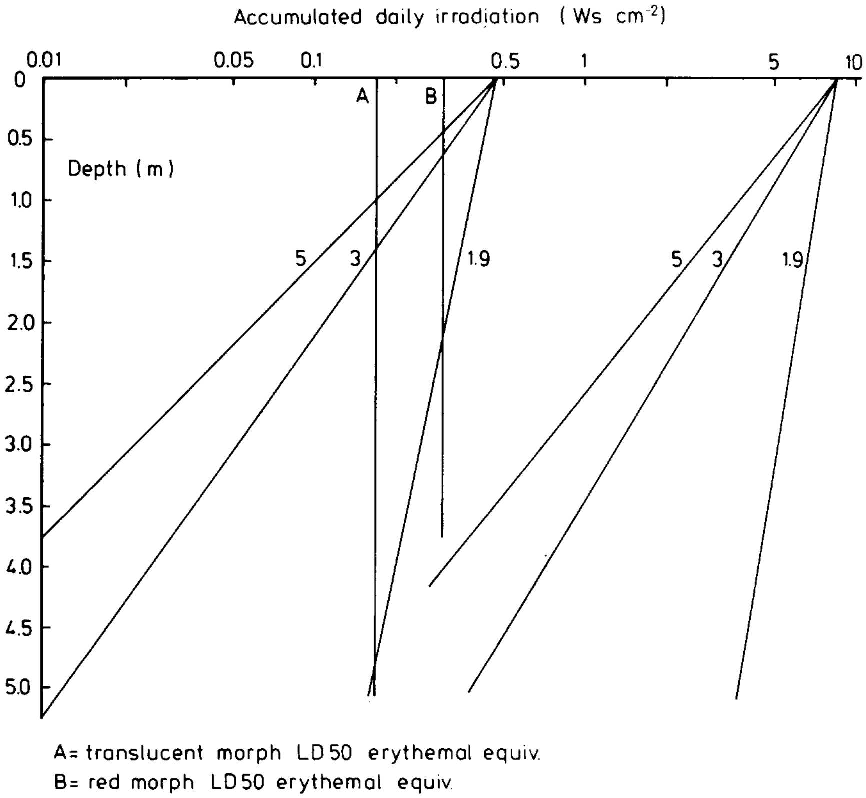

Fig. 2. Calculated U.V.-B irradiation integrated per day between 07 and 17 h at different depths and three concentrations of chlorophyll-a (1.9, 3 and 5 mg m^{-3}) in the water. The left hand set presents lines of integrated erythemal energy equivalents. The two vertical lines represent the LD 50 dose values for red and translucent morphs.

penetration markedly varies with the chlorophyll-a content of the water. Flik *et al* (1973) and Hallegraeff *et al* (1975–76) report a chlorophyll-a concentration in Lac Pavin during the summer months of about 0.3–3 mg m^{-3} in the upper 10 meters with an occasional thin layer of a somewhat higher concentration. The oblique lines on the right in Fig. 2 represent irradiation doses accumulated over a daily period of 10 hours. However, the spectral composition of the ultraviolet radiation from sun and sky differs from that of the fluorescent tubes used to determine the lethal dose. Since the biological photon effectiveness varies with the various wavelengths, it is not possible to compare these experimental lethal levels directly with the doses reached under water. In order to deal with the different spectral compositions of natural radiation and fluorescent tubes, biological effectiveness was calculated for both. Damkaer *et al* (1980) stated effective daily doses for some zooplankton based on the relative erythema (sunburning) effectiveness of the different wavelengths. Smith & Calkins (1976) described a radiation measuring device, equipped with filters to correspond to the erythemal action spectrum with which to measure effective U.V.-B penetration in natural waters. The erythemal curve has a maximum at 297 nm decreasing rapidly on both sides. For a discussion of the ecological relevance of U.V.-B penetration, calculations based on the erythemal effectiveness were preferred above other action spectra, for instance the one published by Caldwell (1971). The effective daily U.V.-B sun + sky dose derived from the Davos measurements is 0.47 Ws cm^{-2} erythemal energy equivalents. Damkaer *et al* (1980) mention a maximal daily dose at sea level (Manchester, Washington, U.S.A.) in July of 0.23 Ws cm^{-2} erythemal equivalents. Although up to 1 500 m the increase of U.V.-B radiation with altitude is slight (at altitudes higher than 2 000 m U.V.-B radiation intensity rapidly increases, see Caldwell, 1971), air turbidity must be much higher in Manchester than at the altitude of either Davos (renowned for its pure air) or Lac Pavin. In any case, it seems that our calculations of the daily dose compare to actual measurements.

From Fig. 2 it appears that at a chlorophyll-a concentration of 3 mg m^{-3}, the 50% lethal dose for the red morph may be exceeded from the surface to 0.6 m and the one for the translucent morph up to a depth of 1.4 m. On a clear day and at very low chlorophyll concentrations, such as can be found in oligotrophic high altitude lakes, ultraviolet hazard, for translucent animals especially, cannot be ruled out within a layer of several meters. On the other hand, a slight decrease in the highly variable U.V.-B radiation intensity, for instance with a factor 2, might very well prevent the lethal dose from being reached within one day of exposure at the surface. Of course, mortality is a very crude criterium to use in studying the ecological relevance of any environmental factor. In any case, few *A. denticornis* are found in depths of less than 10 meters in Lac Pavin, so they are well out of the dangerous zone.

Simple preliminary field experiments showed that animals exposed to surface radiation are liable to be killed. On August 3, 1981 at 18.15 h, plastic bags with red morphs from Lac Pavin and translucent morphs from Lac de Montcineyre were suspended just below the surface of Lac Pavin. On August 6, at 13.45 h, after 2.5 daylight periods, dead and living animals were counted. All the 57 translucent morphs had been dead for some time since many animals were covered by fungus. Of the 138 red morphs, only 32 were dead. The condition of the living individuals was poor: they hardly moved and could easily be sucked up with a pipet. From August 18–21, 1982, plastic bags containing red and translucent morphs were suspended at different depths in Lac Pavin. The results are presented in Table 1. Compared to the mortality in the darkened bags, it may be concluded that the translucent morphs were liable to an additional mortality in depths of less than 5 m. For the red morph, no such additional mortality was found at any depth. It is hard to say which U.V. doses these animals had been subjected to. Nor is it known to what extent

Table 1. Mortality of *A. denticornis* in plastic bags suspended for three days at different depths in Lac Pavin.

Depth	Red morph		Translucent morph	
	Total number	Percentage dead	Total number	Percentage dead
0 m	23[a]	0	77	79
1 m	62	19	91	57
5 m	84	19	112	62
10 m	103	14	109	34
20 m	83	31	114	14
in darkness	116	16	85	29

[a] This bag was damaged and only living animals were found.

recuperation took place at low light intensities in early morning or evening and in darkness at night. A daily radiation during three hours of 0.068 Ws cm^{-2} erythemal equivalents during 9 days resulted in a 50% mortality in *Thysanoessa raschii* (Damkaer *et al,* 1980). The accumulated dose received by then is 0.61 Ws cm^{-2} and not much higher than expected were it given as a single treatment. So there does not seem to have taken place much recovery outside the periods of U.V. irradiation. In any case, zooplankton is prone to U.V.-B damage in clear mountain lakes if they stay too long near the surface in daytime. Diel vertical migration might very well constitute a means to prevent this.

Summary

A morph of the calanoid copepod *Acanthodiaptomus denticornis* with red pigment, and a translucent morph of the same species were treated with different doses of ultraviolet-B radiation. Following irradiation, the animals were either kept in darkness or placed in the light, in order to study a possible photoreactive effect. Mortality was determined 48 h after treatment. The LD 50 irradiation dose proved to be higher in the red morph then in the translucent one.

Based on data from the literature, U.V.-B penetration in an oligotrophic high altitude lake was calculated. On a clear day, U.V.-B hazard may be present in the upper few meters of a lake resembling the one where the red morph was found. Mortality within plastic bags suspended at different depths in this lake confirmed this.

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