Modulators of Immune Responses
The Evolutionary Trail

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Skin Component May Protect Fishes from Sunburn and Fungal Infection Resulting from Exposure to Ultraviolet-B Radiation

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Chapter 20

Skin Component May Protect Fishes from Sunburn and Fungal Infection Resulting from Exposure to Ultraviolet-B Radiation

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ABSTRACT

Elevated levels of ultraviolet-B (UVB) radiation, as a consequence of stratospheric ozone depletion, may cause harmful effects in freshwater fishes. To determine the extent and significance of solar UVB effects on aquatic organisms, we investigated the effects of simulated solar UVB radiation on rainbow trout (*Oncorhynchus mykiss*), Apache trout (*Oncorhynchus apache*), Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), and razorback suckers (*Xyrauchen texanus*). In our solar simulator, fishes received daily 5-hr exposures to UVB (290-320 nm) during a 16-hr photoperiod. Lahontan cutthroat trout and rainbow trout had significant sunburn on day 3 and significant fungal infection on day 6 of exposure to an irradiance of UVB that simulated ambient mid-latitude summer irradiance. When methanol extracts of dorsal skin from unexposed fishes were scanned in a spectrophotometer we observed a large absorbance peak in the UVB wavelength range. The peak area was calculated and the relative amount of this unidentified component was estimated in the dorsal skin of each fish species. Apache trout and razorback suckers had significantly larger amounts of this component than Lahontan cutthroat trout and rainbow trout and did not develop sunburn or fungal infection. These findings indicate that this component may be photoprotective and act as a natural sunscreen.

INTRODUCTION

Increases in UVB at the earth's surface have resulted from stratospheric ozone depletion (Kerr and McElroy, 1993). Aquatic organisms are at risk when UVB penetrates the water column (Smith and Baker, 1979; Smith *et al.*, 1992; Gleason and Wellington, 1993; Hader, 1993; Siebeck *et al.* 1994; Williamson, 1995) causing sunburn in some fishes (DeLong *et al.*, 1958; Dunbar, 1959; Allison, 1960; Bullock and Roberts, 1981; Bullock, 1982; Bullock *et al.*, 1983; Bullock and Coutts, 1985; Bullock, 1988; Fabacher *et al.*, 1994; Little and Fabacher, 1994; Ramos *et al.*, 1994). We compare and discuss the incidence of sunburn and fungal infection in four species of freshwater fishes exposed to simulated solar UVB. We also describe a skin component that may protect some

of these fishes from sunburn and fungal infection resulting from exposure to simulated solar UVB radiation.

METHODS

The solar simulator (0.61 m wide by 1.83 m long) contained ten 160-watt cool white lamps, four 160-watt UVB313 lamps, eight 160-watt UVA365 lamps, two 20-watt cool white lamps, two 20-watt SF20 sun lamps, and eight 75-watt halogen incandescent flood lamps.

UVB lamps were controlled by a recycling 24-hour timer that operated for 5 hours to simulate a total solar daily dose. Cool white and UVA fluorescent lamps were controlled by a second timer that operated for a 16-hour period simulating a midsummer photoperiod. The simulator was suspended over a water bath of similar dimensions and was enclosed with reflective specular aluminum to contain the radiation in the exposure area. Output of the simulator was calibrated as previously described (Fabacher *et al.*, 1994; Little and Fabacher, 1994).

Juvenile fishes (60-75 days post-hatch; mean length 4.8 cm; mean weight 0.95 g) were exposed in 2-L glass 15 x 15 x 23 cm tall airlift chambers (Cleveland *et al.*, 1991) which received a 0.6 L/min flow. The chambers were set in the water bath under the solar simulator. Water quality and temperature (18°C) in the water bath was the same as used in culture. Control conditions, which provided a minimal UVB irradiance of 4.3 $\mu W/cm^2$ and a dose of 0.08 J/cm^2/day, were created by covering the top and bottom of each exposure chamber with 0.76 mm polycarbonate, and then covering the sides with 0.13 mm mylar. The simulated solar UVB irradiance of 190 $\mu W/cm^2$ and a dose of 3.42 J/cm^2/day was generated by covering the top of each exposure chamber with 0.13 mm thick cellulose acetate. Lahontan cutthroat trout, rainbow trout, and Apache trout were exposed for 7 days; razorback suckers for 21 days.

Fish were stocked in groups of 5 per chamber. Three replicate groups per treatment were randomly distributed under the solar simulator, and the entire experiment was repeated. Values obtained from replicates among treatments were pooled to generate a sample size of six per treatment. Fish were examined daily for sunburn, infection, and mortality, and were fed 24-hour old *Artemia sp.* and salmon starter several hours prior to and at the conclusion of each exposure. The effect of treatment level and species on the response of fishes was evaluated using analysis of variance techniques performed with the Statistical Analysis System (SAS, 1989). Cumulative percentages of sunburn and fungal infection were arcsine transformed and analyzed using a randomized block model with a repeated measures ANOVA to account for effects between experimental trials (Snedecor and Cochran, 1980). Mean values were compared using Fisher's protected LSD test ($p \leq 0.05$).

In addition to the exposures, each of five unexposed fish of each species was killed by freezing. A fish was held between a forceps on a watch glass under a binocular dissecting microscope, skin behind the head and opercula was punctured with a pointed forceps, and a small microscissors was used to cut a section of skin from the dorsal surface of the fish. This section of skin encompassed the area from just behind the head to the dorsal fin and just above the lateral lines and was peeled off the underlying musculature. Each skin section was weighed to the nearest milligram, extracted with 100% methanol, and refrigerated. Chilled methanol extracts were scanned in a Beckman 5230

UV/vis recording spectrophotometer. The approximate absorption maximum (λmax) of a skin component was calculated from the recording on the chart paper. Peak area was calculated using the formula 1/2 baseline x height to give a semiquantitative estimate of the amount of skin component. The results were expressed as area units/milligram wet weight of tissue. Statistical significance ($p \leq 0.05$) of the amount of component was determined by t-test and Duncan's multiple range test (Snedecor and Cochran, 1980).

RESULTS

The simulated solar ultraviolet (UV) irradiance produced by the simulator was similar to solar irradiance measured in Columbia, Missouri, on June 21, 1993, at an altitude of 271 m and a latitude of 38.5° N (Figure 1).

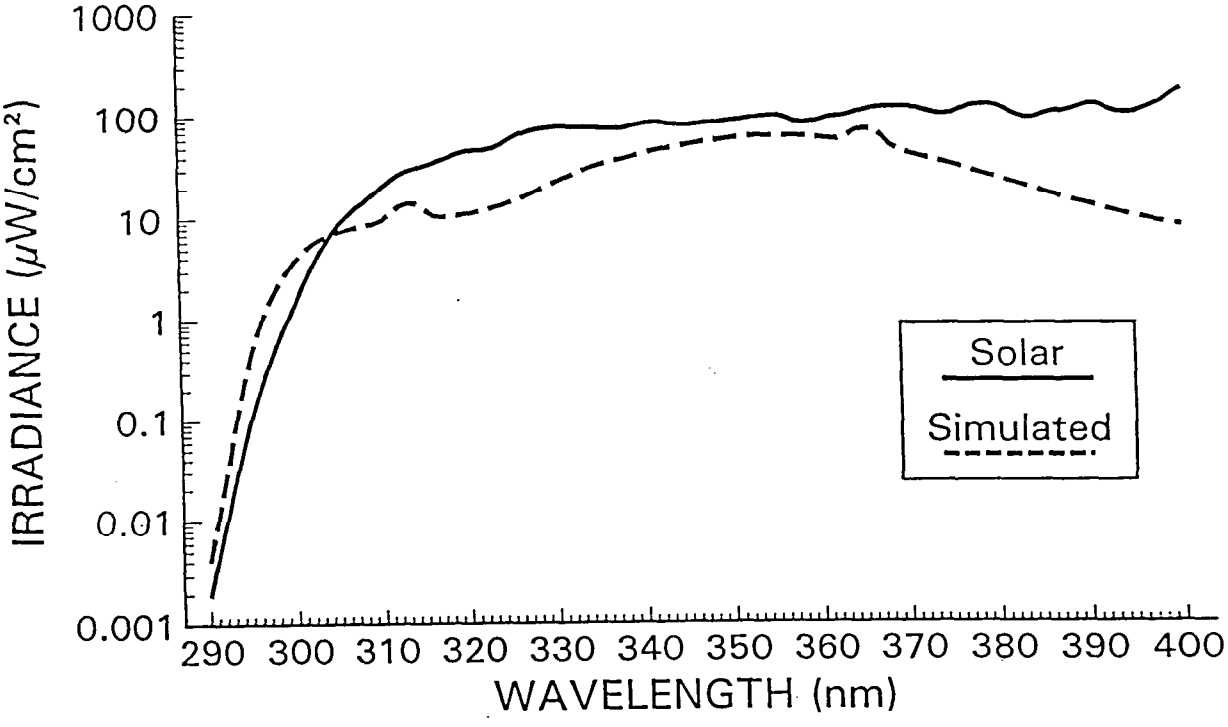

Figure 1. Spectral composition of simulated solar exposure irradiance and solar UV irradiance measured on June 21, 1993 at 38.5°N latitude (modified from Little and Fabacher, 1994).

In order to provide an indication of the wavelength composition and sunburning potential of the simulated solar UV irradiance, we compared the weighted irradiance values for simulated solar and solar UV using the Diffey action spectra (McKinlay and Diffey, 1987) for human erythema (sunburn). Since shorter wavelengths induce erythema more readily than longer wavelengths, this weighting provides a sunburning dose relative to the spectral wavelength composition of the exposure. The Diffey erythemal weighted irradiance reflects the total of the weighted irradiances

for all wavelengths necessary to produce erythema in humans. The Diffey erythemal weighted irradiance was 3.256 x 10^{-5}W/cm^2 for sunlight and 3.021 x 10^{-5}W/cm^2 for the simulated solar irradiance. Thus, the sunburning potential of the simulated solar irradiance was similar to that of ambient solar exposure on June 21, 1993, in Columbia, Missouri.

The presence of sunburn was a qualitative observation which first appeared as a characteristic darkening of the skin on the dorsal surface of the fish between the head and caudal fin and ventrally to about the lateral lines (Fabacher *et al.* 1994; Little and Fabacher, 1994). The largest area of sunburn usually occurred just posterior to the head and anterior to the dorsal fin. After day 2 of the 7-d exposure, incidence of sunburn increased significantly for Lahontan cutthroat trout and rainbow trout exposed to simulated solar UVB (Table 1).

Table 1.
Percent sunburn among fishes during a 7-day exposure to ultraviolet-B radiation

Day of exposure	Lahontan cutthroat trout		rainbow trout	
	control[a] (%)	treated[b] (%)	control[a] (%)	treated[b] (%)
1	0	0	0	0
2	0	16.7[7.4][c]	0	3.3[6.0][c]
3	0	53.3[14.8][c,d]	0	26.6[11.1][c,d]
4	0	73.3[11.0][c,d]	0	33.3[25.2][c,d]
5	0	80.0[8.0][c,d]	0	53.3[11.4]c,d
6	0	86.6[5.1][c,d]	0	83.3[3.7][c,d]
7	6.6 [5.1][c]	90.0[5.5][c,d]	0	93.3[4.7][c,d]

[a]Minimal UVB irradiance of 4.3 μW/cm^2.
[b]Solar simulated irradiance of 190 μW/cm^2.
[c]Mean[SE], n=6.
[d]Significantly different ($p \leq 0.05$) from control.

In addition to sunburn, Lahontan cutthroat trout and rainbow trout also developed fungal infection (Table 2). Fungal infection, observed to be *Saprolegnia* sp. (Fabacher *et al.*, 1994), appeared as mycelia on the darkened areas of the dorsal skin and on the dorsal fin. Later, patches of fungus covered the darkened areas and the dorsal fin giving those areas a white to gray colored appearance. Fungal infection appeared on day 4 and increased significantly from control after day 5 for Lahontan cutthroat trout. Fungal infection was significantly different from control beginning on day 6 for rainbow trout. Apache trout did not develop sunburn or fungal infection within 7 days of exposure. Razorback suckers did not develop sunburn or fungal infection within 21 days of exposure.

In methanol extracts of the dorsal skin from unexposed Lahontan cutthroat trout, rainbow trout, Apache trout, and razorback suckers we observed an unknown skin component (Fabacher and Little, 1995) with an absorption maximum around 292 nm (Figure 2).

Table 2.
Percent fungal infection among fishes during a 7-day exposure to ultraviolet-B radiation

Day of exposure	Lahontan cutthroat trout		rainbow trout	
	control[a] (%)	treated[b] (%)	control[a] (%)	treatedb (%)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	13.3[9.4][c]	0	0
5	0	23.3[13.3][c]	0	0
6	0	46.6[9.4][c,d]	0	20.0[8.1][c,d]
7	0	60.0[8.1][c,d]	0	30.0[7.5][c,d]

[a]Minimal UVB irradiance of 4.3 μW/cm^2.
[b]Solar simulated irradiance of 190 μW/cm^2.
[c]Mean[SE], n=6.
[d]Significantly different ($p \leq 0.05$) from control.

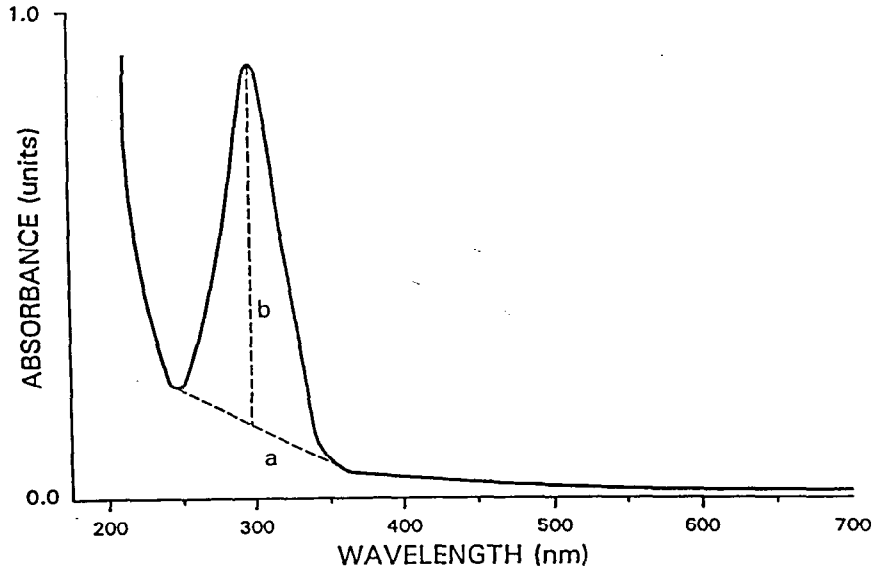

Figure 2. A representative UV-visible light absorption spectrum of dorsal skin methanol extract from a razorback sucker (modified from Fabacher and Little, 1995). Dotted lines are baseline (a) and height (b) of peak.

Apache trout and razorback suckers, which did not develop sunburn or fungal infection, had significantly larger amounts of this component than Lahontan cutthroat trout and rainbow trout (Table 3).

Table 3.
Amount of component in skin of unexposed fishes and day when UVB-exposed fishes were observed with significant sunburn and fungal infection

Species	Amount of component*	Day to sunburn	Day to fungal infection
Lahontan cutthroat trout	23.9[2.8][a+]	3	6
Rainbow trout	23.9[1.2][a]	3	6
Apache trout	49.6[7.8][b]	>7	>7
Razorback suckers	101.2[3.8][c]	>21	>21

*Values are mean[SE] area units/milligram wet weight of tissue for five fish of each species.
+Means with the same letter are not significantly different.

DISCUSSION

We observed a darkening of the dorsal skin within 48 hours of exposure to UVB radiation among Lahontan cutthroat trout and rainbow trout. Widespread dispersion of melanosomes occurred in the dermis of Atlantic salmon (*Salmo salar*) that were thought to have been exposed to direct solar ultraviolet radiation (McArdle and Bullock, 1987). Darkening of the dorsal skin of fishes exposed to simulated solar UVB may have resulted from melanosome dispersion and yielded the first grossly observable symptoms of sunburn.

The appearance of sunburn and subsequent fungal infection we observed are consistent with observations of other investigators. Bell and Hoar (1950) observed changes in pigmentation and fungal infection in irradiated coho salmon (*Oncorhynchus kisutch*) fry and goldfish (*Carassius auratus*). Sunburn and fungal infection were observed in chinook salmon (*Oncorhynchus tshawytscha*) exposed to sunlight (DeLong *et al.*, 1958).

In our study, fungal infection may have resulted from cell necrosis within the sunburned skin (Bullock, 1982), as well as suppression of the immune system by UVB. A wide range of environmental stress factors can depress the immune system in fishes, including chemical contaminants, drugs, and X-radiation (Zeeman and Brindley, 1981; Anderson *et al.*, 1984). Fishes may be more susceptible to infection by pathogens after the immune system has been depressed (Sindermann 1979; Weeks *et al.*, 1986; Anderson, 1990).

We did not observe darkening of the dorsal skin and fungal infection in UVB-exposed Apache trout and razorback suckers. Among Lahontan cutthroat trout and rainbow trout, not all fish in the same exposure chamber experienced sunburn and subsequent fungal infection. Similar to our observations, Bullock (1988) observed considerable variability in response of individual rainbow trout to intense simulated solar radiation and suggested that the ability of some fishes to tolerate UV radiation

may result from elevated levels of a photoprotective factor of genetic origin. The unknown skin component we observed may function as the photoprotective factor suggested by Bullock (1988).

The spectral characteristics of the unknown skin component we observed are similar to mycosporine-like amino acid compounds (MAAs) extracted from a variety of marine organisms, including eggs and eyes of fishes (Chioccara *et al.*, 1980; Dunlap *et al.*, 1989; Karentz *et al.*, 1991; Karentz, 1994). When methanol extracts of tissues containing these low molecular weight, polar MAAs are scanned in a spectrophotometer, absorption maxima can be observed from 310-360 nm. Thus, MAAs may offer organisms protection against UV radiation that occurs around those wavelengths. Even though the λ max of around 292 nm we observed in fish dorsal skin extracts is lower than that normally observed for MAAs, the unknown we observed may be related to MAAs. Preliminary results with high pressure liquid chromatography indicate that the unknown component we observed is a low molecular weight, polar, single compound.

Fish skin is more susceptible than human skin to sunburn damage when exposed to UVB because fish skin lacks a keratinized outer layer, has dividing cells in all layers of the epidermis, and normally does not contain protective epidermal melanin-containing cells (Bullock *et al.*, 1978; Bullock, 1982). The unknown component we observed is probably produced in epidermal cells and concentrated in the mucus. Once in the mucus it could effectively absorb, and thus block, UVB radiation occurring around 292 nm from reaching critical macromolecules in cells of the epidermal and dermal layers of the skin.

In conclusion, we observed an unidentified component in the dorsal skin of unexposed fishes. There appeared to be direct relationship between the amount of this component and the day when UVB-exposed fishes were observed with significant sunburn and fungal infection. This component may protect natural populations of fishes from sunburn and fungal infection resulting from exposure to UVB. The degree of photoprotection would probably depend on the amount of component present as well as on the UVB irradiance. We plan to identify this component, measure the levels of this component in UVB-exposed fishes, and determine if this component can be used as a bioindicator to identify UVB-sensitive fish species.

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