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Histopathology of the skin of UV-B irradiated sole (*Solea solea*) and turbot (*Scophthalmus maximus*) larvae

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Abstract

Larval stages of two economically important flatfish, the sole (*Solea solea*) and turbot (*Scophthalmus maximus*) were exposed to ambient and elevated levels of UV-B. Sole larvae, which naturally occur in the plankton in early spring, demonstrated skin lesions at elevated levels of UV-B. Histopathology of the sole revealed cellular changes in the integument, characteristic of sunburn damage, with a reduction in the size of mucus-secreting cells and an increased epidermal thickening, especially at the highest doses of UV-B (2.15 KJ bio eff/m^2). Pigmentation in the sole is restricted to a few isolated melanocytes. The integrity of the heavily pigmented skin of turbot appeared to be unaffected by comparable doses of UV-B. Both species have protective mechanisms, which minimize the effects of naturally-occurring levels of UV-B. However, sole appear to be poorly adapted to accommodate any further increase in solar radiation.

Keywords: *Solea solea*; Larvae; UV-B radiation; Histopathology

Spawning of many commercially important pelagic and demersal fishes occurs in coastal waters, where larval stages reside in the upper water column, with the potential for exposure to ultra violet-B (UV-B). Their relative inactivity and absence of protective scales enhance their vulnerability to radiation. This highlights the

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potential threat of increasing levels of UV-B for commercial fisheries and the aquaculture industry in temperate regions. Estimates of the maximum decrease in stratospheric ozone are in the order of 16% and it has been predicted that this could result in a 5% loss in global phytoplankton. It is further estimated that this loss of production would cause a reduction in fishery and aquaculture yields of approximately 7% — the equivalent of approximately 7 million tons of fish per annum (Häder, Worrest, Kumar & Smith, 1995).

Solar UV-B radiation, 280–315 nm, can penetrate to significant depths in the water column. Recent studies indicate that levels at the earth's surface are increasing due to ozone depletion (Solomon, 1990). There is evidence that fish are susceptible to UV-B radiation (Bullock, 1988; Hunter, Kaupp & Taylor, 1981), with sensitivity varying within groups (Blazer, Fabacher, Little, Ewing & Kocan, 1997). However, few studies have addressed the relative sensitivity and susceptibility of larval life stages of marine fish. At the molecular and cellular level, the primary products of UV radiation are rapidly formed free radicals, producing biological effects lasting from minutes to years. DNA is the main target for UV radiation-induced damage. The absolute and relative amounts of damage vary according to the specific wavelength of radiation (Kielbassa, Roza & Epe, 1997). The shorter wavelength radiation has more energy and results in greater damage at the molecular level, thus a biological weighting (Setlow DNA weighting function; Setlow, 1974) is applied, based on the measured action spectrum for each treatment group.

Newly hatched yolk-sac larvae of sole (*Solea solea*) and turbot (*Scophthalmus maximus*) were exposed to four levels of UV-B radiation (Table 1). The highest dose, 2.15 KJ bio eff/m^2 (weighted for damage to DNA), was equivalent to that predicted for the sea surface around the UK, on a clear day in July, with an estimated 15%

Table 1
Mean cranial skin thickness and goblet cell diameter (μm) for *Solea solea* larvae exposed to various doses of biologically effective UV-B (KJ bio eff / m^2)[a]

Daily UV-B dose (KJ bio eff /m^2)	Mean (μm) ±2SE	% With epidermal stratification	Daily UV-B dose (KJ bio eff /m^2)			
			0.0	0.41	1.32	2.15
Skin thickness						
0.0	20.39±1.39	0	1			
0.41	25.26±10.19	15	0.975	1		
1.32	27.06±10.66	25	0.881	0.992	1	
2.15	45.50±13.01	75	0.003	0.017	0.025	1
Goblet cell diameter						
0.0	20.23±1.18	–	1			
0.41	17.48±0.72	–	0.000	1		
1.32	17.39±0.82	–	0.000	0.999	1	
2.15	14.42±0.55	–	0.000	0.000	0.000	1

[a] *P*-values are given for the mean skin thickness and goblet cell diameter, derived from an ANOVA, followed by a Tukey HSD multiple comparison.

increase in ozone depletion (SORG, 1996). Exposures were conducted for 6 h daily, on each of five consecutive days after hatching. Simulation of a solar spectrum with up to 15% ozone depletion was obtained with an array of fluorescent lamps fitted with cellulose tre-acetate filters. The control treatment spectrum had no radiation below 315 nm. All treatments had approximately equal ratios of UV-B:UV-A irradiance. The daily dose of biologically effective UV-B (KJ bio eff/m^2) was calculated from the measured irradiance of the lamp array, weighted for the relative biological effect of each wavelength, using an action spectrum for DNA damage normalized to 1 at 300 nm. Twenty individual specimens from each treatment were then anaesthetized, prior to fixation. Macroscopically, none of the larvae had obvious skin lesions.

Larvae were processed for methacrylate embedding, serially sectioned at 2 μm in the sagittal plane and stained in Lees' methylene blue basic fuchsin (McFadzen, Lowe & Coombs, 1994). Skin thickness was measured at 25-μm intervals, from the dorsal surface of the maxilla to the onset of the auditory vesicle. The distance from the outer limit of the basement membrane to the open surface of the epithelium was recorded. The diameter of the epithelial mucus-secreting cells was measured, in a parallel axis to the skin (Table 1), using a filar eyepiece graticule, on sections taken in the dorsal midline.

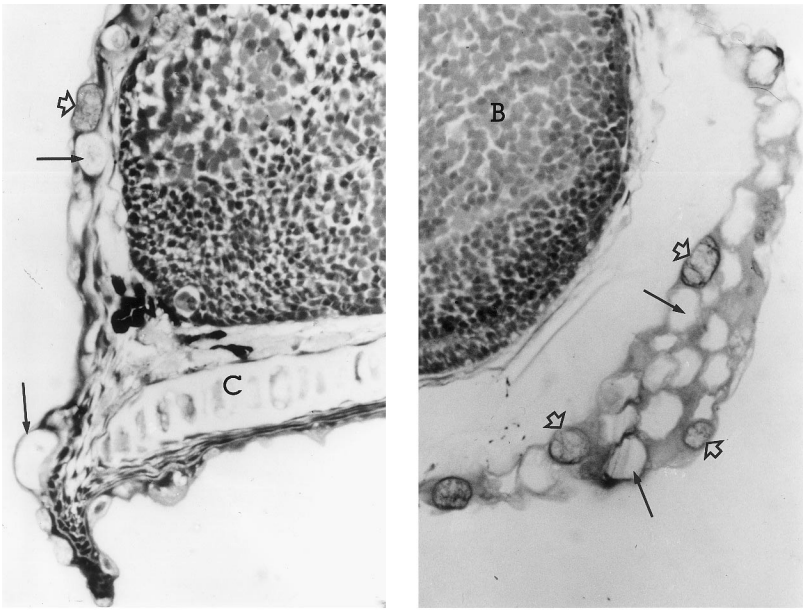

Fig. 1. Two-micrometre sections through the cranial region of sole larvae. Lees' methylene blue basic fuchsin, ×312.5 mag. (a) Normal skin formation at 0.41 (KJ bio eff/m^2). Note the single layer of malpighian cells (m) and the isolated dark mucus secreting cell (open arrows). (b) Abnormal 'sun burned' skin following exposure to elevated levels of UV-B 2.15 (KJ bio eff/m^2). Note the increased layers of malpighian cells (arrows). B, brain; C, cartilage.

The outer region of the normal skin structure of sole larvae is composed of smooth, flattened squamous epithelial cells, predominantly comprising large (~20 μm) malpighian or filament containing cells (Bullock & Roberts, 1992), characterized by a pale cytoplasm and a peripheral nucleus. The epidermis is avascular, in close association with a distinct basement membrane above an ill-defined *stratum spongiosum*. Contained within the basal region of the epidermis are isolated dark-staining, mucus-secreting cells (Fig. 1a), often spanning the epidermis. The only notable cranial melanocytes were in the dermal layer at the apex of the maxilla (Fig. 1a).

In response to elevated UV-B radiation, there was a significant thickening and stratification of the epidermis in 75% of the sole larvae exposed to the highest dose (Table 1), and pronounced separation of the integument from the *stratum spongiosum* (Fig. 1b). There was a highly significant reduction in the diameter of the mucus-secreting goblet cells (Table 1), possibly having exhausted their protective capacity and reducing the future protective capabilities of the skin. At lower doses of UV-B the number of larvae showing epidermal thickening was less (0–25%, Table 1) than at the highest dose, and mean skin thickness was approximately halved (Table 1).

In turbot larvae the skin is a single cell-thick layer of squamous epithelial cells, comprised of a mixture of specialist types; malpighian, and chloride cells (Bullock, 1982). There was no evidence of mucus-secreting cells in the epidermis, but a continuous layer of sub-epithelial pigment cells (melanocytes) covered all larvae.

There was no observed alteration to the structure of the skin of UV-B-exposed turbot larvae, irrespective of the treatment. The epidermis appeared normal, with no increase in cell number or stratification despite the protective pigment cells being sub-epidermal. Therefore no attempt was made to measure the epithelial thickness.

Structural and cellular alterations observed in the skin of the sole from the higher dose treatments are, in part, comparable to those reported for plaice (Bullock, 1982), laboratory-reared trout (Blazer et al., 1997), anchovy (Hunter et al., 1981) and salmon (Berghahn, Bullock & Karakiri, 1993). Kaupp and Hunter (1981) found that anchovy exposed to UV-B radiation alone were more affected than specimens illuminated with white light immediately following the UV-B exposure. This latter group was capable of photo-repair, had greater survival rates, and grew to a greater length. In another experiment, where the larvae were exposed to UV-B for 4 days, surviving specimens exhibited lesions in the brain and eye as well as retarded growth and development (Hunter et al., 1981). Both sole and turbot seem to have evolved protective mechanisms that minimize the effects of UV-B they would naturally encounter in the environment, with no depletion of the stratospheric ozone. Sole are less well adapted to accommodate any increase in UV-B radiation. Turbot appear particularly well adapted, and this is reflected in that they appear in the ichthyoplankton during mid-summer and live high in the water column. However, sole larvae are generally found in the spring in deeper water, where the incident UV-B has been attenuated. This work has shown UV-B radiation can have a direct impact on the integument, which may then have possible deleterious implications for recruitment success.

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