

## Sensitivity of native freshwater fish and invertebrate species and the role of photoprotectant slime

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### Introduction

Levels of ultra-violet (UV) light are naturally high in New Zealand and the exposure of many stream-dwelling aquatic organisms would undoubtedly have increased markedly as a result of widespread deforestation occurring since human colonisation. This extensive landscape modification resulted in reduction of the previously 80-90% forest cover to about 30% remaining today. Additionally, UV levels may be expected to increase in the future as a result of thinning of the stratospheric ozone. The region of influence of the 'ozone hole' is presently largely confined to region over Antarctica, however, recent years (particularly 1998) has seen the hole extend widely to include parts of South America. The seasonal nature of this phenomenon may result in particularly high seasonal peaks in UV exposure. Increased levels of UV radiation have been shown to result in a range of adverse effects on aquatic species.

Overseas studies have demonstrated significant behavioural and physiological effects on sensitive freshwater fish and macroinvertebrates species at UV levels minimally higher than existing levels (e.g., Hurtubise et al. 1998). Notably, studies have also demonstrated a wide range of tolerance levels for different species, prompting the need to understand factors that affect sensitivity.

A number of New Zealand's freshwater fish species are potentially highly susceptible for a number of reasons. Firstly, New Zealand's streams and rivers are largely of high optical clarity that will result in effective UV transmission. Secondly, most of our native fish species are diadromous, with juveniles migrating from the sea to constitute the highly prized 'whitebait' catch. These fish are behaviourally and possibly inherently sensitive to UV exposure. Indeed, species such as the inanga (*Galaxias maculatus*) which lay their eggs on bank-side grasses may be especially susceptible.

Understanding the potential effects of UV on freshwater species is particularly relevant to management of river ecosystems. Many factors affect the exposure of organisms to UV, including shade, dissolved organic matter, organism behaviour and natural photoprotectants. These factors must be

considered in addition to the organism's inherent sensitivity to UV. Manipulation of riparian vegetation provides a practical mechanism to markedly reduce UV exposure in river ecosystems and thus mitigate adverse effects.

UV radiation may affect organisms both directly and by phototoxicity associated with the presence of chemical contaminants in the environment. The damaging effects are wavelength dependent, with direct effects largely attributable to the UVB wavelengths (280-320 nm), while the longer wavelength UVA (320-400 nm) contributes to photoactivation associated with contaminants.

This report addresses the inherent sensitivity of selected freshwater fish and invertebrate species to UVB exposure. Specifically we addressed the following questions:

1. How sensitive are freshwater riverine species to UVB exposure?
2. How do the measured sensitivities compare with organisms from other environments?
3. How do these sensitivities compare with potential field exposures?

### Methods

We measured acute phototoxicity to four freshwater invertebrates and three native fish species. We also measured the sensitivity of inanga eggs to UV exposure (7d). The invertebrate species *Ceriodaphnia dubia* (cladoceran, 1d), *Paracalliope fluviatilis* (amphipod, 2d), *Tenagomysis chiltoni* (mysid, 2d) and *Deleatidium* sp. (mayfly, 4d) were exposed to UV for 24-96h. Native fish species were *Galaxias maculatus* (inanga, 4d) eggs and newly hatched larvae, *Retropinna retropinna* (Common smelt, 4d) juveniles, *Galaxias postvectis* (shortjawed kokopu, 4d) larvae, *Gobiomorphus cotidianus* (common bully, 4d) juveniles and *Anguilla australis* (shortfinned eel, 4d).

The exposures were conducted in an environmental chamber with artificial lighting ('solar simulator'; Little and Fabacher 1996). The simulator consisted of a 1.12 m<sup>2</sup> light cap, suspended over a water bath with a depth of 15 cm. Little and Fabacher (1996) describe the simulation methods in detail. We used a 16h light: 8h dark exposure, with UVB lamps on for

5 hours and UVA lamps for 12 hours. The total daily flux was 27.54 kJ/m<sup>2</sup>/d, which is about 27% of the Auckland summer time (Wubben et al. 2001). The peak UVB in the simulator was 2.5 W/m<sup>2</sup> at 85cm, which compares with about 4 W/m<sup>2</sup> measured in Hamilton on clear summer days. All UV measurements were made with a Macam ultra-violet radiometer (model UV203-3 (Macam Photometrics, Livingston, Scotland). The UVB photometer has a spectral sensitivity of 303-321 nm.

A series of plastic filtering materials were used to create a range of intensity levels of UVB radiation (Table 1). Fifty-millimeter polystyrene cups with aerators were used as the standard exposure chamber for invertebrates and fish larvae. Larger glass beakers were used for juvenile fish. Inanga eggs were exposed in a 20 mL scintillation vial on a glass fibre filter paper that was held above water. The container was sealed with polyethylene foil wrap to maintain a high humidity. The freshly laid eggs were exposed for 7 days then removed to darkness for a further 3 weeks. The eggs were then flooded and agitated to hatch the larvae. All treatments were wrapped in aluminium foil to prevent side light intrusion. All exposures were at 15 °C.

The organisms were observed daily, immediately after the UVA lamps turned off. Mortalities were recorded and the dead organisms removed from the cups. LD<sub>50</sub> values were calculated using the Probit procedure.

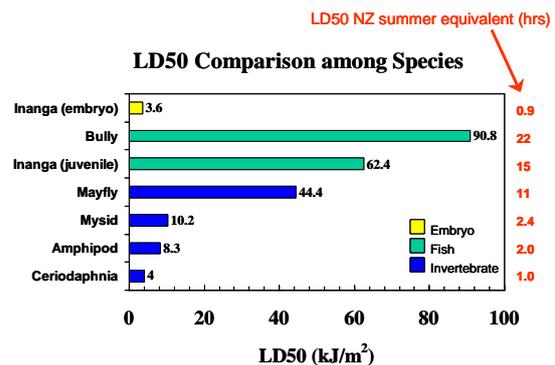
Samples of epidermal slime were removed from adult fish for 'sunscreen' analysis. Slime was dissolved in methanol and absorbance measured at 300 nm. Values are normalised on a dry weight basis.

## Results and Discussion

Phototoxicity to UVB was up to 20 times greater (i.e. LD<sub>50</sub>, the 50% lethality concentration, was lower) for the most sensitive invertebrate (cladoceran, *Ceriodaphnia*) compared with the least sensitive fish species (bully) (Figure 1). Amphipods and mysids were also particularly sensitive to UVB exposure. Juvenile inanga were surprisingly tolerant of UVB exposure, however, their egg/embryo development stage was very sensitive.

These LD<sub>50</sub> values may be converted to time to effects based on exposure to clear summer UVB levels in New Zealand. Based on a value of 100 kJ/m<sup>2</sup>/d (Wubben et al. 2001) these sensitivity values range from 1 h for the most sensitive invertebrate to 22 h for the bully. Both

amphipods and mysids require low exposures (<2.5 h) for significant effects. The inanga eggs/embryos were particularly sensitive requiring less than 1 h equivalent of exposure to cause 50% lethality. These results suggest that inanga eggs will be highly vulnerable to habitat disturbance during development. The removal of vegetation will potentially result in marked increases in UV exposure and, consequent, high lethality for the embryos. This may be the most sensitive life stage for this species. The other sensitive invertebrate species will also require habitats with low UV exposure.

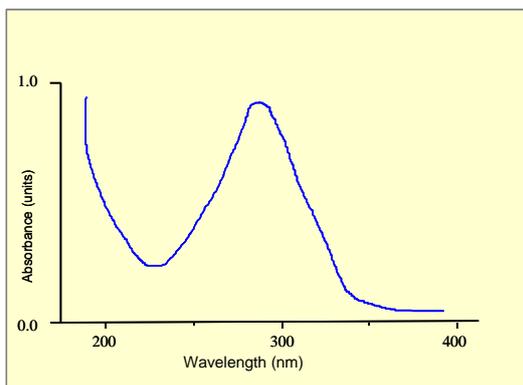


**Figure 1:** Sensitivity of native invertebrate and fish species to UVB exposure

Only limited data is available to compare the sensitivity measured by us with other aquatic species. Our LD<sub>50</sub> data for laboratory reared *C. dubia* (4 kJ/m<sup>2</sup>) show markedly higher sensitivity than that measured for New Zealand field-collected *C. dubia* (36.7 kJ/m<sup>2</sup>) (Wubben et al. 2001) but are similar to laboratory *C. reticulata* (2.07 kJ/m<sup>2</sup>) (Hurtubise et al. 1998). This suggests that marked acclimation to increase tolerance may occur in field populations.

Tolerance of fish populations may be increased by the production of photoprotectants in their slime (Fabacher and Little 1998). Methanol extracts show an absorption peak at 300 nm (Figure 2). We found strong absorption in extracts from native fish species (Table 2). The specific absorption of the shortjawed kokapu slime was markedly higher than that of rainbow trout, while a number of species show no absorption in their slime.

The relationship between slime absorbance and survival threshold for fish was weak. Some species have other mechanisms to protect tissue. Notably, smelt have highly reflective scales which may serve to provide UV protection.



**Figure 2:** Absorption spectra for photoprotectant in fish slime.

### Conclusions

Native freshwater invertebrates were particularly sensitive to UVB exposure, while fish species were more tolerant. Inanga eggs/embryos were found to be particularly sensitive and may be vulnerable to habitat alterations in their natural spawning environment. Together, these data suggest that understanding the environmental and organism (e.g., behavioural) factors which affect UV exposure will be critical to predicting the risks of increased UV exposure scenarios.

**Table 1** Treatments and their corresponding filters along with their UVB irradiance ( $W/m^2$ ) and percent UVB transmittance.

Filter	UVB	UVA	%UVB transmittance	%UVA transmittance	TREATMENT
Polycarbonate	0.0025	0.17	0.11	0.85	Control
Mylar_D	0.21	15	9.4	77	Low
Acrylic	0.66	18	29	93	Med
Acetate	1.91	19	86	97	High
Glad	2.0	19	90	98	Highest
Unfiltered	2.2	20	100	100	

**Table 2** Relative 'sunscreen' absorbance of epidermal slime extracts from New Zealand native fish species and rainbow trout

Species	Amount of sunscreen <sup>a</sup>	Days to Effects <sup>b</sup>	Vulnerability
Shortjawed kokopu (L)	100	1	Low
Inanga (L)	66	2	Low
Rainbow trout (J)	29	>4	Medium
Common bully (J)	<<1	2	High
Smelt (J)	ND	7	Very high
Eels (L)	ND	1.5	Very high

<sup>a</sup> AU 300nm/mgDW – relative values; <sup>b</sup> estimate at 27 kJ/m<sup>2</sup>/d; ND = none detected; L = larvae; J = juveniles.

### References

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