Effects of ultraviolet radiation on the transmission process of an intertidal trematode parasite

A. STUDER[1]*, M. D. LAMARE[2] *and* R. POULIN[1]

[1] *Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand*
[2] *Department of Marine Sciences, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand*

(*Received 24 August 2011; revised 2 November 2011; accepted 3 November 2011; first published online 5 January 2012*)

SUMMARY

The transmission of parasites takes place under exposure to a range of fluctuating environmental factors, one being the changing levels of solar ultraviolet radiation (UVR). Here, we investigated the effects of ecologically relevant levels of UVR on the transmission of the intertidal trematode *Maritrema novaezealandensis* from its first intermediate snail host (*Zeacumantus subcarinatus*) to its second intermediate amphipod host (*Paracalliope novizealandiae*). We assessed the output of parasite transmission stages (cercariae) from infected snail hosts, the survival and infectivity of cercariae, the susceptibility of amphipod hosts to infection (laboratory experiments) and the survival of infected and uninfected amphipod hosts (outdoor experiment) when exposed to photo-synthetically active radiation only (PAR, 400–700 nm; no UV), PAR + UVA (320–700 nm) or PAR + UVA + UVB (280–700 nm). Survival of cercariae and susceptibility of amphipods to infection were the only two steps significantly affected by UVR. Survival of cercariae decreased strongly in a dose-dependent manner, while susceptibility of amphipods increased after exposure to UVR for a prolonged period. Exposure to UVR thus negatively affects both the parasite and its amphipod host, and should therefore be considered an influential component in parasite transmission and host-parasite interactions in intertidal ecosystems.

Key words: transmission, host-parasite interactions, *Maritrema novaezealandensis*, *Paracalliope novizealandiae*, *Zeacumantus subcarinatus*, intertidal soft-sediment ecosystem.

INTRODUCTION

Solar ultraviolet radiation (UVR) (wavelengths; UVB: 280–320, UVA: 320–400 nm) has always been a strong selective force in aquatic communities (Williamson *et al*. 2001; Sommaruga, 2003; Hansson and Hylander, 2009). As an integral part of a complex array of fluctuating environmental factors in these ecosystems, UVR varies considerably in time (e.g. seasonal) and space (e.g. latitude, water depth; Tedetti and Sempere, 2006). Although life on earth has evolved in the presence of UVR, the recent and on-going stratospheric depletion in ozone has altered the selective pressure that UVR, especially UVB, may exert (Lesser and Barry, 2003 and references therein). Moreover, on-going and predicted climate changes are expected to further exacerbate exposure of organisms to UVB in aquatic ecosystems (Haeder *et al*. 2011).

UVR, especially UVB, is predominantly known for its potentially deleterious effects, damaging biological macromolecules and cellular structures including enzymes, membranes, DNA and RNA (e.g. Haeder *et al*. 1998; Vincent and Neale, 2000; Day and Neale, 2002; Dahms and Lee, 2010) (see also Paul and Gwynn-Jones, 2003). Ecologically relevant effects at the whole-organism level include decreases in fecundity, growth, development, mobility and survival rate, all of which can translate into changes in species composition at the community and ecosystem level (e.g. Bothwell *et al*. 1994; Haeder *et al*. 1998; Bancroft *et al*. 2007; Hansson and Hylander, 2009). As a consequence, organisms have evolved a range of behavioural (e.g. migration), physiological (e.g. accumulation of UV-absorbing compounds) and molecular mechanisms (e.g. DNA damage repair), to avoid, minimize or repair damage induced by exposure to UVR (Roy, 2000; Sinha and Haeder, 2002; Hansson and Hylander, 2009; Dahms and Lee, 2010 and references therein).

Biological and ecological effects of UVR have been well studied in aquatic systems (e.g. Haeder *et al*. 1998; de Mora *et al*. 2000; Helbling and Zagarese, 2003; Bancroft *et al*. 2007; Hansson and Hylander, 2009). Organisms vary considerably in their susceptibility to UVR (e.g. Bancroft *et al*. 2007). Hence, it is very likely that at least some of their interactions with other organisms would be influenced by UVR (see examples in Paul and Gwynn-Jones, 2003; Sommaruga, 2003). However, studies on the effects of UVR on aquatic organisms have largely ignored species interactions, particularly those between parasites and their hosts. Despite the fact that parasitism is known to be of high ecological relevance (e.g. Hudson *et al*. 1998; Lafferty *et al*. 2006; Mouritsen and Poulin, 2010), knowledge on the effects of UVR

* Corresponding author: Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand. Tel: +64 3 479 7964 Fax: +64 3 479 7584. E-mail: studeranja@gmail.com

doi:10.1017/S0031182011002174

on parasites and diseases in aquatic systems has remained limited (see Sommaruga, 2003).

The sensitivity of a range of parasites from aquatic environments to UVR, however, has been acknowledged (see Marcogliese, 2001 and references therein). UVR is expected to be particularly detrimental in clear, shallow waters and for parasites with small, delicate free-living stages that are extremely susceptible to environmental conditions (MacKenzie *et al*. 1995). Sublethal and lethal effects of UVR on transmission stages of important human parasites (e.g. *Schistosoma* spp., *Cryptosporidium parvum*) have been investigated using artificial lights (Standen and Fuller, 1959; Ghandour and Webbe, 1975; Prah and James, 1977; Ariyo and Oyerinde, 1990; Connelly *et al*. 2007; Ruelas *et al*. 2006, 2007, 2009). Results from these studies consistently confirm strong negative effects of UVR on survival as well as infectivity of parasites.

However, most of these studies generally do not consider ecologically relevant interactions between a parasite and its host(s). Using a more inclusive approach, Ruelas *et al*. (2006, 2007, 2009) investigated the effects of UVR on the transmission of *Schistosoma mansoni* as well as the parasite's aquatic snail host (*Biomphalaria glabrata*). Their results indicated that not only the parasite, but also juvenile and infected snails were vulnerable to UVR exposure, but that both snail and parasite were also able to repair UV-induced DNA damage. This clearly highlights the importance of direct and indirect effects of UVR on the interaction between parasites and hosts, suggesting that the overall effect of UVR on a particular system may be highly complex and thus difficult to predict.

UVR effects may be particularly relevant in intertidal ecosystems, where conditions for organisms at low tide are almost equivalent to atmospheric conditions (Kramer, 1990; Karentz, 2001), and even more so for those in the Southern hemisphere where incident UVR levels are greatest (Seckmeyer and McKenzie, 1992; McKenzie *et al*. 1999). In intertidal ecosystems, trematode parasites are ubiquitous and important ecological components (e.g. Lauckner, 1984; Sousa, 1991; Mouritsen and Poulin, 2002, 2010). In the present study, the transmission process of the intertidal trematode *Maritrema novaezealandensis* was examined. Like most trematodes, *M. novaezealandensis* has a complex life cycle involving several members of an intertidal community (Martorelli *et al*. 2004). The species in this life cycle are all specific to New Zealand and hence are exposed to the particularly high UVB levels occurring in this region compared to Northern hemisphere regions of the same latitude (Seckmeyer and McKenzie, 1992; McKenzie *et al*. 1999), and to the transient high levels of UVB associated with high latitude ozone depletion in the Southern hemisphere (McKenzie *et al*. 2003, 2007).

The different stages of the parasite throughout a trematode's life cycle experience vastly different conditions in terms of the solar radiation regimes to which they are exposed. *Maritrema novaezealandensis*, for example, lives as an adult worm in the intestine of mudflat affiliated birds (environment with no direct solar irradiation). Eggs pass out with the bird's feces and persist in the environment until ingested by a first intermediate snail host (*Zeacumantus subcarinatus*) foraging on mudflats. Within a snail's opaque shell, a miracidium hatches from an egg and develops into a sporocyst, probably shielded from solar irradiance. Within the sporocysts, asexual reproduction occurs and large numbers of larval transmission stages (cercariae) are produced, which emerge from an infected snail under optimal conditions in order to infect a second intermediate crustacean host. Cercariae are small (approx. 170–200 μm including tail, Martorelli *et al*. 2004; Koehler and Poulin, 2010), short-lived (< 24 h), non-feeding transmission stages and in the case of *M. novaezealandensis*, they are also translucent. These cercariae are directly exposed to and influenced by environmental conditions. After infecting a second intermediate crustacean host, such as the amphipod *Paracalliope novizealandiae* used in this study, the parasite experiences different levels of exposure to solar radiation depending on the opaqueness or transparency of the carapace of its crustacean host. Within a crustacean, the parasite develops into an encysted stage (metacercaria). The life cycle is completed when a crustacean harbouring a mature metacercaria is ingested by a definitive bird host and successfully establishes in its intestine.

In this study, we investigated the effects of ecologically relevant levels of UVR on the transmission process of *M. novaezealandensis* from its first intermediate snail host (*Z. subcarinatus*) to its second intermediate amphipod host (*P. novizealandiae*). In laboratory and outdoor experiments, the effects of UVR on (1) the emergence of cercariae from snail hosts, (2) the survival of cercariae, (3) the infectivity of cercariae, (4) the susceptibility of the amphipod host to infection, and (5) the survival of infected and uninfected amphipods were assessed (for a conceptual figure summarizing these steps see Studer *et al*. 2010). Our aim was to identify steps in this transmission process that are particularly sensitive to UVR, and to evaluate overall net effects of this environmental factor on the transmission of *M. novaezealandensis* from its first to its second intermediate host.

MATERIALS AND METHODS

Parasite and host material

First intermediate snail hosts (*Z. subcarinatus*) were collected from a high prevalence site (Lower

Portobello Bay, Otago Harbour) and screened for infections with *M. novaezealandensis* (see Studer *et al*. 2010 for details). For all experimental infections, uninfected second intermediate amphipod hosts (*P. novizealandiae*) were collected from Hooper's Inlet (Otago Peninsula) several days prior to an experiment in order to allow for acclimatization to laboratory conditions. At Hooper's Inlet, first intermediate snail hosts are absent and therefore, amphipods are not infected naturally (neither by *M. novaezealandensis* nor by any other trematode metacercariae) (Fredensborg *et al*. 2004; Bryan-Walker *et al*. 2007). After their use in experiments (see below), amphipods were stored in small plastic containers containing 300 ml of aerated seawater and a strip of sea lettuce (*Ulva* sp.) until measured (size classes: 2·5, 3·0, 3·5, 4·0, 4·5 ± 0·25 mm), sexed and dissected under a dissecting microscope to assess the number of parasites present (infection status and intensity). All experimental infections as well as the cercarial survival experiment were conducted using 96-well plates (wells 7 × 10 mm; total volume 320 μl). For all experimental infections, a cercarial mixture from 40 infected snails was obtained (see details in Studer *et al*. 2010).

Experimental design and laboratory set-up

All experiments except the amphipod survival experiment (see details below) were conducted as 3 × 2 designs including 3 UV treatments (i.e. exposure to photosynthetically active radiation (PAR) only (no UV; 400–700 nm), PAR + UVA (UVA; 320–700 nm) or PAR + UVA + UVB (UVA + B; 280–700 nm) and 2 exposure durations (i.e. doses). UV treatments were achieved using Plexiglas filters transmitting PAR (81%), only minimal UVA (5·2%) and no UVB (0·0%) (no UV treatment); PAR (77·9%), UVA (46·5%), and only minimal UVB (0·1%) (UVA treatment); PAR (84·5%), UVA (84·6%) and UVB (80·6%) (UVA + B treatment) (see Lister *et al*. 2010 for transmission profiles of filters).

The laboratory set-up included 1 UVB (Atlas 07-29006), 2 UVA (Dr. Kern® excellent RA 80W), and 3 full spectrum light tubes (Phillips Alto TLD 58W/840), which were suspended 45 cm above the bench top and which were wrapped in cellulose acetate to absorb any UVC radiation being emitted. The spectral output of the lamps was measured with a LiCor Li-1800UW spectroradiometer (Table 1, Fig. 1). Data given in Table 1 do not include reductions by filters and only provide lamp output irradiances from 300–700 nm. Compared to ambient local levels of solar irradiance during midday peaks in summer, maximum irradiances from our laboratory set-up were relatively low for UVB, UVA and PAR (Table 1). All doses administered in each experiment can be calculated based on the dose per hour given in Table 1. All low dose exposures (shorter exposures) were administered during the second half of the high dose treatments (longer exposure) and all laboratory experiments were conducted at 20 °C. Statistical analyses are described in each of the following subsections. We checked for differences between replicates and transformed data where necessary to ensure the assumptions of the statistical tests were met.

Table 1. Irradiances in the laboratory set-up: dose per hour (kJ m^{-2}) and maximum irradiance (W m^{-2} s^{-1}) for UVB, UVA and PAR

(Ultraviolet B radiation (UVB), 300–320 nm; ultraviolet A radiation (UVA), 320–400 nm; ultraviolet radiation (UVR), 300–400 nm; photosynthetically active radiation (PAR), 400–700 nm; ratio of ultraviolet B radiation to visible light (UVB:PAR). For comparison, ambient maximum irradiances measured locally. (*Data from Lamare *et al*. 2007).)

	Laboratory set-up		Ambient*
	Dose per hour kJ m^{-2}	Max. irradiance W m^{-2} s^{-1}	Max. irradiance W m^{-2} s^{-1}
UVB	5·84	1·62	3·02
UVA	63·27	17·58	49·72
Total UVR	69·12	19·20	
PAR	185·09	51·41	892·9
UVB:PAR ratio		0·03	

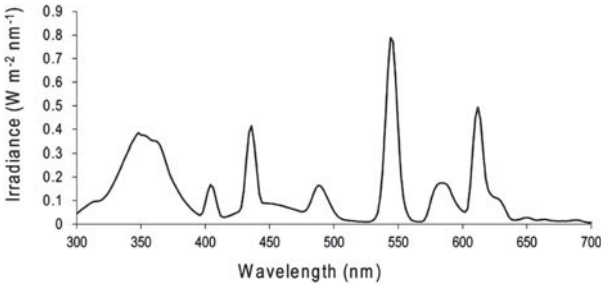

Fig. 1. Spectral output (300–700 nm) of all light tubes combined in the laboratory set-up (W m^{-2} nm^{-1}) as measured by the LiCor Li-1800UW spectroradiometer.

Output of Maritrema novaezealandensis *cercariae from first intermediate snail hosts*

Output of cercariae of *M. novaezealandensis* from infected *Z. subcarinatus* snails was assessed as a short-term response by counting the number of cercariae that emerged from snail hosts during exposure to the different UV treatments at 20 °C. There were 2 trials each with 16 replicate snails per treatment. For the exposures, snails were individually placed in 1·5 ml Eppendorf tubes filled with 1 ml of filtered seawater. Tubes were left open, covered with the respective filters and then exposed for 3 or 6 h. After incubation and removal of the snails, tubes were centrifuged

(5 min, 20 817 ***g***) and 900 μl of the seawater exchanged with 70% ethanol to preserve the samples. The supernatant was checked for the presence of cercariae before disposal. Samples were counted under a dissecting microscope. A General Linear Model (GLM) was used to test for the effects of the trial, UV treatment, trial × UV treatment and dose on the number of emerged cercariae (log transformed).

Survival of cercariae

For this experiment, the survival and activity of cercariae was monitored when exposed to the different UV treatments and doses. At the start of the experiment, 40 μl from a cercarial mixture (average age of cercariae 30 min) were added to 24 replicate wells on two 96-well plates per treatment corresponding to an addition of approximately 45 cercariae per well. Cercariae were then exposed for either 2 or 4 h to one of the UV treatments. After exposure, well plates were placed under visible light (Phillips 40 W) for further monitoring. Survival and activity was categorized visually as fully active, sluggishly motile or immotile/dead. Cercariae were checked at an average age of 3, 5, 7, 9 and 12 h. A repeated measures ANOVA was used to test for the effect of the UV treatment and dose on the proportion of fully active cercariae (arcsine-square root transformed) at an average age of 3, 5, 7 and 9 h. Due to violation of the assumption of sphericity, multivariate results for within-subject effects are reported. Additionally, LD_{50} values were calculated to assess the time in each treatment for 50% of cercariae to lose their full functional activity. This was done by fitting logistic functions to the proportion of fully active cercariae data of each treatment and dose and then calculating the inflection point.

Infectivity of cercariae

Infectivity, i.e. the proportion of cercariae that successfully infects second intermediate amphipod hosts (*P. novizealandiae*), was assessed after exposure of cercariae to the UV treatments and doses. For this, 48 uninfected amphipods ($\geqslant$ 2·25 mm body size) per treatment were put individually into wells on two 96-well plates containing approximately 75 μl of filtered seawater. A cercarial mixture was obtained which was divided into wells of 12-well plates (4 ml per well; 2 wells per treatment) and then exposed for either 30 min or 1 h to respective UV treatments. Based on aliquot counts prior to exposure, 50 μl of the irradiated cercarial mixtures were added to each amphipod, which corresponded to an addition of approximately 18 cercariae per amphipod. Amphipods and irradiated cercariae were then incubated for 2 h at 25 °C under constant illumination with visible light (Philips 40 W). After incubation, amphipods were transferred into plastic containers filled with seawater and dissected under a dissecting microscope 7 days after the experiment. A GLM was used to test for the effect of UV treatment, dose, UV × dose, as well as sex and size of amphipods on the proportion of parasites successfully infecting amphipods (arcsine-square root transformed).

Susceptibility of amphipods to infections

Susceptibility of amphipod hosts was studied by exposing amphipods to the UV treatments before adding non-irradiated cercariae, and then comparing the infection success of the cercariae in these hosts. This experiment consisted of 2 separate trials. In the first trial, amphipods were exposed for 30 and 60 min. In the second trial, amphipods were exposed for 3 and 6 h. In each trial, 48 amphipods per treatment were transferred individually into wells of two 96-well plates per treatment (in approximately 75 μl natural seawater) prior to exposure. A cercarial mixture was prepared which was then added to individual amphipods at the end of the exposure period (first trial: 60 μl of the cercarial mixture corresponding to approximately 13 cercariae per amphipod; second trial: 30 μl corresponding to approximately 20 cercariae per amphipod). Well plates containing irradiated amphipods and cercariae were then incubated for 2 h at 25 °C under visible light. After incubation, amphipods were transferred into plastic containers and dissected 7 days later. The two trials were analysed separately using GLM's assessing the effect of the UV treatment, dose, UV × dose, as well as size and sex of amphipods on the proportion of cercariae (arcsine-square root transformed) successfully infecting the amphipods. In the second trial, this was followed by a Tukey's post-hoc comparison between the UV treatments.

Survival of infected and uninfected amphipods

This experiment was conducted under ambient conditions and assessed the effect of UVR on the survival of uninfected and infected amphipods using the same filters as specified above (treatments: no UV, UVA, UVA + B). The experiment was conducted over a 24 d period at the Portobello Marine Laboratory, Otago Harbour, New Zealand in austral summer. UVA and UVB levels during the experiment were recorded at the laboratory's weather station (UVA SKU 420 and UVB SKU 430 sensors, Skye Instruments Ltd). Mean total daily doses during this period were 1938·67 ± 131·63 kJ m^{-2} of UVA and 113·98 ± 8·12 kJ m^{-2} of UVB, with maximum irradiances of 44·1 (UVA) and 2·83 W m^{-2} s^{-1} (UVB). For the experimental infections, 48 uninfected amphipods per replicate container (3 containers per treatment) were individually put into wells of 96-well plates. A cercarial mixture was

obtained, of which 50 μl were added to half of the amphipods (corresponding to an addition of approximately 28 cercariae per amphipod), whereas the same volume of filtered seawater was added to controls. Amphipods were then incubated for 2 h at 25 °C under constant illumination with visible light. Infected and uninfected amphipods were left in separate plastic containers (1 l; aerated seawater) overnight to allow the parasites to complete infection. Infected and uninfected amphipods (24 each per container, sexes matched) were then distributed into white opaque rectangular plastic containers (1 l) provided with a small strip of sea lettuce (*Ulva* sp.) and covered with the respective filter. Containers were placed outdoors and received filtered flow-through seawater. Survival of amphipods was subsequently monitored and container positions rotated twice a day. Dead amphipods recovered were measured, sexed and dissected under a dissecting microscope to assess the number of parasites present in each amphipod. After 24 days, all remaining amphipods were dissected. The risk of dying during the experimental period was analysed with a Cox proportional hazard regression model with UV treatment, sex, size and infection status of amphipods as predictor variables.

Net effect on transmission

In order to validate the conclusion that UVR, by affecting both the survival of cercariae as well as the susceptibility of amphipods, may overall have a neutral net effect (i.e. the decreased survival of cercariae being compensated by an increased susceptibility of amphipods; see Results and Discussion sections), an additional outdoor experiment was conducted assessing the success of cercariae at infecting amphipods under natural light conditions. For this, cercariae (approximately 25 per amphipod; pooled from 40 infected snails) and amphipods (64 per treatment) were exposed together in wells of 2 replicate 96-well plates to no UV, only UVA or UVA + B for 2 h on a clear summer day using the same filters as described above (total UVB dose 1·607 Jcm^{-2}). Amphipods were dissected the following day and the number of parasites infecting each amphipod was assessed. A GLM was used to assess the effect of UV treatment, size and sex of amphipods on the proportion of parasites successfully infecting the amphipods (arcsine-square root transformed).

RESULTS

Cercarial output from snails

There was substantial variability of cercarial output among individual snails (range 0–2738 cercariae per snail per incubation), with a mean output (± standard error as in all following results) of 152 ± 31 cercariae per snail (at 20 °C). Thus, no clear pattern was found for the effect of the UV treatment or dose on the number of cercariae emerging from infected snails (log number of cercariae; GLM, UV treatment: $F_{2,185} = 1{\cdot}44$, $P = 0{\cdot}239$; dose: $F_{1,185} = 1{\cdot}14$, $P = 0{\cdot}287$). While there was also no significant difference between trials ($F_{1,185} = 3{\cdot}29$, $P = 0{\cdot}072$), there was a significant interaction between trial and UV treatment ($F_{2,185} = 3{\cdot}45$, $P = 0{\cdot}034$), indicating an inconsistency of the UVR effects between trials. The sizes of snails used did not differ between the trials or UV treatments (ANOVA, trials: $F_{1,191} = 1{\cdot}16$, $P = 0{\cdot}284$; UV treatment: $F_{2,191} = 1{\cdot}18$, $P = 0{\cdot}309$).

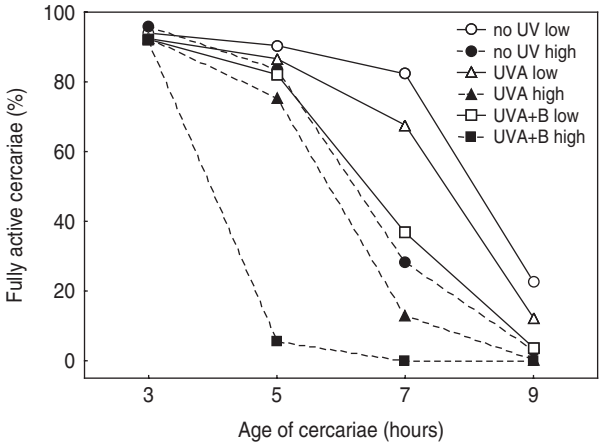

Fig. 2. Survival (i.e. full activity as a percentage) of *Maritrema novaezealandensis* cercariae at an average age of 3, 5, 7 and 9 h post-emergence from infected snails, when exposed to either a low or a high dose of no UV, UVA or UVA + B radiation.

Cercarial survival

UVR negatively influenced the survival of cercariae in a dose-dependent manner. Mortality was highest for cercariae exposed to a high dose of both, UVA + B (all dead at an average age of 7 h), and lowest for the cercariae exposed to a low dose of no UV (23% still fully active at an average age of 9 h (Fig. 2)). Survival after administration of high doses was reduced in all cases when compared to the low dose (Fig. 2), even for exposure to visible light only. This effect was exacerbated when cercariae were exposed to UVR with survival under UVA + B < UVA. The repeated measures ANOVA carried out on the proportions of fully active cercariae (arcsine-square root transformed) indicated a significant effect of all predictor variables and their interactions (between subjects; UV treatment: $F_{2,138} = 301{\cdot}76$; dose: $F_{1,138} = 774{\cdot}62$; UV × dose: $F_{2,138} = 28{\cdot}82$; within subjects; time: $F_{3,414} = 3566{\cdot}28$; time × UV: $F_{6,414} = 93{\cdot}68$; time × dose: $F_{3,414} = 270{\cdot}32$; time × UV × dose: $F_{6,414} = 92{\cdot}77$; for all $P < 0{\cdot}001$). LD_{50} values calculated from these data revealed the following times (i.e.

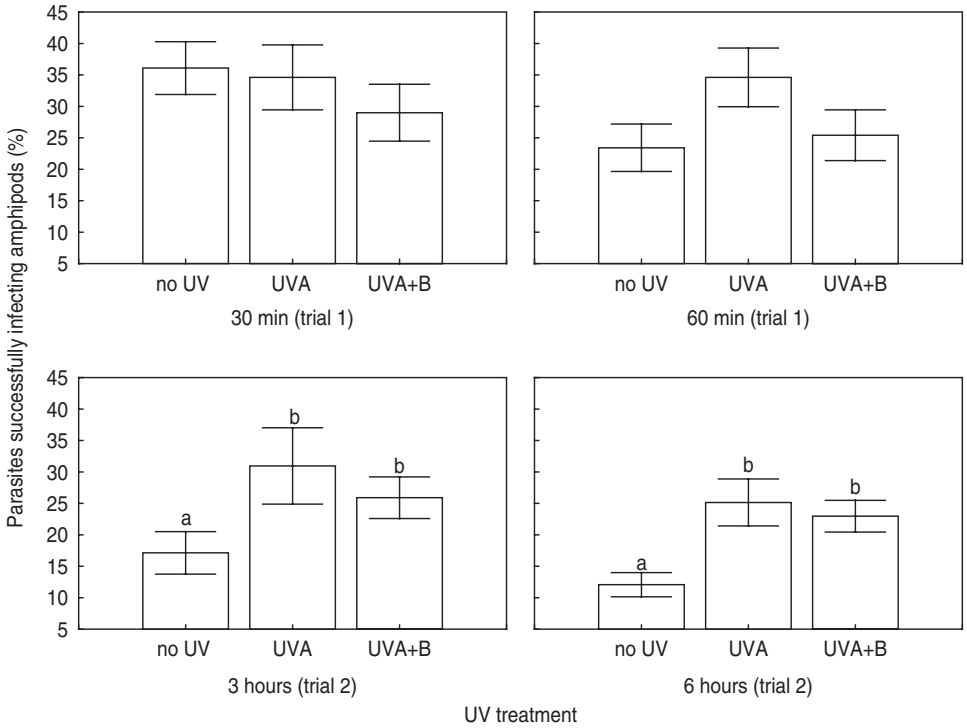

Fig. 3. Susceptibility of *Paracalliope novizealandiae* amphipods to infection with *Maritrema novaezealandensis*, measured as the proportion of parasites successfully infecting the amphipods after a low or a high dose exposure of amphipods to no UV, UVA or UVA + B radiation (mean ± S.E.). Exposure times in trial 1 were 30 and 60 min. In trial 2, amphipods were exposed for 3 and 6 h. Trials are not directly comparable due to differences in numbers of cercariae originally added. The letters denote significant differences between treatments according to Tukey's post-hoc test (significance level $P < 0{\cdot}05$).

ages) at which 50% of cercariae lost their full functional activity: 8·1 h (low no UV dose), 6·3 h (high no UV dose), 7·6 h (low UVA), 5·7 h (high UVA), 6·5 h (low UVA + B) and 3·9 h (high UVA + B).

Infectivity

While UV treatment and dose did not have a significant effect on the proportion of parasites successfully infecting the amphipods (arcsine-square root transformed proportion of successful parasites, GLM; UV treatment: $F_{2,253} = 2{\cdot}65$, $P = 0{\cdot}073$; dose: $F_{1,253} = 0{\cdot}13$, $P = 0{\cdot}725$), their interaction did ($F_{2,253} = 8{\cdot}39$, $P < 0{\cdot}001$). Results indicated that for cercariae exposed to no UV or only UVA, the proportions of successful parasites were higher after a high dose than a low dose (no UV: 0·11 ± 0·02 (low) to 0·13 ± 0·02 (high); UVA: 0·08 ± 0·02 (low) to 0·13 ± 0·02 (high), whereas the proportions for cercariae exposed to UVA + B decreased (from 0·11 ± 0·01 (low) to 0·06 ± 0·01 (high)). While the size of the amphipods did not have a significant effect, sex did, with males being more infected than females ($F_{1,253} = 4{\cdot}11$, $P = 0{\cdot}044$). In total, 264 amphipods were dissected of which 74% were infected. The mean number of parasites per infected amphipod was 1·6 ± 0·2, indicating only low levels of successful transmission overall.

Susceptibility of amphipods to infections

UVR influenced the susceptibility of amphipods in terms of the proportion of cercariae successfully infecting the amphipods, but only after several hours of exposure to UVR (Fig. 3). The pattern observed suggests that, after reaching a certain threshold of exposure to UVR, susceptibility of amphipods increases. After only relatively short exposures (30 and 60 min, trial 1), UV treatment or dose did not have a significant effect on the susceptibility of amphipods (Fig. 3). In this trial, 94% of a total of 155 amphipods dissected were infected (average number of parasites per amphipod: 4·0 ± 0·2). After 3 and 6 h (trial 2), there was a significant effect of the UV treatment, but not of any other factor tested (arcsine-square root transformed proportion of successful parasites, GLM; UV treatment: $F_{2,165} = 6{\cdot}84$, $P = 0{\cdot}001$, dose: $F_{1,165} = 1{\cdot}01$, $P = 0{\cdot}316$; UV × dose: $F_{2,165} = 0{\cdot}20$, $P = 0{\cdot}822$; sex: $F_{1,165} = 0{\cdot}17$, $P = 0{\cdot}683$, size: $F_{4,165} = 0{\cdot}42$, $P = 0{\cdot}794$). The Tukey's post-hoc test revealed a significant difference between the no UV treatment and the UVA and UVA + B treatments ($P < 0{\cdot}05$), but not between the UVA and UVA + B treatments (Fig. 3). In total, 176 amphipods were

Table 2. Results of the Cox proportional hazard model assessing the effects of the UV treatment (no UV, UVA, UVA + B), sex, size and infection status of *Paracalliope novizealandiae* amphipods on the risk of dying during the outdoor experiment

Factor	Cox parameter	S.E.	*P*
UV treatment	−0·13	0·10	0·177
Amphipod sex	−1·42	0·26	< 0·001
Amphipod size	−0·55	0·12	< 0·001
Infection status	0·44	0·16	0·006

dissected in this second trial of which 86·5% (no UV, $n=52$), 96·3% (UVA, $n=54$), 91·4% (UVA + B, $n=70$) were infected. The average number of parasites per amphipods was 3·0 ± 0·4 (no UV), 5·5 ± 0·7 (UVA) and 4·9 ± 0·4 (UVA + B). Due to differences in the number of cercariae originally added to amphipods in the two trials, the resulting proportions are not directly comparable between trials.

Amphipod survival

There was no significant difference in the risk of dying for amphipods exposed to different UV treatments during the 24-day outdoor experiment (Table 2; model $\chi^2=38{\cdot}58$, D.F. = 4, $P<0{\cdot}001$). In contrast, sex, size and infection status had significant effects with infected amphipods, male and larger amphipods having a higher risk of dying than uninfected ones, females or smaller size classes. There was no significant correlation between the number of days alive and the number of parasites present in infected amphipods (Spearman's $P=-0{\cdot}21$, $P=0{\cdot}063$). In total, 293 amphipods were dissected (139 females, 150 males, 4 unknown) of which 43% were infected (no UV: 38% ($n=116$), UVA: 48% ($n=83$), UVA + B: 44% ($n=88$) with a mean infection intensity of 5·1 ± 0·4 parasites per infected amphipod.

Net effect on transmission

There was no significant difference in the proportion of parasites successfully infecting amphipod hosts for cercariae and amphipods simultaneously exposed to ambient conditions under the different UV filters. Moreover, neither size nor sex of amphipods had a significant effect (arcsine-square root transformed proportion of successful parasites, GLM; UV treatment: $F_{2,177}=1{\cdot}30$, $P=0{\cdot}276$, size: $F_{3,177}=0{\cdot}44$, $P=0{\cdot}722$, sex: $F_{1,177}=1{\cdot}77$, $P=0{\cdot}185$). Proportions of successful parasites were 0·20 ± 0·02 (no UV), 0·20 ± 0·01 (UVA) and 0·24 ± 0·02 (UVA + B). In total, 185 amphipods were dissected ($n=63$, 59 and 63 for the no UV, UVA and UVA + B treatments, respectively), which harboured on average 5·1 ± 0·38 (no UV), 5·1 ± 0·35 (UVA) and 6·0 ± 0·43 (UVA + B) parasites.

DISCUSSION

The transmission of parasites *in situ* is taking place under exposure to prevailing environmental conditions, including incident solar irradiance. These conditions can influence the parasite, but also the host, and may thus affect their interaction. In the present study, 2 steps of the transmission process investigated were affected by UVR: the survival of the cercarial transmission stages of *M. novaezealandensis* and the susceptibility of amphipod hosts to infection. This indicates that UVR should be considered an important ecological modulator of parasite transmission in natural systems.

Effects on the parasite

There was a strong negative dose-dependent effect of exposure to UVR on the survival of cercariae. This result is supported by an additional experiment run under ambient conditions on a warm sunny day which confirmed the marked decline in survival and activity when comparing cercariae exposed to no UV, only UVA or UVA + B: after 2 h of exposure in 96-well plates, 97% (no UV), 84% (only UVA) and 14% (UVA + B) of cercariae were fully active, whereas after 3 h of exposure, only 61% (no UV), 4% (only UVA) and 0% (UVA + B) were still fully active. In the laboratory experiment, even exposure to a high dose of no UV (i.e. PAR only) reduced the survival of cercariae when compared to a low dose. This suggests that once cercariae emerge into the environment, an event which does not itself seem to be conclusively influenced by UVR (see Results section), one of the factors determining the life span of these cercariae will be the amount of solar radiation and particularly of UVR that prevails at that time. The high vulnerability and the reduced survival of *M. novaezealandensis* cercariae are consistent with other studies reporting an increased mortality of parasitic transmission stages after exposure to UVR (e.g. Prah and James, 1977; Ariyo and Oyerinde, 1990), and with the general notion of large, negative effects of UVR on the survival of a range of organisms (e.g. Bancroft *et al*. 2007).

In contrast to the clear effect on cercarial survival, results regarding the output and infectivity of cercariae were less straightforward. The effect of UVR on cercarial output from snails may only become apparent after longer exposures, potentially in the order of weeks or months, and we can only conclude here that short-term exposure of the snail host had no clear immediate effect. Regarding infectivity, we expected to find a reduced capacity

of cercariae to complete penetration of amphipod hosts after exposure to UVR, similar to what has been described for miracidia (parasite transmission stage hatching from eggs) of *Schistosoma* sp. These were shown to have an impaired host penetration capability due to the loss of activity after exposure to UVR, whereas the developmental potential of those that successfully infected a host was normal (Prah and James, 1977). Our findings indicated that exposure to a high dose of UVA + B results in lower proportions of cercariae capable of infecting amphipods when compared to cercariae exposed to high doses of no UV or UVA only. The significant interaction was based on the fact that proportions of successful parasites were higher for no UV and UVA-exposed cercariae and lower for UVA + B-exposed cercariae compared to the low dose treatments. This may indicate a stimulating effect of PAR and UVA on cercarial infectivity promoting their successful transmission to the next host when exposed to ambient conditions, whereas a high dose of UVA + B has detrimental effects on the cercariae. However, we have been unable to confirm the responses observed in the present experiment in any subsequent trial conducted (A. Studer, *unpublished data*) and thus no consistent effect of UVR has been found on the subtle functional aspect of infectivity of *M. novaezealandensis*. It is possible that UVR may not harm cercariae in their functionality until a certain threshold is reached, after which not just infectivity, but survival as such is impaired. However, different approaches may be needed to reach more conclusive results on these aspects of the transmission process.

Effects on the host

The susceptibility of *P. novizealandiae* amphipods to infection was increased after a prolonged exposure to UVR compared to prolonged exposure to no UV. A range of mechanisms could account for this observation. For example, exposure to UVR may exert immediate behavioural or physiological stress reactions that allow for easier location of the host and subsequent penetration by the cercariae. Alternatively, UVB has been shown to cause epidermal tissue damage in fish (e.g. McArdle and Bullock, 1987; Ewing *et al*. 1999; McFadzen *et al*. 2000; Sommaruga, 2003 and references therein). This may increase a host's susceptibility to bacterial or parasitic infections which may cause further tissue damage, increasingly weakening the functionality of the epidermal tissue as a physical barrier (Kramer, 1990 and references therein). Similar effects may facilitate penetration of cercariae in organisms such as crustaceans. Alternatively, UVR is known to be immunosuppressive in some organisms (e.g. Patz *et al*. 1996; Salo *et al*. 1998); thus stress reactions in crustacean hosts due to the exposure to UVR may suppress immune responses and thus limit the ability of a host to deal with parasites after their successful penetration.

Various species of zooplankton and benthic invertebrates are known to be sensitive to UVR (e.g. Bothwell *et al*. 1994; Leech and Williamson, 2000). UVR has also been shown to affect the survival, fecundity and sex ratio in some intertidal copepods, but these effects were highly species-specific (Chalker-Scott, 1995). Amphipods are translucent to UVR and therefore intertidal species are thought to be particularly prone to stress due to the fluctuating exposure levels (Obermueller *et al*. 2005). Nonetheless, no effect of UVR was found on the survival of *P. novizealandiae* amphipods in our study. Infected amphipods had, however, an increased risk of dying during this experiment. Organisms have evolved strategies to cope with UVR and the occurrence of these mechanisms often depends on the radiation levels to which organisms are normally exposed to (e.g. Siebeck *et al*. 1994; Gleason and Wellington, 1995; Helbling *et al*. 2002*b*). *Paracalliope novizealandiae* lives in high UVR environments, i.e. shallow intertidal soft-sediment habitats and tidal pools during low tide, and thus should be adapted to high levels of UVR. Several protective mechanisms may contribute to this. Vegetation present in this habitat allows for behavioural adaptations (i.e. seek protective shading). Amphipods may be capable of photo-repairing damage therefore mitigating the effect of UVR on their survival (e.g. Obermueller *et al*. 2005). Also, these amphipods contain photo-protective compounds such as mycosporine-like amino acids (A. Studer and V. Cubillos, *unpublished data*) and it is likely that other protective compounds (e.g. carotenoids) or repair mechanisms are present, similar to what has been described for other marine, herbivore amphipod species (e.g. Helbling *et al*. 2002*a*; Obermueller *et al*. 2005).

Net effects

The net effect of UVR on the transmission process of a parasite from one host to the next depends on the differential response and sensitivity of each species and step involved. Based on our results, we suggest that the negative effect of UVR on the survival of *M. novaezealandensis* cercariae is the most pronounced response, but that this negative effect on cercarial survival might be compensated, at least to some degree, by the increased susceptibility of amphipod hosts to infection. While this research was not designed to quantify total net effects, it is possible that no clear impact of UVR on the transmission process would be expected, a conclusion which was supported by the additional outdoor experiment conducted to assess the net effect of UVR on the transmission process (see Results section).

UVR does not act in isolation under natural conditions (see e.g. Przeslawski *et al*. 2005). Our study is therefore limited by the fact that interactive effects with other environmental factors, especially temperature, were not incorporated and that many experiments were only conducted under laboratory conditions. For example, during optimal conditions for the transmission of *M. novaezealandensis* in the field, i.e. during low tide on warm sunny days when water in the shallow tide pools warms up, temperature and UVR may synergistically affect the organisms and their interaction differently than what has been described here. In particular, cercarial survival is likely to be strongly negatively affected by synergistic effects of UVR and temperature; however, increased infectivity at an optimum temperature level (Studer *et al*. 2010) may, on the other hand, counterbalance this reduced survival of cercariae. Such interactions, as well as many other aspects of the ecological role of UVR for parasitism and disease transmission in marine ecosystems (as well as other ecosystems), remain to be elucidated. Appropriate experiments should be conducted in laboratory settings closely matching relevant ambient field conditions, and preferably, whenever feasible, should be repeated under natural conditions.

In summary, we found that UVR negatively influenced both the parasite and its amphipod host. The survival of cercariae was reduced and the susceptibility of amphipod hosts to infection increased. UVR should therefore be considered an important ecological component in the transmission process of intertidal and possibly other parasites. Although it remains unclear how these effects may manifest in nature, the overall net effect of UVR on the host-parasite system studied here may be considered neutral, with the negative effect on cercarial survival being compensated by the increased susceptibility of amphipods.

ACKNOWLEDGEMENTS

We are grateful to an anonymous reviewer for constructive comments that have considerably improved the manuscript. We would also like to thank the staff at the Portobello Marine Laboratory for their technical and logistical support for this study. We also like to thank David J. Burritt for advice and helpful discussions. This research was funded by the University of Otago, New Zealand, and the Department of Zoology for postgraduate research by A.S.A.S. also acknowledges the support from a Postgraduate Publishing Bursary for the preparation of this manuscript.

REFERENCES

Ariyo, A. A. and Oyerinde, J. P. O. (1990). Effect of ultraviolet radiation on survival, infectivity and maturation of *Schistosoma mansoni* cercariae. *International Journal for Parasitology* **20**, 893–897.

Bancroft, B. A., Baker, N. J. and Blaustein, A. R. (2007). Effects of UVB radiation on marine and freshwater organisms: a synthesis through meta-analysis. *Ecology Letters* **10**, 332–345. doi: 10.1111/j.1461-0248.2007.01022.x.

Bothwell, M. L., Sherbot, D. M. J. and Pollock, C. M. (1994). Ecosystem response to solar ultraviolet-B radiation: Influence of trophic-level interactions. *Science* **265**, 97–100.

Bryan-Walker, K., Leung, T. L. F. and Poulin, R. (2007). Local adaptation of immunity against a trematode parasite in marine amphipod populations. *Marine Biology* **152**, 687–695. doi: 10.1007/s00227-007-0725-x.

Chalker-Scott, L. (1995). Survival and sex ratios of the intertidal copepod, *Tigriopus californicus*, following ultraviolet-B (290–320 nm) radiation exposure. *Marine Biology* **123**, 799–804.

Connelly, S. J., Wolyniak, E. A., Williamson, C. E. and Jellison, K. L. (2007). Artificial UV-B and solar radiation reduce *in vitro* infectivity of the human pathogen *Cryptosporidium parvum*. *Environmental Science & Technology* **41**, 7101–7106. doi: 10.1021/es071324r.

Dahms, H. U. and Lee, J. S. (2010). UV radiation in marine ectotherms: molecular effects and responses. *Aquatic Toxicology* **97**, 3–14. doi: 10.1016/j.aquatox.2009.12.002.

Day, T. A. and Neale, P. J. (2002). Effects of UV-B radiation on terrestrial and aquatic primary producers. *Annual Review of Ecology and Systematics* **33**, 371–396. doi: 10.1146/annurev.ecolysis.33.010802.150434.

de Mora, S., Demers, S. and Vernet, M. (2000). *The Effects of UV Radiation in the Marine Environment*. Cambridge University Press, Cambridge, UK.

Ewing, M. S., Blazer, V. S., Fabacher, D. L., Little, E. E. and Kocan, K. M. (1999). Channel catfish response to ultraviolet-B radiation. *Journal of Aquatic Animal Health* **11**, 192–197.

Fredensborg, B. L., Mouritsen, K. N. and Poulin, R. (2004). Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda: Crustacea) infected by a trematode: experimental infections and field observations. *Journal of Experimental Marine Biology and Ecology* **311**, 253–265. doi: 10.1016/j.jembe.2004.05.011.

Ghandour, A. M. and Webbe, G. (1975). Effect of ultraviolet radiation on cercariae of *Schistosoma mansoni* and *Schistosoma haematobium*. *Journal of Helminthology* **49**, 153–159.

Gleason, D. F. and Wellington, G. M. (1995). Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient. *Marine Biology* **123**, 693–703.

Haeder, D. P., Kumar, H. D., Smith, R. C. and Worrest, R. C. (1998). Effects on aquatic ecosystems. *Journal of Photochemistry and Photobiology B Biology* **46**, 53–68.

Haeder, D. P., Helbling, E. W., Williamson, C. E. and Worrest, R. C. (2011). Effects of UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences* **10**, 242–260. doi:10.1039/C0PP90036B.

Hansson, L. A. and Hylander, S. (2009). Effects of ultraviolet radiation on pigmentation, photoenzymatic repair, behavior, and community ecology of zooplankton. *Photochemical & Photobiological Sciences* **8**, 1266–1275. doi: 10.1039/b908825c.

Helbling, E. W., Menchi, C. F. and Villafane, V. E. (2002*a*). Bioaccumulation and role of UV-absorbing compounds in two marine crustacean species from Patagonia, Argentina. *Photochemical & Photobiological Sciences* **1**, 820–825. doi: 10.1039/b206584c.

Helbling, E. W. and Zagarese, H. (2003). *UV Effects in Aquatic Organisms and Ecosystems*. The Royal Society of Chemistry, Cambridge, UK.

Helbling, E. W., Zaratti, F., Sala, L. O., Palenque, E. R., Menchi, C. F. and Villafane, V. E. (2002*b*). Mycosporine-like amino acids protect the copepod *Boeckella titicacae* (Harding) against high levels of solar UVR. *Journal of Plankton Research* **24**, 225–234.

Hudson, P. J., Dobson, A. P. and Newborn, D. (1998). Prevention of population cycles by parasite removal. *Science* **282**, 2256–2258.

Karentz, D. (2001). Chemical defences of marine organisms against solar radiation exposure: UV absorbing mycosporine-like amino acids and scytonemin. In *Marine Chemical Ecology* (ed. McClintock, J. and Baker, B.), pp. 481–520. CRC Press, Boca Raton, FL, USA.

Koehler, A. V. and Poulin, R. (2010). Host partitioning by parasites in an intertidal crustacean community. *Journal of Parasitology* **96**, 862–868. doi: 10.1645/ge-2460.1.

Kramer, K. J. M. (1990). Effects of increased solar UVB radiation on coastal marine ecosystems – an overview. In *Expected Effects of Climatic Change on Marine Coastal Ecosystems* (ed. Beukema, J. J., Wolff, W. J. and Brouns, J. J. M. W.), pp. 195–210. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Lafferty, K. D., Dobson, A. P. and Kuris, A. M. (2006). Parasites dominate food web links. *Proceedings of the National Academy of Sciences, USA* **103**, 11211–11216. doi: 10.1073/pnas.0604755103.

Lamare, M. D., Barker, M. F. and Lesser, M. P. (2007). *In situ* rates of DNA damage and abnormal development in Antarctic and non-Antarctic sea urchin embryos. *Aquatic Biology* **1**, 21–32.

Lauckner, G. (1984). Impact of trematode parasitism on the fauna of a North Sea tidal flat. *Helgoländer Meeresuntersuchungen* **37**, 185–199.

Leech, D. M. and Williamson, C. E. (2000). Is tolerance to UV radiation in zooplankton related to body size, taxon, or lake transparency? *Ecological Applications* **10**, 1530–1540.

Lesser, M. P. and Barry, T. M. (2003). Survivorship, development, and DNA damage in echinoderm embryos and larvae exposed to ultraviolet radiation (290–400 nm). *Journal of Experimental Marine Biology and Ecology* **292**, 75–91. doi: 10.1016/s0022-0981(03)00141-2.

Lister, K. N., Lamare, M. D. and Burritt, D. J. (2010). Sea ice protects the embryos of the Antarctic sea urchin *Sterechinus neumayeri* from oxidative damage due to naturally enhanced levels of UV-B radiation. *Journal of Experimental Biology* **213**, 1967–1975. doi: 10.1242/jeb.039990.

MacKenzie, K., Williams, H. H., Williams, B., McVicar, A. H. and Siddall, R. (1995). Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *Advances in Parasitology* **35**, 85–144.

Marcogliese, D. J. (2001). Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology – Revue Canadienne de Zoologie* **79**, 1331–1352.

Martorelli, S. R., Fredensborg, B. L., Mouritsen, K. N. and Poulin, R. (2004). Description and proposed life cycle of *Maritrema novaezealandensis* N. sp (Microphallidae) parasitic in red-billed gulls, *Larus novaehollandiae scopulinus*, from Otago Harbor, South Island, New Zealand. *Journal of Parasitology* **90**, 272–277.

McArdle, J. and Bullock, A. M. (1987). Solar ultraviolet radiation as a causal factor of summer syndrome in cage-reared Atlantic salmon, *Salmo salar* L – a clinical and histopathological study. *Journal of Fish Diseases* **10**, 255–264.

McFadzen, I., Baynes, S., Hallam, J., Beesley, A. and Lowe, D. (2000). Histopathology of the skin of UV-B irradiated sole (*Solea solea*) and turbot (*Scophthalmus maximus*) larvae. *Marine Environmental Research* **50**, 273–277.

McKenzie, R., Conner, B. and Bodeker, G. (1999). Increased summertime UV radiation in New Zealand in response to ozone loss. *Science* **285**, 1709–1711.

McKenzie, R. L., Aucamp, P. J., Bais, A. F., Bjorn, L. O. and Ilyas, M. (2007). Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Photochemical & Photobiological Sciences* **6**, 218–231. doi: 10.1039/b700017k.

McKenzie, R. L., Bjorn, L. O., Bais, A. and Ilyasd, M. (2003). Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Photochemical & Photobiological Sciences* **2**, 5–15. doi: 10.1039/b211155c.

Mouritsen, K. N. and Poulin, R. (2002). Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* **124**, S101–S117. doi: 10.1017/s0031182002001476.

Mouritsen, K. N. and Poulin, R. (2010). Parasitism as a determinant of community structure on intertidal flats. *Marine Biology* **157**, 201–213. doi: 10.1007/s00227-009-1310-2.

Obermueller, B., Karsten, U. and Abele, D. (2005). Response of oxidative stress parameters and sunscreening compounds in Arctic amphipods during experimental exposure to maximal natural UVB radiation. *Journal of Experimental Marine Biology and Ecology* **323**, 100–117. doi: 10.1016/j.jembe.2005.03.005.

Patz, J. A., Epstein, P. R., Burke, T. A. and Balbus, J. M. (1996). Global climate change and emerging infectious diseases. *Jama-Journal of the American Medical Association* **275**, 217–223.

Paul, N. D. and Gwynn-Jones, D. (2003). Ecological roles of solar UV radiation: towards an integrated approach. *Trends in Ecology & Evolution* **18**, 48–55.

Prah, S. K. and James, C. (1977). Influence of physical factors on survival and infectivity of miracidia of *Schistosoma mansoni* and *Schistosoma haematobium*. 1. Effect of temperature and UV light. *Journal of Helminthology* **51**, 73–85.

Przeslawski, R., Davis, A. R. and Benkendorff, K. (2005). Synergistic effects associated with climate change and the development of rocky shore molluscs. *Global Change Biology* **11**, 515–522. doi: 10.1111/j.1365-2486.2005.00918.x.

Roy, S. (2000). Strategies for the minimisation of UV-induced damage. In *The effects of UV radiation in the marine environment*. (ed. de Mora, S., Demers, S. and Vernet, M.), pp. 177–205. Cambridge University Press, Cambridge, UK.

Ruelas, D. S., Karentz, D. and Sullivan, J. T. (2006). Lethal and sublethal effects of UVB on juvenile *Biomphalaria glabrata* (Mollusca: Pulmonata). *Journal of Invertebrate Pathology* **93**, 192–200. doi: 10.1016/j.jip.2006.08.001.

Ruelas, D. S., Karentz, D. and Sullivan, J. T. (2007). Sublethal effects of ultraviolet-B radiation on miracidia and sporocysts of *Schistosoma mansoni*: Intramolluscan development, infectivity, and photoreactivation. *Journal of Parasitology* **93**, 1303–1310.

Ruelas, D. S., Karentz, D. and Sullivan, J. T. (2009). Effects of UVB on interactions between *Schistosoma mansoni* and *Biomphalaria glabrata*. *Journal of Invertebrate Pathology* **101**, 140–142. doi: 10.1016/j.jip.2009.04.001.

Salo, H. M., Aaltonen, T. M., Markkula, S. E. and Jokinen, E. I. (1998). Ultraviolet-B irradiation modulates the immune system of fish (*Rutilus rutilus*, Cyprinidae). I. Phagocytes. *Photochemistry and Photobiology* **67**, 433–437.

Seckmeyer, G. and McKenzie, R. L. (1992). Increased ultraviolet radiation in New Zealand (45°S) relative to Germany (48°N). *Nature* **359**, 135–137.

Siebeck, O., Vail, T. L., Williamson, C. E., Vetter, R., Hessen, D., Zagarese, H., Little, E., Balseiro, E., Modenutti, B., Seva, J. and Shumate, A. (1994). Impact of UV-B radiation on zooplankton and fish in pelagic freshwater ecosystems. *Advances in Limnology* **43**, 101–114.

Sinha, R. P. and Haeder, D. P. (2002). UV-induced DNA damage and repair: a review. *Photochemical & Photobiological Sciences* **1**, 225–236. doi: 10.1039/b201230 h.

Sommaruga, R. (2003). UVR and its effects on species interactions. In *UV Effects in Aquatic Organisms and Ecosystems* (ed. Helbling, E. W. and Zagarese, H.), pp. 485–508. The Royal Society of Chemistry, Cambridge, UK.

Sousa, W. P. (1991). Can models of soft-sediment community structure be complete without parasites? *American Zoologist* **31**, 821–830.

Standen, O. D. and Fuller, K. A. (1959). Ultraviolet irradiation of the cercariae of *Schistosoma mansoni* – inhibition of development to the adult stage. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **53**, 372–379.

Studer, A., Thieltges, D. W. and Poulin, R. (2010). Parasites and global warming: net effects of temperature on an intertidal host-parasite system. *Marine Ecology-Progress Series* **415**, 11–22.

Tedetti, M. and Sempere, R. (2006). Penetration of ultraviolet radiation in the marine environment. A review. *Photochemistry and Photobiology* **82**, 389–397. doi: 10.1562/2005-11-09-ir-733.

Vincent, W. F. and Neale, P. J. (2000). Mechanisms of UV damage to aquatic organisms. In *The Effects of UV Radiation in the Marine Environment*. (ed. de Mora, S., Demers, S. and Vernet, M.), pp. 149–176. Cambridge University Press, Cambridge, UK.

Williamson, C. E., Neale, P. J., Grad, G., De Lange, H. J. and Hargreaves, B. R. (2001). Beneficial and detrimental effects of UV on aquatic organisms: Implications of spectral variation. *Ecological Applications* **11**, 1843–1857.