International Journal for Parasitology 42 (2012) 453–461

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Effects of ultraviolet radiation on an intertidal trematode parasite: An assessment of damage and protection

A. Studer [a,*], V.M. Cubillos [b,c], M.D. Lamare [c], R. Poulin [a], D.J. Burritt [b]

[a] Department of Zoology, P.O. Box 56, Dunedin 9054, New Zealand
[b] Department of Botany, P.O. Box 56, Dunedin 9054, New Zealand
[c] Department of Marine Sciences, P.O. Box 56, Dunedin 9054, New Zealand

ARTICLE INFO

Article history:
Received 9 December 2011
Received in revised form 18 February 2012
Accepted 20 February 2012
Available online 3 April 2012

Keywords:
Maritrema novaezealandensis
Cercariae
Transmission
DNA damage
Cyclobutane–pyrimidine dimers
Oxidative stress
Photoprotective compounds
Mycosporine-like amino acids

ABSTRACT

Trematode parasites are integral components of intertidal ecosystems which experience high levels of ultraviolet radiation. Although these parasites mostly live within hosts, their life cycle involves free-living larval transmission stages such as cercariae which are directly exposed to ambient conditions. UV has previously been shown to considerably reduce the survival of cercariae. Here, we investigated potential mechanisms of protection and damage related to UV in the intertidal trematode *Maritrema novaezealandensis*. Firstly, the presence of sunscreen compounds (i.e. mycosporine-like amino acids) was quantified in the parasite tissue producing cercariae within a snail host, as well as in the free-swimming cercariae themselves. Secondly, levels of oxidative stress in cercariae after exposure to UV were investigated (i.e. protein carbonyls, catalase and superoxide dismutase). Thirdly, the DNA damage (i.e. cyclobutane–pyrimidine dimers) was compared between cercariae exposed and not exposed to UV. Lastly, functional aspects (survival and infectivity) of cercariae were assessed, comparing cercariae under light conditions versus dark after exposure to UV. We confirmed the presence of mycosporine-like amino acids in cercariae-producing tissue from within snail hosts, but were unable to do so in cercariae directly. Results further suggested that exposure to UV induced high levels of oxidative stress in cercariae which was accompanied by a reduction in the levels of protective antioxidant enzymes present. We also identified higher levels of DNA damage in cercariae exposed to UV, compared with those not exposed. Moreover, no clear effect of light condition was found on survival and infectivity of cercariae after exposure to UV. We concluded that cercariae are highly susceptible to UV damage and that they have very little scope for protection against or repair of UV-induced damage.

1. Introduction

Ultraviolet radiation is an important environmental factor fluctuating at various spatial and temporal scales (e.g. Hansson and Hylander, 2009). In biological terms, UV (UVB: 280–320, UVA: 320–400 nm) represents the most reactive part of incident solar radiation and is known to have a broad spectrum of deleterious genetic and cytological effects in aquatic organisms (e.g. Häder et al., 1998, 2007; Vincent and Neale, 2000; Sinha and Häder, 2002; Dahms and Lee, 2010). Of all aquatic environments, the intertidal zone is among the most physiologically stressful, with organisms living exposed to multiple fluctuating stressors including high levels of UV (e.g. Kramer, 1990; Karentz, 2001; Przeslawski et al., 2005). In intertidal ecosystems, there is little or no attenuation of UV through the water column and consequently, conditions at low tide are similar to atmospheric conditions. Trematode parasites are an integral component in these ecosystems, being the most common parasite group (Lauckner, 1984; Mouritsen and Poulin, 2002, 2010). Trematodes have complex life cycles involving several members of an intertidal community as hosts. Their transmission from one host to the next relies on free-living larval stages directly exposed to and influenced by prevailing environmental conditions (Pietrock and Marcogliese, 2003). Despite the biological and ecological importance of both parasites and UV, little is known about the damaging effects of UV on parasites, or the latter's capacity to protect against, or cope with, UV-induced damage or stress.

The damaging effects of UV occur at the molecular, cellular and physiological levels. Direct absorption of UV can degrade or transform molecules such as proteins, lipids or nucleic acids, which may result in the impairment or complete loss of their biological function (Vincent and Neale, 2000). For example, direct absorption of UVB by DNA molecules results predominantly in the formation of cyclobutane–pyrimidine dimers (CPDs) and to a lesser extent in 6–4 photoproducts (e.g. Malloy et al., 1997; Lesser et al., 2003;

* Corresponding author. Tel.: +64 3 479 7964; fax: +64 3 479 7584.
E-mail address: studeranja@gmail.com (A. Studer).

0020-7519/$36.00
http://dx.doi.org/10.1016/j.ijpara.2012.02.014

MacFadyen et al., 2004; Lamare et al., 2006). The formation of CPDs is dose-dependent and varies between taxa (Lamare et al., 2007). CPD levels resulting from sub-lethal doses of UVB may inhibit normal development of embryos and larvae, and thus affect their survival and fitness (Dahms and Lee, 2010). Indirectly, UV can be absorbed by molecules, leading to an accelerated production of reactive oxygen species (ROS) (Lesser, 2006). ROS are powerful oxidants, some of which can diffuse through cellular membranes and react with other components, leading to cytological damage. If the production of ROS exceeds a certain threshold, oxidation of DNA, proteins and membrane fatty acids occurs (Lesser, 2006). This damage is also known as oxidative stress and is considered to be a highly sensitive biomarker for a range of environmental stressors including UV (Burritt and MacKenzie, 2003; Lesser, 2006; Burritt, 2008; Lister et al., 2010a). Oxidative stress can further translate into ecologically relevant effects, including impaired development and reduced survival of damaged organisms (Lesser, 2006).

Most organisms have evolved a range of mechanisms for protection against UV, such that the net effect of UV on an organism is a combination of damage, repair and the energetic costs associated with the protective strategies (Vincent and Neale, 2000). The range of strategies to cope with UV and its potentially deleterious effects include the intracellular accumulation of UV screening compounds such as mycosporine-like amino acids (MAAs) or other UV absorbing compounds (e.g. carotenoids) (Roy, 2000; Shick and Dunlap, 2002; Dahms and Lee, 2010; Rastogi et al., 2010). MAAs play a dual role, protecting not only against UV, but also against oxidative damage (Shick and Dunlap, 2002). These compounds are synthesised by bacteria, fungi and algae and are transferred trophically to other organisms (Shick and Dunlap, 2002; Rastogi et al., 2010 and references therein). MAA transfer is also known to take place via symbiotic associations or through maternal provision to eggs (e.g. Carroll and Shick, 1996; Adams and Shick, 2001; Karentz, 2001; Shick and Dunlap, 2002; Rastogi et al., 2010). Other preventive mechanisms include antioxidants (e.g. the enzymes catalase and superoxide dismutase), which scavenge ROS and help reduce oxidative stress, and the capacity to repair UV-induced DNA damage (e.g. Kim and Sancar, 1993; Malloy et al., 1997; Sinha and Häder, 2002; Lesser, 2006; Dahms and Lee, 2010). The two primary repair mechanisms for UV-induced DNA damage are photo-reactivation and the light-independent nucleotide excision repair which can also take place in the dark (e.g. Sancar, 1994; Sinha and Häder, 2002; Dahms and Lee, 2010). Photo-reactivation is likely the simplest and oldest form of DNA repair, and is widespread in aquatic organisms (Häder and Sinha, 2005; Weber, 2005). Photo-reactivation repairs primarily CPDs via the enzyme photolyase which depends on light as its source of energy.

Most studies combining the effects of UV and parasites or pathogens are studies of induced immune reactions, vaccine development or sterilisation of drinking water or food, which are typically done in vitro using unrealistic levels of UV (e.g. Bintsis et al., 2000; Lagapa et al., 2001; Rochelle et al., 2004; Hijnen et al., 2006; Allam and Hadid, 2009). The role of UV as an ecological factor has only rarely been investigated in host-parasite interactions (but see recent studies by Perrot-Minnot et al., 2011; Overholt et al., 2011; Studer et al., 2012). The study by Overholt et al. (2011) combined laboratory and field experiments, indicating that UV may reduce levels of parasitism in *Daphnia dentifera* by reducing the infectivity of the fungal parasite, *Metschnikowia bicuspidata*. Studies by Ruelas et al. (2006, 2007, 2009) on effects of UVB irradiation on an important human parasite, the trematode *Schistosoma mansoni* and its snail host (*Biomphalaria glabrata*), have shown negative effects of UV for both the parasite and the snail. This included reduced survival of snails as well as abnormal and reduced development of parasites after penetration of the snail host following irradiation. Moreover, these authors provided evidence that not only was the snail able to repair DNA damage by photo-reactivation (Ruelas et al., 2006), but that the parasite was also able to do so (Ruelas et al., 2007). Their results indicated that photo-reactivation, rather than nucleotide excision repair, was the primary repair mechanism of UVB-induced DNA damage.

Here, we used the intertidal microphallid trematode parasite, *Maritrema novaezealandensis*, as a model system. The complex life cycle of this parasite includes the first intermediate snail host, *Zeacumantus subcarinatus*, second intermediate crustacean hosts such as the amphipod *Paracalliope novizealandiae* and definitive bird hosts (Martorelli et al., 2004). The transmission process from the first to the second intermediate host is via translucent, free-living, non-feeding, short-lived (<24 h) larval transmission stages called cercariae (approximately 100 μm body length, 100 μm tail length, 20 μm body depth and 10 μm tail depth, Koehler et al., 2011). These cercariae are produced asexually in the parasite's sporocysts within infected snails and emerge into the environment to infect second intermediate hosts. It is thought that the optimal conditions for transmission of this parasite occur on warm days during low tides when shallow soft-sediment tide pools warm up (e.g. Fredensborg et al., 2004; Bates et al., 2010); conditions typically coinciding with maximal levels of UV (i.e. spring to late summer). A previous study investigating *M. novaezealandensis* showed that the survival of cercariae was strongly reduced by UV in a dose-dependent manner (Studer et al., 2012).

Because the survival of cercariae is a key step in the life cycle of this parasite, the aims of this study were to investigate routes of damage, as well as potential protective mechanisms available to cercariae during the free-living phase when they are exposed to UV. Firstly, we searched for protective UV absorbing compounds (especially MAAs) in sporocyst tissue within snail hosts as well as in cercariae directly. Secondly, we investigated oxidative damage to proteins in cercariae after exposure to UV as a biomarker of UV-induced oxidative stress, as well as activity of protective antioxidant enzymes. Thirdly, we assessed differences in the relative concentrations of CPDs in cercariae exposed or not exposed to UV. Lastly, we evaluated differences in survival and infectivity of cercariae when kept under light or dark conditions after exposure to UV. The data provided here substantially increase our mechanistic understanding of the direct effects of UV on trematodes, in particular their free-living cercarial transmission stage, in an ecologically relevant context.

2. Materials and methods

The UV treatments in all experiments described in detail below were achieved by covering experimental units with Plexiglas filters either blocking UV (i.e. photosynthetically active radiation (PAR; 400–700 nm) only, the no UV treatment), transparent to PAR and some UVA (UVA treatment), or transparent to PAR, UVA and UVB (UVA + B treatment). The filters used had the following transmission characteristics. No UV treatment: 81% PAR, 5.2% UVA, 0.0% UVB; UVA treatment: 77.9% PAR, 46.5% UVA, 0.1% UVB; UVA + B treatment: 84.5% PAR, 84.6% UVA, 80.6% UVB (see Lister et al., 2010b for the transmission profiles of the filters).

2.1. UV absorbing compounds

In a preliminary HPLC–mass spectrophotometry analysis, the presence of UV absorbing compounds, and MAAs in particular, was detected in a small number of samples of cercariae-producing tissue from within snail hosts (sporocysts). Therefore, an outdoor experiment was conducted to assess temporal changes in the concentrations of UV absorbing compounds in sporocysts of infected snails over a 6 month period. For this, white rectangular 1 L plastic

containers were placed outdoors at the end of austral winter (July 2009). Containers (three replicate containers per treatment) were filled with a thin layer of sediment and 30 snails infected with *M. novaezealandensis* were added to each container. Snails had a constant supply of sea lettuce, *Ulva* sp., a species of algae not containing MAAs (Carefoot et al., 2000; Lamare et al., 2004). Containers were covered with the filters and randomly distributed (and occasionally rotated) on an outdoor shelf receiving flow-through filtered seawater. After 3 (October 2009) and 6 months (January 2010), snails were randomly sub-sampled, dissected and the parasite tissue from within the snails frozen at −80 °C. Additionally and because experimental snails did not shed enough cercariae, samples of cercariae were obtained by incubating snails collected from the field in January 2010 (i.e. austral summer) at 25 °C under constant illumination. Seawater containing emerged cercariae was transferred into Eppendorf tubes and centrifuged in order to remove excess seawater, then also frozen at −80 °C until processing.

For the extraction and analysis of UV absorbing compounds, samples (five replicate tissue samples per container and five samples containing cercariae) were dried using a SpeedVac (Savant Thermo Electon Corporation, USA) (45 °C for 24 h), then ground and weighed. Samples were frozen at −80 °C until extraction of MAA was carried out by adding 1 ml of 100% HPLC grade methanol (MeOH) per 10 mg of sample. Due to varying amounts of dry material per sample, the volume of MeOH added was adjusted according to each sample weight. Samples were then sonicated in an ultrasonic bath for 8 min and stored at 4 °C wrapped in aluminium foil for approximately 24 h. This was followed by filtering of samples using nylon syringe filters (0.22 μm). An aliquot of 20 μl of the methanolic extraction from each sample was injected into an HPLC (Dionex Ultimate 3000, Thermo Scientific, USA) using a C8 Phenosphere analytical column (Phenomnenx, USA; 250 × 4.6 mm; pore size 5 μm) and eluted in a mobile phase composed of 89.9% MilliQ water + 10% MeOH + 0.1 formic acid at a flow rate of 1 ml min^{-1}. All samples injected into the HPLC were analysed at 310 and 334 nm using a UV/visible light detector. Peaks identified in the chromatograms were corroborated by mass spectrometry (Bruker Daltonics TOF, USA).

HPLC revealed a mixture of two to three compounds making up the main peak at 310 nm (Fig. 1). One compound was identified as mycosporine-glycine (λ_{max} = 310; molecular weight = 245 Da, ε (molar extinction coefficient) = 28,100 M^{-1} cm^{-1}), whereas another main compound had a molecular weight of 261 Da (hereafter referred to as unknown UV-absorbing compound UC-261). To estimate the concentration of these compounds, the entire peak area was used, thus combining all compounds making up the main peak. At 334 nm, the main compound identified was the MAA porphyra-334 (hereafter referred to as P-334; λ_{max} = 334; molecular weight 346 DA; ε = 42,300 M^{-1} cm^{-1}). Concentrations were estimated using calibration curves which were generated from isolated standards obtained through semi-preparative HPLC (C18 column, Phenomenex – Luna 250 × 10 mm; pore size 5 μm). For this, UC-261 was extracted from the parasite tissue of infected *Z. subcarinatus* snails and P-334 was isolated from *Nyctiphanes australis* (krill). The concentration of each isolated standard was then estimated using the respective molar extinction coefficient (ε) and the corresponding absorbance at their maximum wavelength.

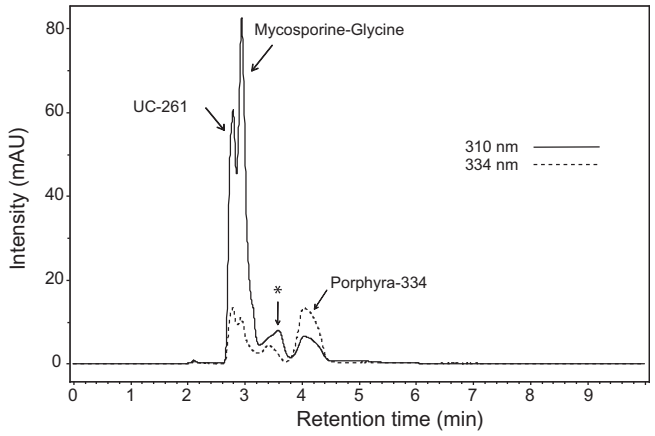

Fig. 1. HPLC chromatogram of a methanol extract of *Maritema novaezealandensis* tissue (sporocysts) from within an infected *Zeacumantus subcarinatus* snail host. Four absorbance peaks with different retention times (min) are present: at 310 nm (solid line), the main peak was mainly composed of an unidentified UV absorbing compound (UC-261) and mycosporine-glycine, whereas at 334 nm, the main peak was porphyra-334. Another small peak (∗) was apparent with a higher absorbance at 310 nm.

For the statistical analysis, the concentrations of the compounds in sporocyst tissue obtained from infected snails were square root transformed in order to meet the assumption of normality and homogeneity of variances. A repeated measures ANOVA was then used to assess the effect of the UV treatment on the concentration of the compounds absorbing at 310 nm and at 334 nm after 3 and 6 months, followed by Tukey's post hoc tests (significance level $P \leqslant 0.05$ as in all following analyses). Container was initially included as a factor but had no significant effect and thus was omitted from the analysis and the results presented.

2.2. Oxidative stress and antioxidant enzymes

The extent of oxidative stress in cercariae experiencing different regimes of UV was assessed in an experiment conducted under ambient conditions. To achieve the UV treatments, the three types of Plexiglas filters were used as described earlier. To obtain free-living cercariae, 40 snails infected with *M. novaezealandensis* were put into 50 ml UV opaque plastic jars filled with seawater (four replicate samples per treatment). Snails were then incubated for 1 h at 25 °C under constant illumination (using cold light). After incubation, the seawater containing the emerged cercariae was transferred into new 50 ml plastic jars. Four replicate baseline samples were processed immediately (see below). The remaining samples were exposed outdoors on a cloudless summer day under one of the three types of filters (no UV, UVA, or UVA + B) for 2 h (UVB dose: 16.1 kJ m^{-2}; not considering the reductions by the filters).

Samples were processed by centrifuging the seawater containing cercariae in 50 ml tubes (Nalgene Centrifuge Ware, USA) for 30 min at 38,724*g* (4 °C). Seawater was removed and cercariae re-suspended in 5 ml of 50 mM potassium phosphate buffer (pH 7.4). Samples were subsequently analysed for total protein content, protein oxidation (i.e. protein carbonyls) and the levels of activity of antioxidant enzymes (i.e. catalase (CAT) and superoxide dismutase (SOD)). For this, cercariae were centrifuged as above and re-suspended in 0.5 ml of 100 mM potassium phosphate buffer (pH 7.4) containing 1.5% NaCl and 1 mM phenylmethylsulphonyl, transferred into 1.5 ml Eppendorf tubes and then lysed by sonication at 4 °C. The protein extracts were again centrifuged for 30 min at 21,000*g* in a microfuge and the supernatants were frozen at −80 °C prior to analysis. The protein content of the extracts was determined using a Lowry protein assay (Fryer et al., 1986). Protein carbonyl levels in the protein extracts were analysed via reaction with 2,4-dinitrophenylhydrazine (Reznick and Packer, 1994) with minor adaptations (Burritt, 2008). CAT (EC 1.11.1.6) was assayed using the chemiluminescent method of Maral et al. (1977), as adapted by Janssens et al. (2000). SOD (EC 1.15.1.1) was analysed using the microplate assay described by Banowetz et al. (2004) with minor modifications. All assays were carried out with a

PerkinElmer (Wallac) 1420 multilabel counter (Perkin Elmer, USA), controlled by a computer and fitted with a temperature control cell (set to 25 °C) and an auto-dispenser. Data were acquired and processed using the WorkOut 2.0 software package (Perkin Elmer).

Differences in concentrations of protein carbonyls, CAT and SOD between the different UV treatments were analysed using ANOVAs followed by Tukey post hoc tests. Data were transformed where necessary (log transformation of protein carbonyl concentrations) to meet the assumptions of the parametric test.

2.3. DNA damage in cercariae

In this outdoor experiment, the amount of DNA damage in terms of the relative concentration of CPDs per megabase of DNA was measured after exposing cercariae to ambient conditions. The concentration of CPDs formed was compared between cercariae exposed or not exposed to UV. In order to obtain the cercariae, 35 infected snails were placed in individual 50 ml UV opaque plastic jars filled with seawater and then incubated at 25 °C under constant illumination (cold light) for 1 h. After incubation, the seawater containing the cercariae was transferred to new 50 ml plastic jars. Two samples were used as a baseline and processed immediately (see below). The remaining samples were either exposed to ambient conditions under a UV-blocking filter (no UV), or UV transparent filters (UVA + B; four samples per treatment). After 1 h of exposure (corresponding to a UVB dose of 12.0 kJ m^{-2}), samples were processed by centrifuging the seawater containing cercariae twice in sequence; first in 50 ml Nalgene tubes for 25 min at 38,724*g* (4 °C) and then for 10 min in 1.5 ml Eppendorf tubes at 20,817*g* (4 °C). After removal of seawater and addition of 1 ml of salt-saturated DMSO buffer to each tube, samples were stored at −80 °C until further analysis.

DNA damage in terms of CPDs was quantified in an ELISA. For this, DNA was isolated from the cercariae using a commercially available extraction kit and protocol (Bioline, UK). The ELISA was carried out following Mori et al. (1991), as modified by Schmitz-Hoerner and Weissenböck (2003). Briefly, 10 ng of DNA from each sample was used per well in protamine sulphate (0.003%)-coated 96-well polyvinyl chloride microtiter plates, with three replicate wells assayed per sample. In each well, DNA was added to PBS denatured by boiling at 100 °C for 10 min followed by 10 min on ice and incubation overnight. Plates were washed five times before 150 µl of 2% FBS in PBS–Tween (PBS–T) was added followed by 1.5 h of incubation and rinsing. To each well, 100 µl of 0.001% TDM-2 primary monoclonal antibody was added and incubated for 30 min, after which wells were rinsed. Rabbit anti-mouse IgG secondary antibody (100 µl) was added to each well and incubated for 30 min followed by rinsing. Then, 100 µl of 0.0001% streptavidin horseradish peroxidase conjugate was added to each well and incubated for 30 min followed by rinsing. All rinsing steps were done five times using 0.05% Tween-20 in PBS and all incubations were at 38 °C. Colour development was achieved by addition of Sigma Fast reagents (Sigma) and colour was read (at 405 nm) after 5 min using a plate reader. Absorption readings were adjusted for background absorption levels. The CPD concentrations from the DNA of cercariae exposed (UVA + B) and not exposed (no UV) were compared with a *t*-test.

2.4. Effect of light condition on survival and infectivity of cercariae

When free-living cercariae are exposed to environmental conditions, light-dependent repair mechanisms were hypothesised to potentially ameliorate the damage caused by UV. Two separate laboratory experiments were thus conducted to assess differences in survival and infectivity of cercariae of *M. novaezealandensis* after exposure to UV. After UV treatment of cercariae (dose administered during a 1 h exposure, not considering reductions by filters: UVB: 5.84, UVA: 63.27, PAR: 185.09 kJ m^{-2}), cercariae were either incubated under white fluorescent light (Phillips 40 W) (using a transparent plastic bag) or kept in the dark (using a black, non-transparent plastic bag). The same laboratory set-up as described in Studer et al. (2012) and the same filters (UVA + B treatment) were used as described above.

2.4.1. Survival experiment

A cercarial mixture (60 µl; prepared using 40 snails; for details on preparation see Studer et al., 2010) was added to 12 replicate wells on two replicate 96-well plates per treatment (corresponding to an addition of approximately 35 cercariae per well). Cercariae were then exposed for 1 h to UVA + B and subsequently placed in either a transparent (light treatment) or a black plastic bag (dark treatment) under white fluorescent light. Functional activity of the cercariae was monitored at an average cercarial age of 2, 4, 6, 10 and 12 h. Functional activity categories distinguished were fully active, sluggishly motile and immotile/dead. The effect of the light/dark treatment on the proportion of fully active cercariae (arcsine-square root transformed) at an average age of 2, 4, 6 and 10 h was statistically analysed with a repeated measures ANOVA.

2.4.2. Infectivity experiment

Uninfected *P. novizealandiae* amphipods were placed individually into wells of 96-well plates (48 amphipods on two replicate well plates per treatment). A cercarial mixture prepared from 40 snails was distributed into four small Petri dishes (7 ml per dish) and exposed to UVA + B for 1 h. After exposure, the mixture was combined again and 50 µl was added to each amphipod (corresponding to an addition of approximately 20 cercariae per amphipod). Amphipods and cercariae were then incubated for 3 h under light or dark conditions (temperature in both plastic bags was approximately 21–22 °C). After incubation, amphipods were transferred into plastic containers (300 ml) filled with seawater (aerated) and provided with sea lettuce, *Ulva* sp. After 8 days, surviving amphipods were measured, sexed and dissected under a dissecting microscope. The number of parasites per amphipod was counted. The effect of the light/dark treatment, as well as sex and size of amphipods, on the proportion of cercariae successfully infecting amphipods (arcsine-square root transformed) was statistically analysed with a General Linear Model (GLM).

3. Results

3.1. UV absorbing compounds

In the vast majority of samples, four peaks were identified by HPLC (see Fig. 1 for retention times). Two of those were MAAs, namely mycosporine-glycine and porphyra-334 (P-334). Two peaks were not identified (including UC-261). For the main compounds with a maximum absorbance at 310 nm, no differences in the concentrations were found after 3 months of exposure between treatments (Fig. 2). After 6 months, concentrations were significantly lower in the no UV and UVA treatments, but not in parasite tissue from snails exposed to UVA + B, in which concentrations remained highest and were not significantly different from the concentration after 3 months (Tukey's post hoc, $P = 0.932$) (see also Table 1). Compared with the mean concentrations of these compounds in the parasite tissue of UV unexposed snails after 3 months, mean concentrations after 6 months had decreased to 44.7% (no UV), 49.7% (UVA) and 82.0% (UVA + B).

For P-334, UV treatment and time both had a significant effect on concentrations (Table 1). However, the only significant difference was between the concentrations in parasite tissue from snails

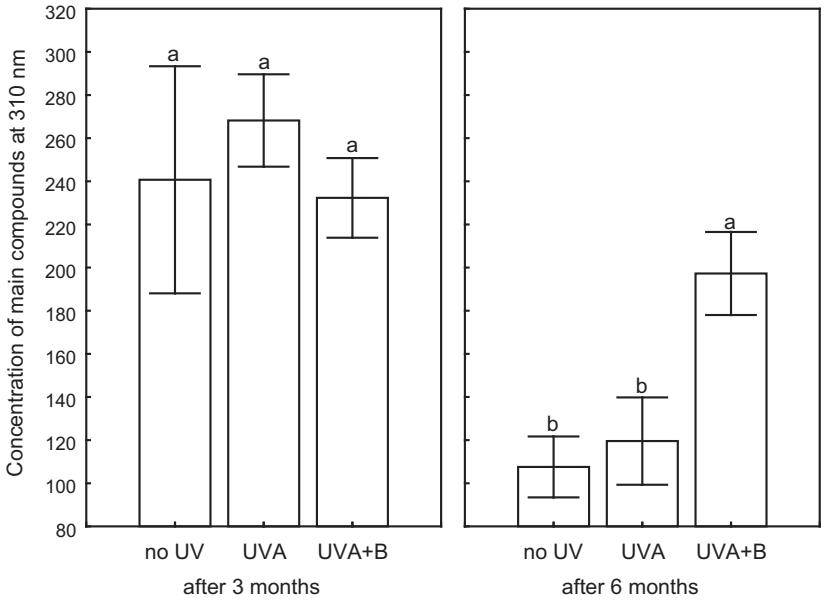

Fig. 2. Concentrations (mean ± S.E.) of the compounds with a maximum absorbance at 310 nm (mycosporine-glycine, UC-261 and in few samples another unidentified compound; nmol mg^{-1} dry weight) in *Maritrema novaezealandensis* tissue in *Zeacumantus subcarinatus* snail hosts after 3 and 6 months exposure to UV treatments (no UV, UVA, UVA + B; $n = 15$ per treatment) under ambient conditions. The data were analysed with a repeated measures ANOVA followed by a Tukey's post hoc test: the letters denote significant differences between treatments if not equal (significance level $P < 0.05$).

Table 1
Results of the repeated measures ANOVA testing the effect of the UV treatment (no UV, UVA or UVA + B) on the concentration of compounds absorbing at 310 nm and 334 nm, respectively, in *Maritrema novaezealandensis* tissue (sporocysts) in infected *Zeacumantus subcarinatus* snail hosts after 3 and 6 months of exposure ($n = 15$ per treatment).

	Factor	df	MS	*F*	*P*
310 nm	*Between subjects*				
	UV	2	27.58	3.24	0.050
	Error	40	8.51		
	Within subjects				
	Time	1	329.05	25.31	<0.001
	Time × UV	2	42.76	3.29	0.048
	Error	40	13.00		
334 nm	*Between subjects*				
	UV	2	1.17	3.89	0.029
	Error	40	0.30		
	Within subjects				
	Time	1	3.09	10.40	<0.001
	Time × UV	2	0.38	1.28	0.288
	Error	40	0.30		

not exposed to UV after 3 months and those exposed to UVA + B after 6 months (Tukey's post hoc, $P = 0.001$). Mean concentrations of P-334 compared with the mean concentration in parasite tissue from snails not exposed to UV after 3 months increased to 126.6% (no UV), 142.5% (UVA) and 178.1% (UVA + B) (Fig. 3). Concentrations of P-334 were, however, much lower than concentrations of compounds absorbed at 310 nm.

In contrast to the presence of MAAs in sporocysts, these compounds could not be detected in cercariae directly. While HPLC revealed the presence of unidentified UV absorbing compounds in three out of five samples, no MAAs were identified by mass spectrometry.

3.2. Oxidative stress

Exposure of cercariae to the UV treatments resulted in significantly different concentrations of protein carbonyls, as well as activity levels of CAT and SOD enzymes (ANOVA; log transformed, protein carbonyls: $F_{3,12} = 100.42$; CAT: $F_{3,12} = 14.01$; SOD: $F_{3,12} = 63.65$; all $P < 0.001$). Levels of protein carbonyls increased by more than fivefold in cercariae exposed to UV compared with baseline levels, and both UVA and UVB radiation induced protein oxidation in these organisms (Fig. 4). However, in the no UV (i.e. PAR only) treatment there was already a considerable increase in protein carbonyl levels compared with baseline levels. A similar pattern emerged for the activity levels of antioxidant enzymes (i.e. CAT and SOD). When comparing the UVA + B treatment to the baseline, enzyme activity was reduced by almost fourfold (CAT) and by more than sixfold (SOD). Again, both UVA and UVB negatively affected these enzymes, but the no UV (PAR only) treatment also caused a substantial decrease in activity levels of these enzymes (Fig. 4).

3.3. DNA damage in cercariae

There was a significant increase in the concentration of CPDs per megabase of DNA for cercariae exposed to UVA + B compared with cercariae not exposed to UV (Fig. 5) (*t*-test; $t = -17.71$, df = 6, $P < 0.001$), with the average concentration of the two baseline samples (0.19) being similar to the concentration in cercariae not exposed to UV.

3.4. Effect of light condition on survival and infectivity of cercariae

There was no significant difference in the survival of cercariae kept under light versus dark after exposure to UV (repeated measures ANOVA; arcsine-square root transformed proportion of fully active cercariae; between subjects, effect of light/dark: $F_{1,46} = 0.85$, $P = 0.362$; within subjects, time: $F_{3,138} = 2471.33$, $P < 0.001$, time × light/dark: $F_{3,138} = 0.64$, $P = 0.591$). Full activity of cercariae ceased within 10 h regardless of treatment. There was a slight trend in terms of infectivity, i.e. a higher proportion of cercariae successfully infecting amphipods, for infections taking place under light (21.9% successful in the light treatment versus 17.5% in the dark), which was however also not significant (GLM, arcsine-square root transformed proportion of successful parasites;

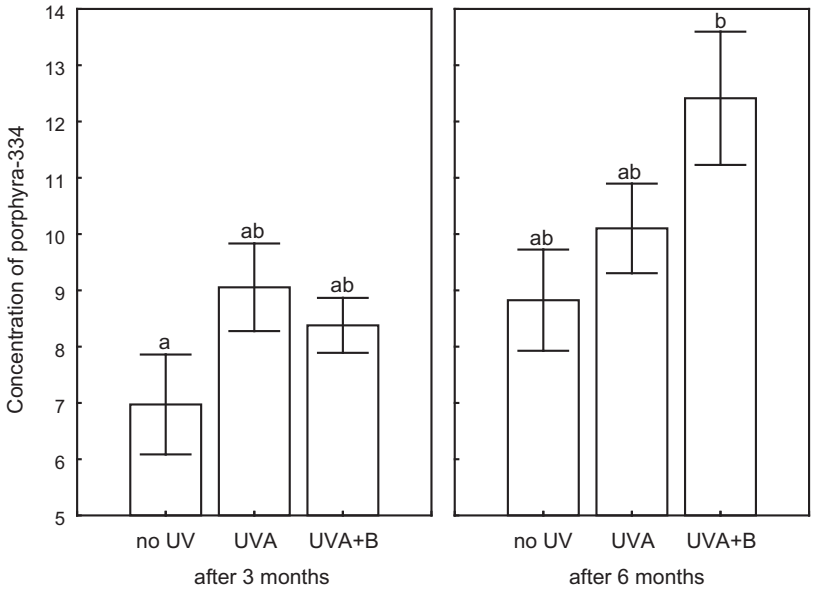

Fig. 3. Concentrations (mean ± S.E.) of porphyra-334 (nmol mg^{-1} dry weight) in *Maritrema novaezealandensis* tissue in *Zeacumantus subcarinatus* snail hosts after 3 and 6 months exposure to ambient conditions in the UV treatments (no UV, UVA, UVA + B; $n = 15$ per treatment). The data were analysed with a repeated measures ANOVA followed by a Tukey's post hoc test: the letters denote significant differences between treatments (significance level $P < 0.05$).

$F_{1,44} = 1.55$, $P = 0.220$). Amphipod sex significantly influenced the proportion of successful cercariae, with males being more heavily infected than females ($F_{1,44} = 4.62$, $P = 0.037$), while size of amphipods did not ($F_{3,44} = 0.31$, $P = 0.815$).

4. Discussion

Larval stages and planktonic eggs of aquatic organisms are ecologically very important but are also particularly at risk of UV-induced damage (Adams and Shick, 1996, 2001; Lister et al., 2010b). However, organisms have a range of mechanisms and strategies to counteract the potentially detrimental effects of UV (e.g. Roy, 2000; Shick and Dunlap, 2002; Dahms and Lee, 2010). In this study, the larval cercarial transmission stage of the intertidal trematode parasite, *M. novaezealandensis*, was investigated in terms of UV related mechanisms of protection, damage and repair. In contrast to larval stages of marine organisms, cercariae were shown to have only limited capacity to prevent UV-induced damage through the provision of MAAs and to cope with oxidative stress. In addition, DNA damage was increased in cercariae exposed to UV and no difference was found in the functional aspects of survival and infectivity of cercariae if, after exposure to UV, they were kept under light or dark conditions.

Significant differences in the concentrations of UV absorbing compounds in sporocyst tissue in snail hosts emerged only after 6 months of exposure to ambient conditions. After this period, concentrations of the compounds with a maximum absorbance at 310 nm remained high, and concentrations of P-334 were slightly increased only in parasite tissue from snails exposed to UVA + B. Increases in MAA content in response to ambient UV (and temperature in some cases) has been shown in a number of organisms (e.g. algae, sponges, corals, see reviews by Shick and Dunlap (2002) and Sinha et al. (1998)), but it has also been shown that this may not be a universal pattern in marine organisms (e.g. Adams and Shick, 1996; Hoyer et al., 2001; Shick and Dunlap, 2002; Shick et al., 2002). During the experiment, snails had to acquire MAAs through their algal diet and parasites through uptake from the snail host. In an infected snail host, the sporocysts replace the gonads and may divert any resources that the snail would allocate to this organ. Both loss and accumulation of MAAs in the parasite tissue is thus likely to reflect the respective concentrations in the snail itself and hence the concentrations in periphyton growing in the containers under the different light conditions over the entire period of the experiment (see Whitehead et al., 2001). Alternatively, due to changing conditions (in particular temperature) over the 6 month period, it is possible that snails were selectively metabolising certain compounds, thus accounting for some of the observed patterns. It is also possible that changes in sporocyst metabolism over time may cause differential uptake of MAAs independent of concentrations in the snail. Unfortunately, snail tissue was not assessed in this study and neither was the dietary content within the experimental units (e.g. microalgae growing in the containers). The lack of this data clearly limits our understanding of the potential pathways and mechanisms behind the observed patterns in the parasite tissue within the snails.

Importantly, despite the presence of these MAAs in sporocysts, we could not find any evidence that these may be transferred to cercariae for protection against UV during their free-living transmission phase. The dual protection these compounds provide (absorption of UV as well as antioxidant activity) (Dunlap and Yamamoto, 1995; Shick and Dunlap, 2002), hence does not seem to be available to cercariae, at least not in concentrations high enough to be detectable by the methods used. The small size of the *M. novaezealandensis* cercariae and consequently the small amount of tissue we were working with may be responsible for our limited ability to detect the low concentrations in which these compounds may be present. In any case, the small size of cercariae appears to limit the beneficial use of sunscreen compounds: the model of Garcia-Pichel (1994) describes the degree to which organisms can self-shade based on their size and the concentration of sunscreen compounds. Using this model under the assumption that it is also applicable to organisms such as parasitic transmission stages, we estimated (assuming a body radius of 10 μm, a tail radius of 4.7 μm and a water content of cercariae of 70%), that even if cercariae contained the same amount of MAAs as present in the sporocyst tissue (mycosporine-glycine (245 Da) and porphyra-334 (346 Da)), this would only protect them against ⩽6% of the incom-

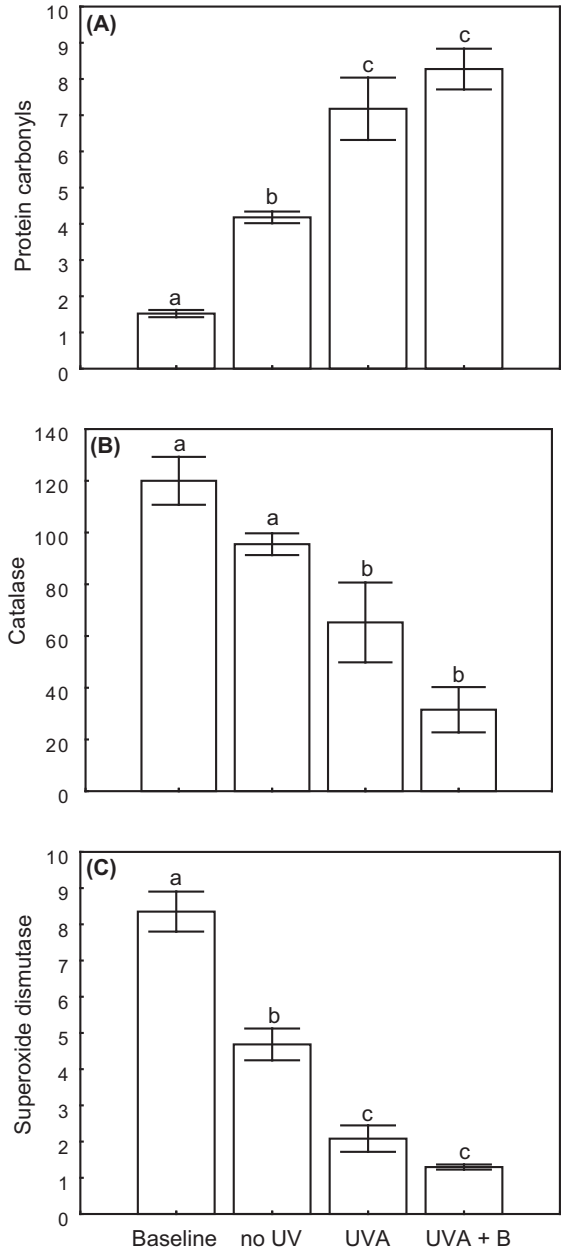

Fig. 4. Concentrations (mean ± S.E.) of protein carbonyls (nmol mg^{-1} of protein) (A) and antioxidant enzyme activity of (B) catalase (μmol H_2O_2 mg^{-1} protein min^{-1}) and (C) superoxide dismutase (U mg^{-1} protein min^{-1}) in cercariae of *Maritrema novaezealandensis* in baseline samples and after exposure to the different UV treatments (no UV, UVA or UVA + B radiation; $n = 4$ per treatment). The letters denote significant differences between treatments if not equal, according to Tukey's post hoc tests (significance level $P < 0.05$).

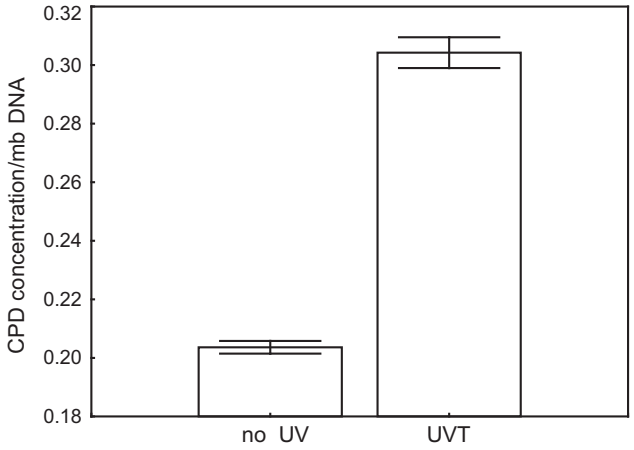

Fig. 5. Concentrations (mean ± S.E.) of cyclobutane pyrimidine dimers per megabase of DNA in samples containing cercariae of *Maritrema novaezealandensis* pooled from several *Zeacumantus subcarinatus* snail hosts, exposed (i.e. filter used transparent to UVA + B; UVT) and not exposed to UV (no UV) ($n = 4$).

ing radiation. Hence for the cercariae studied here, their small size may mean that MAAs would be ineffective self-shading sunscreen compounds unless they were present in unrealistically high concentrations.

As indicated by the increasing levels of protein oxidation, exposure to UV induced oxidative stress in cercariae. Compared with the baseline samples, even exposure to no UV (i.e. only PAR) increased levels of protein carbonyls and this was further exacerbated under exposure to UVA and UVA + B. This increase in oxidative damage of proteins was, however, not accompanied by an increase in the activity of antioxidant enzymes. While CAT and SOD activity levels in baseline samples were still mostly comparable with those found in other marine organisms (e.g. Buchner et al., 1996; Korkina et al., 2000; Lister et al., 2010b), no increase could be detected in the cercariae of *M. novaezealandensis*, despite the fact that activity levels of antioxidant enzymes are usually increased in response to oxidative stress (Dykens and Shick, 1982; Shick et al., 1995). Rather than an increase in activity, activities of both CAT and SOD declined in response to UV, indicating that enzymes lost their functionality during exposure and that they could no longer be produced or regenerated to required levels. These results from an outdoor experiment were repeated in a laboratory experiment, confirming the observed patterns (A. Studer and D.J. Burritt, unpublished data). Therefore, UV-induced damage to cercariae after reaching a certain threshold is bound to be substantial and is highly likely to contribute to the high mortality of cercariae after exposure to UV (Studer et al., 2012).

It has been observed in a behavioural study, that cercariae of *M. novaezealandensis* during their first hour after emergence from snail hosts prefer "light" microhabitats, whereas afterwards they prefer "dark" ones (Koehler et al., 2012). Thus, it is possible that oxidative stress accumulating during this first hour of exposure to ambient conditions is perceived by the parasite and serves as a signal to induce avoidance of light, i.e. to move into the dark. Ultimately, this is what the parasite needs to do in order to locate crustacean hosts such as crabs, inside which it would thereafter live in a more or less opaque internal environment. If this transition does not take place, then the negative effects of UV-induced damage may exceed a threshold with direct consequences for the survival of cercariae.

In addition to the oxidative stress parameters, deleterious effects may also include damage to DNA, as observed in the increased concentrations of CPDs in cercariae exposed compared with those not exposed to UV. This increase in CPDs in response to UV is consistent with other studies on a range of marine organisms (e.g. Malloy et al., 1997; Karentz et al., 2004; Lamare et al., 2007). Cercariae were exposed to natural sunlight for only 2 h, hence the low concentrations of CPDs. However, concentrations (i.e. <0.5 CPD Mb^{-1} DNA) are relatively comparable if values from other organisms exposed for several days are converted to an hourly accumulation concentration (e.g. 16 CPDs Mb^{-1} DNA in *Evechinus chloroticus* sea urchin embryos exposed in Otago harbour, New Zealand and at the surface over 4 days in Lamare et al., 2007).

No evidence for an effective or efficient photo-dependent mechanism could be detected in cercariae after exposure to UV to prolong the survival and hence the chance of successful transmission. Both survival and infectivity of cercariae in our experiments did not differ significantly between cercariae kept under light and those kept under dark conditions after exposure to UV. Based on the observations described above, this is not unexpected. Nonetheless, experiments were conducted using light from a 40 W light bulb and the quality of this light may not have been optimal; exposing cercariae to natural light might be a better approach. Moreover, other experimental designs (e.g. allowing time for a potential recovery or assessing DNA repair rates) may be required to yield further information on these functional aspects. These methodological approaches are, however, beyond the scope of the present study. Nonetheless, it is possible that light-independent repair mechanisms were responsible for the non-significant differences observed. In particular the dark excision of DNA damage clearly warrants further research as it should be the preferred mechanism for a parasitic organism living in an opaque environment after successful penetration of a host.

The results presented here indicate that negative effects of UV on these free-living parasitic larvae may be even more pronounced than effects on non-parasitic marine larvae or eggs. Cercariae are small, equipped with only limited energy reserves (e.g. Johnson et al., 2010) and their survival is usually less than 24 h (depending on environmental conditions). Due to these limited resources and the high energetic costs of some of the protective and repair mechanisms, no costly investments may be made in this relatively short-lived cercarial transmission stage. Our observations may thus be more consistent with what has been described for sperm of marine free spawners, which is more susceptible to damage caused by UV than eggs, embryos or larvae (Lu and Wu, 2005; Dahms and Lee, 2010). Sperm lack UV-absorbing compounds such as MAAs, possess limited antioxidant potential and have a comparatively low DNA repair capacity (e.g. Aitken et al., 1998; Adams et al., 2001), and a decline in motility after exposure to UV can contribute to reduced fertility (Dahms and Lee, 2010). In the case of cercariae, their reduced survival and activity when exposed to UV may limit their chance of successful transmission and therefore the continuation of their life cycle.

The high vulnerability of the small and translucent *M. novaezealandensis* cercariae may be offset by the parasite producing them in large numbers. This strategy is common in nature, especially for parasites. Moreover, for the transmission process taking place in nature, certain environmental characteristics may further increase the number of successful transmission events. For example, vegetation in tide pools where snail and crustacean hosts are aggregated provides some shelter from direct insolation. On the other hand, temperature in particular is bound to play an important role, affecting the transmission process on many levels (Studer et al., 2010), and conditions of increased temperature are highly likely to further increase the negative effect of UV on cercariae (Studer et al., 2012). Interestingly, other intertidal trematode cercariae are not produced in similarly large numbers as *M. novaezealandensis* and cercariae of some species are pigmented (e.g. other trematode species infecting *Z. subcarinatus* such as *Acanthoparyphium* sp.; Martorelli et al., 2008; A. Studer, personal observation). Moreover, one of the trematode species infecting *Z. subcarinatus* snails, *Philophthalmus* sp., does not seek a second intermediate host but encysts on hard substrates (Lei and Poulin, 2011), therefore remaining exposed to ambient conditions once emerged from a snail host awaiting ingestion by the next host.

It is concluded that the free-living transmission stage of the intertidal trematode *M. novaezealandensis* is clearly at the mercy of environmental conditions, including the strong effects of UV. The cercariae neither contain detectable levels of UV absorbing compounds for protection against UV, nor do they appear capable of dealing with increasing oxidative stress during exposure to UV. Moreover, exposure of cercariae to UV also induced DNA damage and no evidence was found for a light-dependent recovery capacity which may possibly prolong their functionality in the environment and thus increase their transmission success. Results presented here thus consistently confirmed that UV has the potential to impair cercariae and cause disruption of cellular processes, and that cercariae of *M. novaezealandensis* possess only little, if any, capacity to prevent and cope with such effects.

Acknowledgements

We wish to thank the reviewers for their constructive comments that helped to considerably improve the manuscript. We also thank staff at the Departments of Zoology, Botany and the Portobello Marine Laboratory (University of Otago, New Zealand) for technical and logistical support throughout the study. We also wish to thank the Chemistry Department of the University of Otago, in particular Ian Stewart, for use of the HPLC–mass spectrophotometer. We are grateful to Amanda Bates for her help with snail collections. A.S. further acknowledges funding through a University of Otago Postgraduate Scholarship.

References

Adams, N.L., Shick, J.M., 1996. Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin *Strongylocentrotus droebachiensis*. Photochem. Photobiol. 64, 149–158.

Adams, N.L., Shick, J.M., 2001. Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin *Strongylocentrotus droebachiensis*. Mar. Biol. 138, 267–280.

Adams, N.L., Shick, J.M., Dunlap, W.C., 2001. Selective accumulation of mycosporine-like amino acids in ovaries of the green sea urchin *Strongylocentrotus droebachiensis* is not affected by ultraviolet radiation. Mar. Biol. 138, 281–294.

Aitken, R.J., Gordon, E., Harkiss, D., Twigg, J.P., Milne, P., Jennings, Z., Irvine, D.S., 1998. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol. Reprod. 59, 1037–1046.

Allam, G., Hadid, S.M.A., 2009. The protective capacity of UV-exposed *Clinostomum complanatum* metacercariae against challenge infections in *Ardeola ibis ibis*. J. Helminthol. 83, 361–367.

Banowetz, G.M., Dierksen, K.P., Azevedo, M.D., Stout, R., 2004. Microplate quantification of plant leaf superoxide dismutases. Anal. Biochem. 332, 314–320.

Bates, A.E., Poulin, R., Lamare, M.D., 2010. Spatial variation in parasite-induced mortality in an amphipod: shore height versus exposure history. Oecologia 163, 651–659.

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R.K., 2000. Existing and potential applications of ultraviolet light in the food industry – a critical review. J. Sci. Food Agric. 80, 637–645.

Buchner, T., Abele-Oeschger, D., Theede, H., 1996. Aspects of antioxidant status in the polychaete *Arenicola marina*: tissue and subcellular distribution, and reaction to environmental hydrogen peroxide and elevated temperatures. Mar. Ecol. Prog. Ser. 143, 141–150.

Burritt, D.J., MacKenzie, S., 2003. Antioxidant metabolism during acclimation of *Begonia x erythrophylla* to high light levels. Ann. Bot. 91, 783–794.

Burritt, D.J., 2008. The polycyclic aromatic hydrocarbon phenanthrene causes oxidative stress and alters polyamine metabolism in the aquatic liverwort *Riccia fluitans* L.. Plant Cell Environ. 31, 1416–1431.

Carefoot, T.H., Karentz, D., Pennings, S.C., Young, C.L., 2000. Distribution of mycosporine-like amino acids in the sea hare *Aplysia dactylomela*: effect of diet on amounts and types sequestered over time in tissues and spawn. Comp. Biochem. Phys. C 126, 91–104.

Carroll, A.K., Shick, J.M., 1996. Dietary accumulation of UV-absorbing mycosporine-like amino acids (MAAs) by the green sea urchin (*Strongylocentrotus droebachiensis*). Mar. Biol. 124, 561–569.

Dahms, H.U., Lee, J.S., 2010. UV radiation in marine ectotherms: molecular effects and responses. Aquat. Toxicol. 97, 3–14.

Dunlap, W.C., Yamamoto, Y., 1995. Small-molecule antioxidants in marine organisms – antioxidant activity of mycosporine-glycine. Comp. Biochem. Phys. B 112, 105–114.

Dykens, J.A., Shick, J.M., 1982. Oxygen production by endosymbiotic algae controls superoxide-dismutase activity in their animal host. Nature 297, 579–580.

Fredensborg, B.L., Mouritsen, K.N., Poulin, R., 2004. Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda: Crustacea) infected by a trematode: experimental infections and field observations. J. Exp. Mar. Biol. Ecol. 311, 253–265.

Fryer, H.J.L., Davis, G.E., Manthorpe, M., Varon, S., 1986. Lowry protein assay using an automatic microtiter plate spectrophotometer. Anal. Biochem. 153, 262–266.
Garcia-Pichel, F., 1994. A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. Limnol. Oceanogr. 39, 1704–1717.
Häder, D.P., Kumar, H.D., Smith, R.C., Worrest, R.C., 1998. Effects on aquatic ecosystems. J. Photochem. Photobiol. B 46, 53–68.
Häder, D.P., Sinha, R.P., 2005. Solar ultraviolet radiation-induced DNA damage in aquatic organisms: potential environmental impact. Mutat. Res. Fundam. Mol. Mech. Mutag. 571, 221–233.
Häder, D.P., Kumar, H.D., Smith, R.C., Worrest, R.C., 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem. Photobiol. Sci. 6, 267–285.
Hansson, L.A., Hylander, S., 2009. Effects of ultraviolet radiation on pigmentation, photoenzymatic repair, behavior, and community ecology of zooplankton. Photochem. Photobiol. Sci. 8, 1266–1275.
Hijnen, W.A.M., Beerendonk, E.F., Medema, G.J., 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. Water Res. 40, 3–22.
Hoyer, K., Karsten, U., Sawall, T., Wiencke, C., 2001. Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages. Mar. Ecol. Prog. Ser. 211, 117–129.
Janssens, B.J., Childress, J.J., Baguet, F., Rees, J.F., 2000. Reduced enzymatic antioxidative defense in deep-sea fish. J. Exp. Biol. 203, 3717–3725.
Johnson, P.T.J., Dobson, A., Lafferty, K.D., Marcogliese, D.J., Memmott, J., Orlofske, S.A., Poulin, R., Thieltges, D.W., 2010. When parasites become prey: ecological and epidemiological significance of eating parasites. Trends Ecol. Evol. 25, 362–371.
Karentz, D., 2001. Chemical defenses of marine organisms against solar radiation exposure: UV absorbing mycosporine-like amino acids and scytonemin. In: McClintock, J., Baker, B. (Eds.), Marine Chemical Ecology. CRC Press, Boca Raton, Florida, pp. 481–520.
Karentz, D., Bosch, I., Mitchell, D.M., 2004. Limited effects of Antarctic ozone depletion on sea urchin development. Mar. Biol. 145, 277–292.
Kim, S.T., Sancar, A., 1993. Photochemistry, photophysics, and mechanism of pyrimidine dimer repair by DNA photolyase. Photochem. Photobiol. 57, 895–904.
Koehler, A.V., Springer, Y.P., Keeney, D.B., Poulin, R., 2011. Intra- and interclonal phenotypic and genetic variability of the trematode *Maritrema novaezealandensis*. Biol. J. Linn. Soc. 103, 106–116.
Koehler, A.V., Springer, Y.P., Rhandhawa, H.S., Leung, T.L.F., Keeney, D.B., Poulin, R., 2012. Genetic and phenotypic influences on clone-level success and host specialization in a generalist parasite. J. Evolut. Biol. 25, 66–79.
Korkina, L.G., Deeva, I.B., De Biase, A., Iaccarino, M., Oral, R., Warnau, M., Pagano, G., 2000. Redox-dependent toxicity of diepoxybutane and mitomycin C in sea urchin embryogenesis. Carcinogenesis 21, 213–220.
Kramer, K.J.M., 1990. Effects of increased solar UVB radiation on coastal marine ecosystems – an overview. In: Beukema, J.J., Wolff, W.J., Brouns, J.J.M.W. (Eds.), Expected Effects of Climatic Change on Marine Coastal Ecosystems. Kluwer Academic Publishers, Dordrecht, pp. 195–210.
Lagapa, J.T.G., Konno, K., Oku, Y., Nonaka, N., Kamiya, M., 2001. Inhibitory effect of different UV lamps on the infectivity of taeniid eggs. Parasitol. Res. 87, 593–597.
Lamare, M.D., Lesser, M.P., Barker, M.F., Barry, T.M., Schimanski, K.B., 2004. Variation in sunscreen compounds (mycosporine-like amino acids) for marine species along a gradient of ultraviolet radiation transmission within Doubtful Sound, New Zealand. NZ J. Mar. Freshwater Res. 38, 775–793.
Lamare, M.D., Barker, M.F., Lesser, M.P., Marshall, C., 2006. DNA photorepair in echinoid embryos: effects of temperature on repair rate in Antarctic and non-Antarctic species. J. Exp. Biol. 209, 5017–5028.
Lamare, M.D., Barker, M.F., Lesser, M.P., 2007. *In situ* rates of DNA damage and abnormal development in Antarctic and non-Antarctic sea urchin embryos. Aquat. Biol. 1, 21–32.
Lauckner, G., 1984. Impact of trematode parasitism on the fauna of a North Sea tidal flat. Helgoländer Meeresun. 37, 185–199.
Lei, F.Y., Poulin, R., 2011. Effects of salinity on multiplication and transmission of an intertidal trematode parasite. Mar. Biol. 158, 995–1003.
Lesser, M.P., Kruse, V.A., Barry, T.M., 2003. Exposure to ultraviolet radiation causes apoptosis in developing sea urchin embryos. J. Exp. Biol. 206, 4097–4103.
Lesser, M.P., 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol. 68, 253–278.
Lister, K.N., Lamare, M.D., Burritt, D.J., 2010a. Oxidative damage in response to natural levels of UV-B radiation in larvae of the tropical sea urchin *Tripneustes gratilla*. Photochem. Photobiol. 86, 1091–1098.
Lister, K.N., Lamare, M.D., Burritt, D.J., 2010b. Sea ice protects the embryos of the Antarctic sea urchin *Sterechinus neumayeri* from oxidative damage due to naturally enhanced levels of UV-B radiation. J. Exp. Biol. 213, 1967–1975.
Lu, X.Y., Wu, R.S.S., 2005. UV induces reactive oxygen species, damages sperm, and impairs fertilisation in the sea urchin *Anthocidaris crassispina*. Mar. Biol. 148, 51–57.
MacFadyen, E.J., Williamson, C.E., Grad, G., Lowery, M., Jeffrey, W.H., Mitchell, D.L., 2004. Molecular response to climate change: temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulicaria*. Glob. Change Biol. 10, 408–416.
Malloy, K.D., Holman, M.A., Mitchell, D., Detrich, H.W., 1997. Solar UVB-induced DNA damage and photoenzymatic DNA repair in Antarctic zooplankton. Proc. Natl. Acad. Sci. USA 94, 1258–1263.
Maral, J., Puget, K., Michelson, A.M., 1977. Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochem. Biophys. Res. Commun. 77, 1525–1535.
Martorelli, S.R., Fredensborg, B.L., Mouritsen, K.N., 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. J. Parasitol. 90, 272–277.
Martorelli, S.R., Poulin, R., Mouritsen, K.N., 2008. Four trematode cercariae from the New Zealand intertidal snail *Zeacumantus subcarinatus* (Batillariidae). NZ. J. Zool. 35, 73–84.
Mori, T., Nakane, M., Hattori, T., Matsunaga, T., Ihara, M., Nikaido, O., 1991. Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or (6–4)-photoproduct from the same mouse immunized with ultraviolet-irradiated DNA. Photochem. Photobiol. 54, 225–232.
Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124, S101–S117.
Mouritsen, K.N., Poulin, R., 2010. Parasitism as a determinant of community structure on intertidal flats. Mar. Biol. 157, 201–213.
Overholt, E.P., Hall, S.R., Williamson, C.E., Meikle, C.K., Duffy, M.A., Caceres, C.E., 2011. Solar radiation decreases parasitism in *Daphnia*. Ecol. Lett. 15, 47–52.
Perrot-Minnot, M.J., Gaillard, M., Dodet, R., Cezilly, F., 2011. Interspecific differences in carotenoid content and sensitivity to UVB radiation in three acanthocephalan parasites exploiting a common intermediate host. Int. J. Parasitol. 41, 173–181.
Pietrock, M., Marcogliese, D.J., 2003. Free-living endohelminth stages: at the mercy of environmental conditions. Trends Parasitol. 19, 293–299.
Przeslawski, R., Davis, A.R., Benkendorff, K., 2005. Synergistic effects associated with climate change and the development of rocky shore molluscs. Glob. Change Biol. 11, 515–522.
Rastogi, R.P.R., Sinha, R.P., Singh, S.P., Häder, D.P., 2010. Photoprotective compounds from marine organisms. J. Ind. Microbiol. Biotechnol. 37, 537–558.
Reznick, A.Z., Packer, L., 1994. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. In: Abelson, J.N., Simon, M.I., Sies, H. (Eds.), Oxygen Radicals in Biological Systems Part C, Methods in Enzymology. Elsevier Science and Technology Books, San Diego, CA, pp. 357–363.
Rochelle, P.A., Fallar, D., Marshall, M.M., Montelone, B.A., Upton, S.J., Woods, K., 2004. Irreversible UV inactivation of *Cryptosporidium* spp. despite the presence of UV repair genes. J. Eukaryot. Microbiol. 51, 553–562.
Roy, S., 2000. Strategies for the minimisation of UV-induced damage. In: de Mora, S., Demers, S., Vernet, M. (Eds.), The Effects of UV Radiation in the Marine Environment. Cambridge University Press, Cambridge, UK, pp. 177–205.
Ruelas, D.S., Karentz, D., Sullivan, J.T., 2006. Lethal and sub-lethal effects of UVB on juvenile *Biomphalaria glabrata* (Mollusca: Pulmonata). J. Invertebr. Pathol. 93, 192–200.
Ruelas, D.S., Karentz, D., Sullivan, J.T., 2007. Sublethal effects of ultraviolet-B radiation on miracidia and sporocysts of *Schistosoma mansoni*: intramolluscan development, infectivity, and photoreactivation. J. Parasitol. 93, 1303–1310.
Ruelas, D.S., Karentz, D., Sullivan, J.T., 2009. Effects of UVB on interactions between *Schistosoma mansoni* and *Biomphalaria glabrata*. J. Invertebr. Pathol. 101, 140–142.
Sancar, A., 1994. Mechanisms of DNA excision repair. Science 266, 1954–1956.
Schmitz-Hoerner, R., Weissenböck, G., 2003. Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. Phytochemistry 64, 243–255.
Shick, J.M., Lesser, M.P., Dunlap, W.C., Stochaj, W.R., Chalker, B.E., Won, J.W., 1995. Depth-dependent responses to solar ultraviolet-radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. Mar. Biol. 122, 41–51.
Shick, J.M., Dunlap, W.C., 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu. Rev. Physiol. 64, 223–262.
Shick, J.M., Dunlap, W.C., Pearse, J.S., Pearse, V.B., 2002. Mycosporine-like amino acid content in four species of sea anemones in the genus *Anthopleura* reflects phylogenetic but not environmental or symbiotic relationships. Biol. Bull. 203, 315–330.
Sinha, R.P., Klisch, M., Gröningner, A., Häder, D.-P., 1998. Ultraviolet absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae. Photochem. Photobiol. 47, 83–94.
Sinha, R.P., Häder, D.P., 2002. UV-induced DNA damage and repair: a review. Photochem. Photobiol. Sci. 1, 225–236.
Studer, A., Lamare, M.D., Poulin, R., 2012. The effects of ultraviolet radiation on the transmission process of an intertidal trematode parasite. Parasitology. http://dx.doi.org/10.1017/S0031182011002174 (available on CJO).
Studer, A., Thieltges, D.W., Poulin, R., 2010. Parasites and global warming: net effects of temperature on an intertidal host–parasite system. Mar. Ecol. Prog. Ser. 415, 11–22.
Vincent, W.F., Neale, P.J., 2000. Mechanisms of UV Damage to Aquatic Organisms. In: de Mora, S., Demers, S., Vernet, M. (Eds.), The effects of UV Radiation in the Marine Environment. Cambridge University Press, Cambridge, UK, pp. 149–176.
Weber, S., 2005. Light-driven enzymatic catalysis of DNA repair: a review of recent biophysical studies on photolyase. Biochim. Biophys. Acta 1707, 1–23.
Whitehead, K., Karentz, D., Hedges, J.I., 2001. Mycosporine-like amino acids (MAAs) in phytoplankton, a herbivorous pteropod (*Limacina helicina*), and its pteropod predator (*Clione antarctica*) in McMurdo Bay. Antarctica Mar. Biol. 139, 1013–1019.