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Ultraviolet absorbing compounds provide a rapid response mechanism for UV protection in some reef fish

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ABSTRACT

The external mucus surface of reef fish contains ultraviolet absorbing compounds (UVAC), most prominently Mycosporine-like Amino Acids (MAAs). MAAs in the external mucus of reef fish are thought to act as sunscreens by preventing the damaging effects of ultraviolet radiation (UVR), however, direct evidence for their protective role has been missing. We tested the protective function of UVAC's by exposing fish with naturally low, *Pomacentrus amboinensis*, and high, *Thalassoma lunare*, mucus absorption properties to a high dose of UVR (UVB: 13.4 $W * m^{-2}$, UVA: 6.1 $W * m^{-2}$) and measuring the resulting DNA damage in the form of cyclobutane pyrimidine dimers (CPDs). For both species, the amount of UV induced DNA damage sustained following the exposure to a 1 h pulse of high UVR was negatively correlated with mucus absorbance, a proxy for MAA concentration. Furthermore, a rapid and significant increase in UVAC concentration was observed in *P. amboinensis* following UV exposure, directly after capture and after ten days in captivity. No such increase was observed in *T. lunare*, which maintained relatively high levels of UV absorbance at all times. *P. amboinensis*, in contrast to *T. lunare*, uses UV communication and thus must maintain UV transparent mucus to be able to display its UV patterns. The ability to rapidly alter the transparency of mucus could be an important adaptation in the trade off between protection from harmful UVR and UV communication.

1. Introduction

Ultraviolet radiation (UVR, 280–400 nm), specifically short-wavelength UVB radiation (280–315 nm) causes damage to DNA. The formation of cyclobutane pyrimidine dimers (CPDs) between adjacent pyrimidine bases [1] is one of the key consequences of UVB exposure leading to structural changes in the DNA double helix, which can inhibit polymerases thus arresting replication and transcription of the DNA sequence [2]. If left unrepaired, UV-induced DNA damage can lead to mutations [3] and apoptosis of affected cells [4,5]. In fish, the effects of UVR exposure include behavioural changes (e.g. in trout [6] and salmon [7]), damage to tissues of the skin (Japanese medaka [8]) and the brain (Northern Pike [9]), DNA damage [10] MAAs can be found in fish eggs [11], larvae [10] and in the ocular media as well as the external mucus of reef fish [12,13] and can lead to increased mortality (Zebrafish [14], Atlantic cod [15] and Sea Bream [16]).

In the tropics, where levels of UVR are among the highest on Earth [17], the clear and shallow waters around coral reefs allow UVR to penetrate farther than in other aquatic ecosystems [18,19] leading to a high risk of UVB induced DNA damage. Due to changes in ozone levels [20], aerosols, greenhouse gases and cloud cover [21,22] as well as loss of coral complexity due to increased cyclone intensity [23] and severe coral bleaching [24], UVR around coral reefs is likely to continue to increase [22]. These changes can be mediated wither directly by increases in irradiance [21,22], or indirectly by the increases in water clarity and loss of shelter [24].

Protection from harmful UVR in marine organisms can arise from physical barriers (e.g. shells and scales) as well as from UV-absorbing compounds (UVACs) like carotenoids [25] and Mycosporine-like amino acids (MAAs), [26]. Over twenty MAAs with absorbance maxima between 309 and 360 nm have been found in the tissues of hundreds of marine species from all trophic levels and all latitudes, and together with Gadusol (absorbance maximum ~290 nm) cover the UVB and UVA spectrum [27]. This variety of MAA compounds [28] is synthesized by microbes, fungi and plants via the shikimate pathway [29] and alternatively the pentose phosphate pathway [30,31]. Although some of the genes from the shikimate pathway have been found in the sea anemone *Nematostella vectensis* [32] and corals [33], MAAs cannot be synthesized by animals [29] and are likely of dietary origin [34,35]. In reef fish, over 100 species (of 137 studied) show UV absorbing mucus [13], and the tissues where MAAs can be found are as varied as the number of compounds [36]. MAAs have been detected in fish eggs [11], larvae [10] and in the ocular media as well as the external mucus of reef fish [12,13], all tissues which are vulnerable and exposed to UVR.

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MAAs as part of all UVACs in fish mucus are widely recognized to act as sunscreens due to their absorbance properties, the tissues in which they are found [17,27,34,37], and due to their ability to prevent sunburn when topically applied to the skin of mice [38]. MAAs have been shown to protect against cleavage delay in sea urchins [39] and have recently been linked to reduced DNA damage in an intertidal gastropod [40]. In corals and other marine organisms, MAA concentrations in exposed tissues are linked environmental levels of UVR, as reviewed by Shick and Dunlap [26]. There is circumstantial evidence that MAAs may also have a protective function in reef fish [36]. The MAA concentration in the external mucus of reef fish correlates with the levels of UVR in their habitat [41,42]. In captivity, the UVR absorbance of mucus of Hawaiian wrasse that were provided with an MAA-rich diet decreased under conditions that lacked UVR, suggesting that there is an energetic cost to the maintenance of MAA protection in the external mucus [43]. In the presence of UVR and under the same dietary conditions, MAA levels in mucus remained at pre-capture levels. The MAA profiles detected using laboratory methods (HPLC) and the absorbance of whole mucus samples measured in the field both vary between species and geographical locations [41,44]. Mucus absorption has been established as a proxy for MAA concentration [42] in the external mucus of reef fish, and can be easily quantified in the field [45] by measuring UV absorbance in mucus samples.

Here, we address the sunscreen hypothesis, specifically that a higher level of UVACs lead to reduced UV-induced DNA damage. Therefore, fish with different known mucus absorbances were exposed to a high pulse of UVB radiation in order to induce UV-specific DNA damage in the skin. If UVACs, of which MAAs are an integral part, indeed acted as sunscreens, it is expected to find higher DNA damage (CPDs) in fish that have lower levels of UVACs in their mucus. Consequently, we tested for a sunscreen function of UVACs shortly after capture, assuming unchanged mucus absorbance, and after a period of captivity, which is shown to reduce mucus absorbance.

2. Methods

2.1. Location and Experimental Animals

The study was carried out at the end of the Australian summer in March and April 2013 at Lizard Island Research Station (LIRS, 14°40′5″ S, 145°27′47″E). *Pomacentrus amboinensis* (Bleeker 1868, n = 50, SL = 6.9 cm, SD = 0.63 cm) and *Thalassoma lunare* (Linnaeus 1758, n = 30, SL = 11.08 cm, SD = 1.84 cm) were caught at two shallow (water depth <2 m) sites inside the Lizard Island lagoon using hand and barrier nets. Both species were caught in locations that had a maximum depth of 2 m. Both species are active during the day, and occur in the same habitat at Lizard Island. Damselfish such as *P. amboinensis* are highly territorial [46], and although wrasses such as *T. lunare* are more mobile than substrate-associated Damselfish, individuals were observed in the same habitat over several days (C. Braun, pers. observation). It is therefore highly likely that both species experience similar environmental levels of UVR. For the transport back to the research station by boat (<5 min), the fish were held in plastic tanks (23 × 21 × 21 cm) filled with seawater and the lid closed. Upon arrival at the station, the size (SL) of the fish was measured to the nearest mm by transferring individuals to a sealable plastic bag with little seawater and gently placing the bag on a mat that had a ruler taped to it. Only fish of similar sizes (+/− 2 cm difference in SL) were used in the experiments since Zamzow and Siebeck [47] showed an effect of body length on mucus absorbance in *P. amboinensis*. All procedures were conducted with permission from the Queensland Government (General fisheries permit 162472 to U.E.S.), the Great Barrier Reef Marine Park Authority (permit G11/34453.1 to C.B. and U.E.S.) and the animal ethics commission of the University of Queensland (permit SBMS/091/11 to C.B. and U.E.S.).

2.2. Holding Conditions

Test 1 — natural mucus absorbance levels: All fish were randomly assigned to the following treatments and holding conditions. Thirty *P. amboinensis* and twenty *T. lunare* were subjected to the UV challenge (see below) within 24 h of capture. These fish were held in plastic tanks (*P. amboinensis*: 23 × 21 × 21 cm, water depth 20 cm; *T. lunare*: 40 × 30 × 30 cm, water depth 20 cm) with flow through seawater inside an aquarium room of LIRS.

Test 2 — following manipulation of mucus absorbance levels: Twenty *P. amboinensis* and ten *T. lunare* were held in captivity for ten days before being subjected to the treatments of the UV exposure challenge. These fish were different individuals than the fish that were used in Test 1. The aim of this was to manipulate mucus MAA levels to increase their variability within each species and hence manipulate mucus absorbance. Previous studies showed that both, MAA-rich food and exposure to UV is required to maintain high mucus UV absorbance [43]. Here all fish were fed the same diet (MAA rich food (see below and Fig. S1) and relied on the presence/absence of UV for the manipulation of mucus absorbance levels.

The fish were randomly assigned to an experimental tank (same dimensions per species as above), which was linked to the seawater flow-through system and contained a small PVC pipe that served as shelter. *P. amboinensis* were either shielded from natural sunlight (inside an aquarium room of LIRS), or exposed to natural sunlight, with equal number of fish being held in each condition. All ten individuals of *Thalassoma lunare* were held inside an aquarium, shielded from natural sunlight. The aquaria exposed to natural sunlight were placed on two benches which were aligned on an east–west axis to maximize sun exposure during the day and to prevent shading from nearby trees and buildings. On a daily basis, tanks were cleaned to prevent build-up of algae, detritus and leftover food.

The water temperature for each holding condition was recorded every 15 min by an immersed HOBO Datalogger (Onset Computer Corporation, Bourne, MA, U.S.A.), placed in an additional tank, which was also linked to the flow-through system. Mean water temperature for the outside condition was 29.53 °C (SD ± 1.57), which was slightly higher than for the inside condition (28.79 °C, SD ± 0.59) and the water in the Lizard Island Lagoon (28.52 °C, SD ± 0.24; measured by an oceanographic mooring (Australian Institute of Marine Science (AIMS), 14°68'S, 145°45'E at 0.6 m, data provided by Integrated Marine Observing System (IMOS)).

2.3. Diet During Captivity

Fish were fed twice daily with approximately 0.5 g food paste made of frozen prawns and whitebait, supplemented with 10% (*w*/w) ground *Acanthophora spicifera*, a rhodophyte rich in MAAs [48]. The algae were collected (GBRMPA permit G11/33857.1 to LIRS) from a shallow site (water depth <2 m) in the lagoon. The food paste was prepared before the start of the experiment, aliquoted to small portions, and frozen at −20 °C. A new aliquot was used each day and leftover food discarded. Characteristic signatures of eight known MAAs were detected in samples of *A. spicifera* using HPLC-MS (Fig. S1).

2.4. Experimental Treatments (UV Exposure Challenge)

The experiment was conducted inside the aquarium room. Each light treatment (see below) lasted for 1 h. Fish were placed individually in one of five plastic tanks (23 × 21 × 21 cm, water depth 20 cm) connected to the seawater flow-through system.

Within 24 h of capture (test 1), ten *P. amboinensis* were challenged with exposure to a high dose of UVB radiation (treatment "UVB +"). Ten control individuals (treatment "UVB −") were exposed to the fluorescent lights of the room only and handled in the same way as the treatment fish (Fig.). In order to ensure that any changes in UV-