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Distribution of Ultraviolet-Absorbing Sunscreen Compounds Across the Body Surface of Two Species of Scaridae

Elizabeth Cerny-Chipman
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**Distribution of Ultraviolet-Absorbing Sunscreen Compounds Across
the Body Surface of Two Species of Scaridae**

Elizabeth Cerny-Chipman
World Learning, SIT Study Abroad, Cairns, Australia
December 2007
Advisor: Maxi Eckes, PhD Candidate, University of Queensland

Figures and Tables.....	iii
Abstract.....	iv
Acknowledgements.....	v
1. INTRODUCTION.....	1
1.1 Purpose and Research Objectives	3
1.2 Background.....	4
1.2.1 Ultraviolet radiation and its effects.....	4
1.2.2 Mycosporine-like amino acids (MAAs)	5
2. METHODOLOGY	6
2.1 Data Collection.....	6
2.1.1 Study species	8
2.2 Mucus preparation	9
2.2.1 Homogenization.....	9
2.2.2 Drying and resuspension.....	10
2.2.3 Sample weight measurement	10
2.2.4 UV spectra analysis	11
3. RESULTS	11
3.1 Distribution of MAAs over fish body surface	11
3.2 Size of fish and UV absorbance of mucus.....	13
3.3 Species of fish and mucus absorbance	15
3.4 Possible MAA compounds in study fish.....	16
3.6 Background UV results.....	17
4.0 DISCUSSION	17
4.1 Distribution of MAAs across body surface.....	17
4.2 Size of fish and UV absorbance	19
4.3 Species of fish and MAA absorbance	20
4.4 Potential MAAs present	20
5.0 CONCLUSION	21
5.1 investigation of study aims.....	21
5.2 Future research.....	21
5.3 Significance	22
6.0 REFERENCES.....	23

Figures and Tables

Figure 1. Diagram of mucus sampling areas on fish. A is dorsal surface, B is ventral surface, C is caudal area, and D is head. Drawing courtesy of Maxi Eckes.....	7
Figure 2. Removing mucus from a parrotfish using a dull scalpel. Photo courtesy of Maxi Eckes.....	8
Figure 3. <i>S. schlegeli</i> on the left (photo by Dr. Andy Lewis). <i>C. sordidus</i> on the right (photo by Maxi Eckes).....	9
Figure 4. Average UV spectrum of <i>C. sordidus</i>	12
Figure 5. Average UV spectrum of <i>S. schlegeli</i>	13
Figure 6. Average integrated absorbance of <i>C. sordidus</i> organized by fish size class.....	14
Figure 7. Average integrated absorbance of <i>S. schlegeli</i> mucus from dorsal (blue) and ventral (green) areas.....	15
Figure 8. Average absorbance spectra of <i>C. sordidus</i> and <i>S. schlegeli</i>	16

Abstract

Ultraviolet radiation has far-reaching effects in marine ecosystems, but many marine organisms have UV-absorbing compounds that protect them from sun-induced damage. The mucus of coral reef fish has been found to contain mycosporine-like amino acids that absorb UV light from 309-360 nm. Using UV spectrophotometry, we examined whether fish are able to allocate these MAA sunscreen compounds to areas of the body that receive the most UV radiation. We compared absorbance spectra of mucus from the body surface of dorsal, ventral, caudal and head areas in two species of Scaridae (*Scarus schlegeli* and *Chlorurus sordidus*) from Coral Bay, Western Australia. All fish analyzed showed signs of MAAs, and results suggested that fish can increase UV absorbance in mucus over the dorsal area, which receives the brunt of UV radiation. Less radiation was found in mucus from the ventral area, which receives the least radiation. We also analyzed integrated absorbance and standard length and found a relationship in *C. sordidus*. Significance was not found between standard length and integrated absorbance, indicating the need for further study. Overall, results suggest that fish are highly responsive to UV levels in relation to mucus secretion and that MAAs may be ecologically expensive to acquire and utilize.

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1. Introduction

Ultraviolet radiation is an important factor affecting both terrestrial and marine ecosystems (Mason 1998, Jokiel 1980). UV radiation damages DNA, lowers growth rates, releases harmful reactive oxygen species, and can cause cell apoptosis in many different types of marine organisms from phytoplankton to teleost fish (Häder et. al 2005). Coral reef systems are particularly susceptible to damage from UV radiation as they are found at lower latitudes where solar radiation levels are high, water is oligotrophic and the atmosphere is thinner (Dunlap et. al 1998). During the summer months on the Great Barrier Reef, corals in shallow water can experience 30 times the minimum dosage of UV radiation capable of causing sunburn in humans each day (Dunlap et. al 2000). All organisms on the reef must in some way protect themselves from the high levels of UV radiation to avoid the detrimental results of prolonged exposure. For fish in particular, sunburning can be of fatal consequence, so protection is of the utmost importance (Zamzow 2003).

There are four major ways that organisms on the reef avoid UV damage. These strategies include chemical defenses like sequestering UV-absorbing compounds to prevent damage, DNA repair mechanisms to fix the damage that has been done, avoidance behaviors such as seeking shelter or migrating to deeper water for motile organisms, and also gaining protective morphological features such as scales that block some UVR (Dunlap et. al 1998, Eckes in press).

Most studies on chemical defenses like the production and sequestration of UV-absorbing compounds have been conducted on corals (Dunlap et. al 1998, Shick et. al 1996, Häder et. al 2005). Relatively little is known about what vertebrate fish on the reef do for protection, but a study by Zamzow and Losey (2002) showed that 90%

of 137 reef fish species surveyed had chemical compounds that absorbed UV light in their epithelial mucus (2002). These UV-absorbing compounds are primarily made up of a group of structurally similar molecules called mycosporine-like amino acids (MAAs) that absorb light in the 309-360 nm range, which includes much of the UVB and UVA spectra (Dunlap et. al 1998).

Whether or not fish have the ability to allocate these UV-absorbing compounds in mucus to different surfaces on the body is unknown. Fish likely sequester MAAs from their diet as metazoans are unable to synthesize them (Dunlap et. al 1998). Once the UV-absorbing compounds are taken in, sunscreens are processed, possibly converted to structurally different MAAs, and then excreted into the epithelial mucus as a protective method from ultraviolet radiation (Dunlap and Shick 2000).

Depending on the optical clarity of the water, UV radiation can penetrate the water column down to several hundred meters (Tedetti et. al 2007). As UV light passes through the water it is attenuated and eventually it is completely absorbed by particles and dissolved organic carbon in the water (Bancroft et. al 2007). UV radiation on the reef changes on hourly scales based on cloud cover and turbidity of the water (Jokiel et. al 1996), though the ability of fish to respond to changes in UV is on longer time scales on the order of days and weeks (Zamzow 2004).

Regardless differences in total UV attenuation in the water, specific areas of the fish receive more UV radiation than others (Eckes, unpubl). The dorsal surface and pectoral fins of the fish tend to receive the brunt of the UV-radiation, while the underside likely receives less, although reflection off of the bottom influence the underside (Eckes, unpublished). It has already been shown that the levels of absorbance in mucus are responsive to the overall amount of UV-radiation in the

environment overall, indicating a level of control with the secretion of MAAs in mucus (Zamzow and Losey 2002).

For this study we used parrotfish (Scaridae) to determine whether they have the ability to selectively secrete MAA sunscreens into their mucus according to UV exposure levels on the reef. Parrotfish are unusual because they have two kinds of mucus glands each sequestering a different mucus type; the nighttime mucus cocoon and the mucus constantly excreted during the day. It has been shown that the mucus that makes up the cocoon at night does not contain MAAs (Zamzow and Losey 2002). As the cocoon is secreted at night in the absence of UV, the difference in MAA concentration of the two kinds of mucus glands is a response to the UV light level, suggesting some control of MAA secretion on the part of the fish. Such results could mean that parrotfish can also differentially distribute MAAs across the body surface.

1.1 Purpose and Research Objectives

The purpose of this study was to examine the distribution of UV-blocking compounds in mucus on the surface of fishes from the family Scaridae. Research aims stemming from the purpose were as follows:

1. Determine if mucus from different regions of the fish that receive the highest amounts of ultraviolet radiation have higher UV absorbance (and thus more MAAs) than mucus from areas that receive less radiation. Analyze four areas of the fish (dorsal surface, ventral surface, area from caudal peduncle through caudal fin and head area) evaluate the UV absorbance of mucus.
2. Assess whether the size of the fish (standard length) influences integrated absorbance of ultraviolet radiation.
3. Determine possible differences in the mucus make-up (profile) between two species of Scaridae through UV spectral analysis

4. Use UV absorbance spectral peak analysis to determine MAA presence in mucus of parrotfish from Coral Bay in Western Australia.

These aims fell under the PhD research being conducted by Maxi Eckes on MAAs and coral reef fish from Australia. The primary aim of determining the distribution of MAAs across the fish body surface was an aim in Eckes' PhD proposal. Other aims of Maxi's project were examined over the course of the ISP informally.

1.2 Background

1.2.1 Ultraviolet radiation and its effects

Ultraviolet radiation is light in the non-visible area of the spectrum that is of shorter wavelength and higher energy; it ranges roughly from 150 nm to 400 nm (Sparling 2001). Most of the highest energy UV radiation (UVC radiation at wavelengths less than 280 nm) is absorbed by ozone and stratospheric oxygen (Sparling 2001). UVB radiation comprised of wavelengths from 280-320 nm and UVA radiation made up of wavelengths from 320-400 nm are the two significant causes of damage in organisms. UVB is particularly harmful to organisms because its absorption by DNA creates cyclobutane pyrimidine dimers, which do damage to other DNA, lipids and proteins within the body (Häder et. al 2005). It is a common cause of skin cancer in terrestrial mammals (Mason et. al 1998). In marine biota, UVB has large-scale ecological effects like decreasing the overall biomass productivity because it decreases productivity, reproduction, development, and increases the mutation rate in individual organisms (Häder et. al 2003).

The amount of UV radiation received by organisms depends on elevation, latitude, cloud cover, and for aquatic organisms, the optical qualities of the water column (Sparling 2001, Zamzow 2007). UVR levels are also significant in areas like

the Antarctic where the ozone hole allows for increased UV penetration. As anthropogenic inputs to our atmosphere increase and the ozone hole continues to get larger because of the lag time of inputs like CFCs, organisms will have to deal with elevated levels of UV radiation, and it is unknown whether many organisms will be able to adapt quickly enough for survival.

1.2.2 Mycosporine-like amino acids (MAAs)

MAAs are UV-absorbing compounds, but unlike pigments like melanin, MAAs are translucent. The primary structural component of MAAs is a cyclohexenone or cyclohexenimine chromophore with a conjugated nitrogen substituent from an amino acid, amino alcohol or an amino group (Carreto et. al. 2005). The compound works as a UV-protectant for organisms by absorbing incoming UV radiation and dissipating it as heat (Mason et. al 1998). MAAs also have antioxidant properties that help repair damage (Dunlap et. al 2002). There are now 26 primary structural MAAs that have been classified, and they have a range of absorption maxima from 290 nm to 360 nm. The first MAA was discovered by Shinbata in 1969, who observed a water-soluble compound in corals that had a broad absorption maximum at 320nm and called it “S-320” (Dunlap et. al 1998). Since that time, many MAAs have been found in a diverse range of marine taxa and are considered to be ubiquitous (Dunlap et. al 2002).

Though recent studies show MAAs to be highly important for many marine organisms (Zamzow 2003), the response of organisms to UV radiation and utilization of MAAs varies greatly. In fishes, MAAs are found in the mucus, which gets continuously excreted over the body surface (Zamzow and Losey 2002). Because there is a relationship between water depth and UV penetration, fish and other organisms residing in deeper waters receive less UV-radiation than those living closer

to the surface. It has been found that organisms living in deeper water are less tolerant to UV radiation (Shick et. al 1996) and have fewer MAAs in their mucus (Zamzow 2003). Some fish can also respond to changes in UV-radiation by adjusting levels of MAAs in their mucus over short time scales so long as MAAs are available in their diet (Zamzow 2004). The primary producers of MAAs must be photosynthetic organisms that use the light induced Shikimate pathway (Dunlap et. al 2000). On reef ecosystems, zooxanthellae are believed to produce MAAs and transfer them to coral hosts (Shick et. al 1996). Parrotfish feed on coral and cleaner wrasse “cheat” and feed on parrotfish mucus, indicating trophic MAA transfer (Grutter and Bshary 2004).

2. Methodology

2.1 Data Collection

Mucus samples were collected by Maxi Eckes on Ningaloo reef at the Coral Bay Marine Research Station, Western Australia. The collection dates were from October 14 through October 22 2007. Fish were caught using barrier nets and kept in a bucket underwater until just prior to mucus removal. Mucus was removed no more than 10 minutes after fish were brought onto the boat in a holding bucket. Using a dull scalpel blade, mucus was gently taken from four different areas of the fish and frozen in liquid nitrogen at a temperature of -196°C soon after (Fig. 1). The first area, labeled A, was the area directly under the dorsal fin, the second area B was the ventral or underside surface, the third area C was the caudal fin and the caudal peduncle, and the fourth area D was the head (fig. 1). Fewer mucus samples from area D were taken because of the difficulty of getting sufficient enough mucus from the head of the fish. The size of the each fish was recorded (standard length and total length) in order to standardize quantities of mucus.

The fish that were taken ranged in size from 8.7cm to 29.5cm. The fish that collected were of all different life phases and previous published work has shown that the UV absorbance of the mucus increases with increasing fish size (Zamzow et. al 2006).

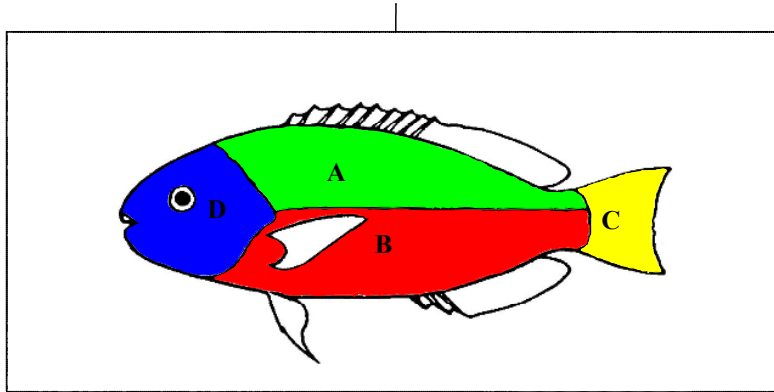


Figure 1. Diagram of mucus sampling areas on fish. A is dorsal surface, B is ventral surface, C is caudal area, and D is head. Drawing courtesy of Maxi Eckes.

In addition to collecting mucus samples, it was also necessary to determine the UV light that was penetrating the water column and reflecting off the sand and reef from the areas where the fish were caught. For this reason, UV transmission data was recorded using an underwater spectrophotometer (Ocean Optics 2000) from three different sites and subsurface directions on the reef. UV transmission was measured at a depth of 1.5m from different directions. Measurements of light were recorded directly from above (the surface), from several subsurface directions (at many angles underwater) and also from the bottom as reflectance off the sand and off different species of coral on the reef flat. Recordings were done on a cloudless day.



Figure 2. Removing mucus from a parrotfish using a dull scalpel. Photo courtesy of Maxi Eckes.

2.1.1 Study species

The two study species were *Chlorurus sordidus*, the Bullethead parrotfish, and *Scarus schlegeli*, the Yellowbar parrotfish. Parrotfish were used as the model species for this study because of the large volumes of mucus they produce that have easily detectible high MAA levels. Parrotfish (Scaridae) are some of the most distinctive fish on the reef because they have numerous sex change strategies, display many morphological differences based on sex and age, and are specialized to feed on corals (Bellwood et. al 1990). There are two different feeding strategies of Scaridae: some like *S. schlegeli* are scrapers, which eat only the live coral tissue and algae, and others like *C. sordidus* are excavators, which feed on the coral skeleton in addition to the living tissue (Steelman et. al 2002).

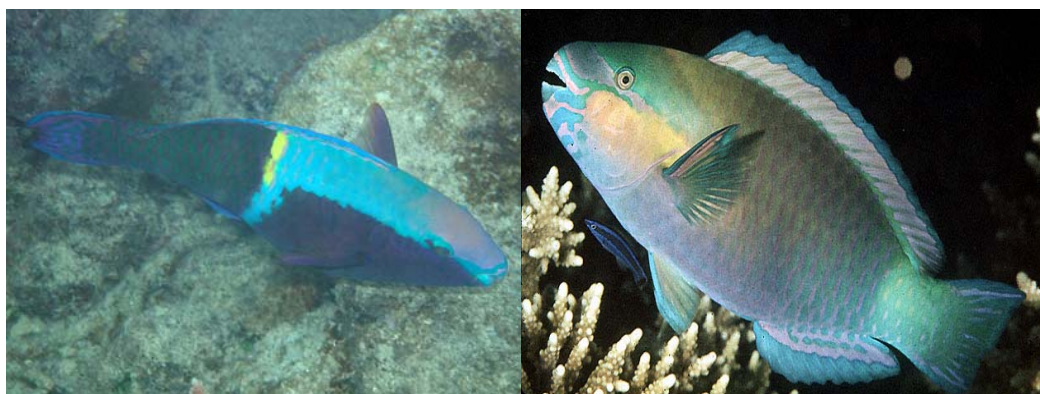


Figure 3. *S. schlegeli* on the left (photo by Dr. Andy Lewis). *C. sordidus* on the right (photo by J.E. Randall).

The two species of parrotfish were ideal for this study because they have abundant mucus that allows for good swabbing across different areas of the fish (Eckes, pers. comm.). In addition, they are diurnal, meaning they are exposed to UV radiation during most of the day, and can occupy shallow habitats; these behaviors put them at direct risk for UVR damage. They feed on corals and algae, which are known to contain MAAs, so diet is not a likely limiting MAA factor.

2.2 Mucus preparation

2.2.1 Homogenization

Because raw samples of mucus are very cohesive and can contain saltwater and other compounds, it is important to homogenize the mucus for consistency throughout the sample. This ensures that sample data will be characteristic of the whole sample and not of any heterogeneity within the sample. Samples were homogenized using the following method:

1. 100% HPLC-grade methanol was added in two 500 μ L aliquots to each sample.

2. With the first addition of methanol, mucus was broken up as much as possible into the methanol. Mucus and methanol were then transferred to a tissue grinder or homogenizer.
3. The remaining 500 μ L were used to remove any remaining sample from the walls or bottom of the tube. Contents of the tube were then transferred to the homogenizer.
4. Sample was ground until homogenous in consistency. The liquid was then divided into two 1.5mL Eppendorf tubes with 500 μ L in each tube. One tube was for HPLC analysis and the other was for the UV spectral analysis.

2.2.2 Drying and resuspension

After samples were divided into two 1.5 μ L tubes, they were dried in a speed evaporation vacuum for several hours to remove excess methanol and saltwater. Once dry, samples needed to be resuspended for use with UV and HPLC analysis. Another 500 μ L of methanol was added to the dry sample, and the sample was re-suspended primarily by the use of a sonicator and eppendorf grinding pestle.

2.2.3 Sample weight measurement

Due to time constraints it was not possible to do a High Performance Liquid Chromatography (HPLC) analysis that would show quantity and type of MAAs in mucus by looking at retention times of the sample as it is run through a column. In order to standardize the UV spectral data of each sample to get data on the relative concentrations of MAAs in our samples, dry weight measurements of the sample were recorded. Dry samples were measured by zeroing the scale using a test tube of the same type and make number, and then weighing the sample in its test tube.

2.2.4 UV spectra analysis

Using spectrophotometry is an accepted method for measuring the absorbance of a sample over a set of wavelengths. For our samples, we were looking at the absorbance of mucus samples over UV wavelengths (280-400 nm). The resuspended aqueous samples were prepared for UV spectral testing in the following method:

1. To make a starting dilution of 20%, 160 μL of 100% pure HPLC-grade methanol was added to 40 μL of resuspended and mixed mucus samples for a total sample volume of 200 μL . The samples were first done at a dilution of 20% as an estimate to find the optimum scale for absorbance.
2. Samples were vortexed then loaded in duplicate onto a standard 96-well plate in sets of 11 plus two blanks in order to minimize evaporation of samples in wells during loading. Total volume in each well was 200 μL .
3. A spectrum between 280-400 nm was recorded from each sample in increments of 2 nm. There was a temperature regulation of 23°C to minimize evaporation during the run. Samples were auto-mixed for 5 seconds prior to reading. All samples were run on a SpectraMax M2 spectrophotometer supplied by Molecular Devices.

3. Results

3.1 Distribution of MAAs over fish body surface

After recording UV spectra for approximately 10 individual fish of each species and standardizing the absorbance using dry weight measurements, it appeared that there were visual differences in mucus UV absorbance graphs between the different body surfaces of the fish. Mucus from the dorsal surface of the fish—the

area receiving the highest UV radiation—consistently had the highest absorbency. The mucus from the caudal fin and peduncle of the fish had the second highest UV absorption. Mucus from the ventral area and from the head both had relatively low absorbencies for *C. sordidus* (fig. 4) and *S. schlegeli* (fig. 5).

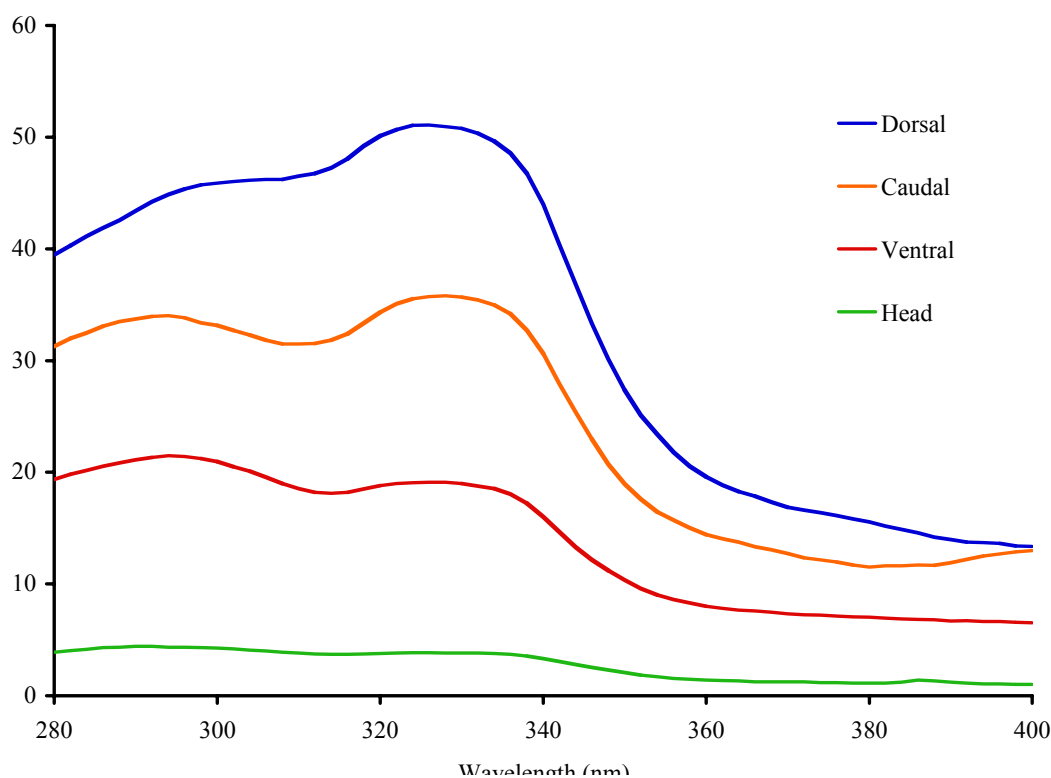


Figure 4. Average UV absorption spectrum of *C. sordidus*.

Because no HPLC analysis was done, the exact concentration of MAAs in the mucus samples is still unknown. Due to the relatively small dataset (around 10 fish of each species) the variation in the absorbance spectra was large, with high variation in UV absorbance between individual fish of the same species. No statistics were conducted on the dataset at this time, but large standard deviation suggest that further research would need to be conducted to quantify the relationship between MAAs in mucus of different body surfaces and the among of UV radiation they receive.

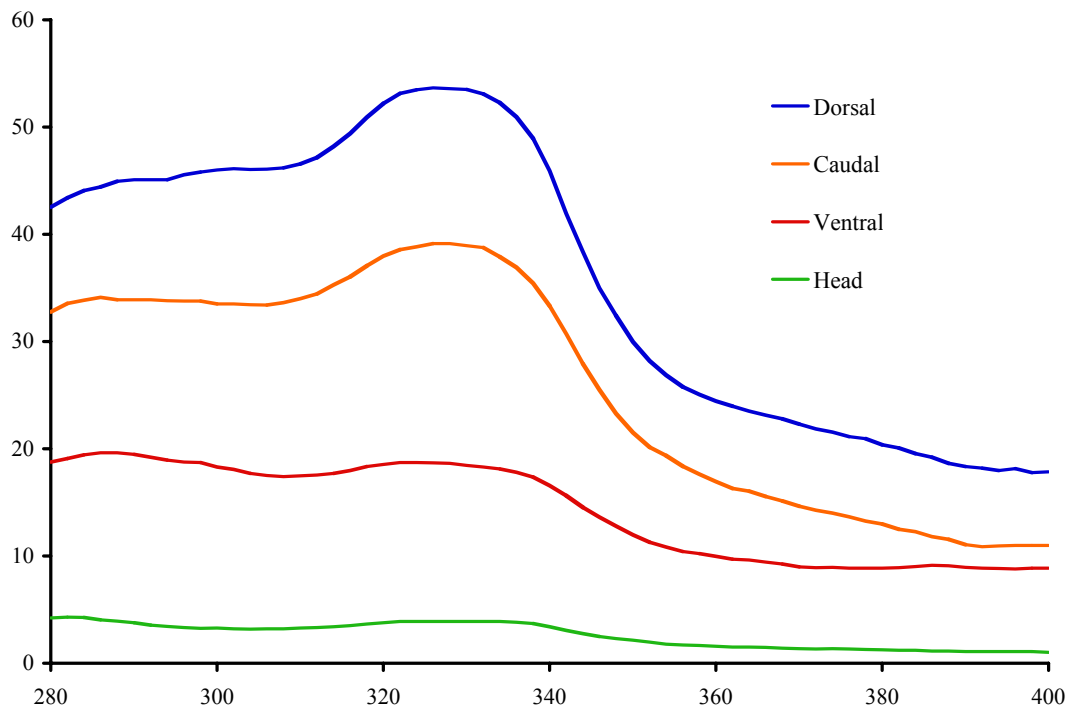


Figure 5. Average UV absorption spectrum of *S. schlegeli*.

3.2 Size of fish and UV absorbance of mucus

Fish were categorized into four size classes based on standard length. These categories were 15-18cm, 18+ to 21cm, 21+ to 24cm and 24+ to 27cm. Data shows that for dorsal and ventral areas of *C. sordidus*, mucus absorbance changes with fish size (fig. 6). Results are not significant between any size classes but 15 to 18cm and 24+ to 27cm, but this may be due to large the intraspecific variation between samples.

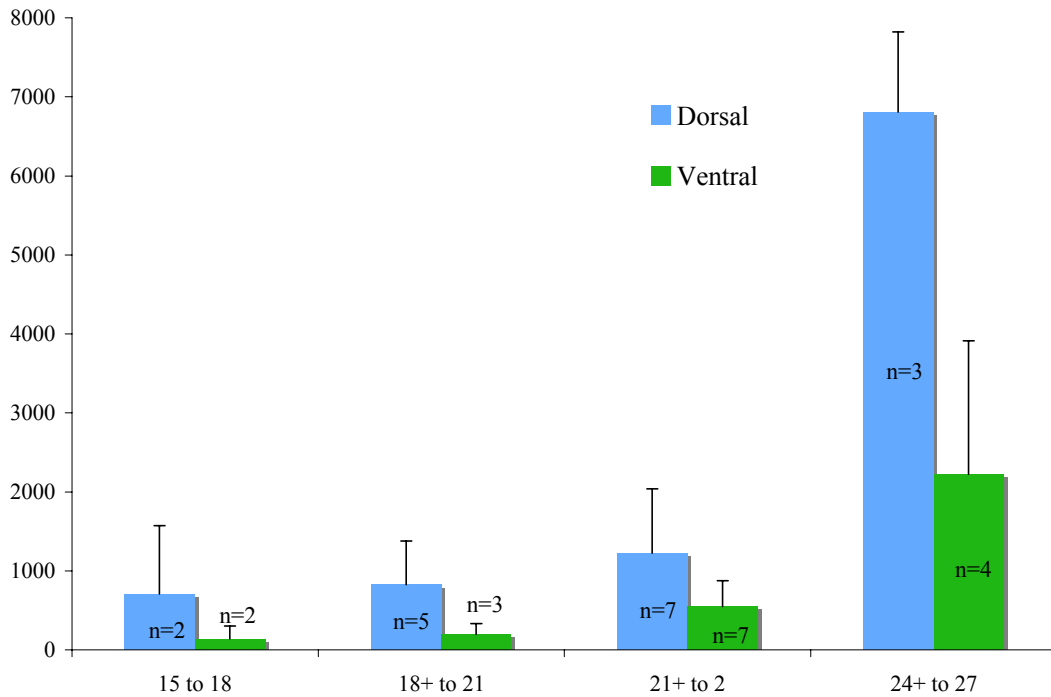


Figure 6. Average integrated absorbance of *C. sordidus* mucus from the dorsal (blue) and ventral (green) areas of the fish organized by fish size class.

Integrated absorbance (total area underneath the spectral curve) was used to represent the findings. As the size of the fish increases, the integrated absorbance increases as well, correlating well to the study done by Zamzow and Siebeck in 2006 on damselfishes that found UV absorbance was related to fish size. For *S. schlegeli*, the relationship between size class and integrated absorbance was less consistent (fig. 7). For the size class 21+ to 24, the ventral surface has higher UV absorption than the dorsal surface, however this result could be related to small sample size for *S. schlegeli* in that size class. There was no apparent correlation for either species of fish between size and integrated absorbance of mucus from the caudal area and the head, and so the data from the caudal area and the head were not included in the data analysis. Though no statistical tests were run to show significance, error bars suggest that results are inconclusive, but a visual trend is still apparent in the data.

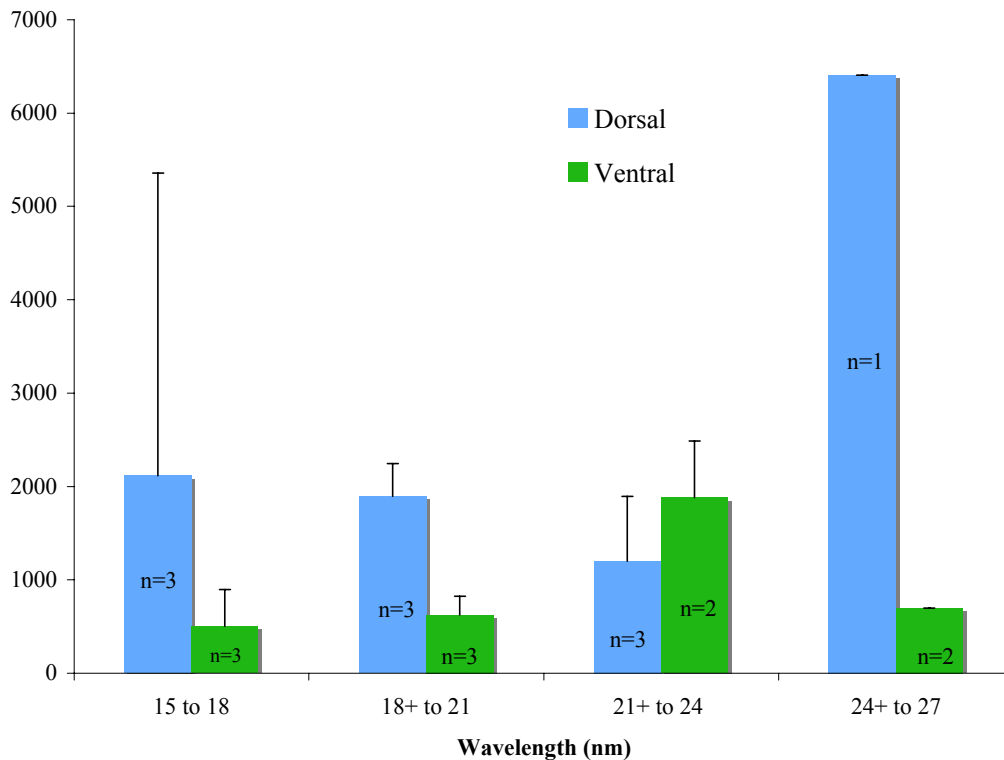


Figure 7. Average integrated absorbance of *S. schlegeli* mucus from dorsal (blue) and ventral (green) areas.

3.3 Species of fish and mucus absorbance

Though different species are known to show different UV spectra and different combinations of MAAs, the data shows that the absorbance for both *C. sordidus* and *S. schlegeli* were similar (fig. 8). It is possible that different MAAs are present because the two species had spectra that varied slightly, with the *S. schlegeli* having a small peak slightly after 280nm and *C. sordidus* having a small peak nearer to 300nm. With just the UV-spectra analysis, it is not possible to show exactly what different MAAs could be present in the two species, but the spectra do show that absorbance is similar and thus the level of sun protection and concentration of MAAs is similar. Because it is believed that fish acquire MAAs from dietary sources and both species were caught on Ningaloo reef, the spectra could look very similar

because the fishes are utilizing the same food sources containing the same MAA compounds.

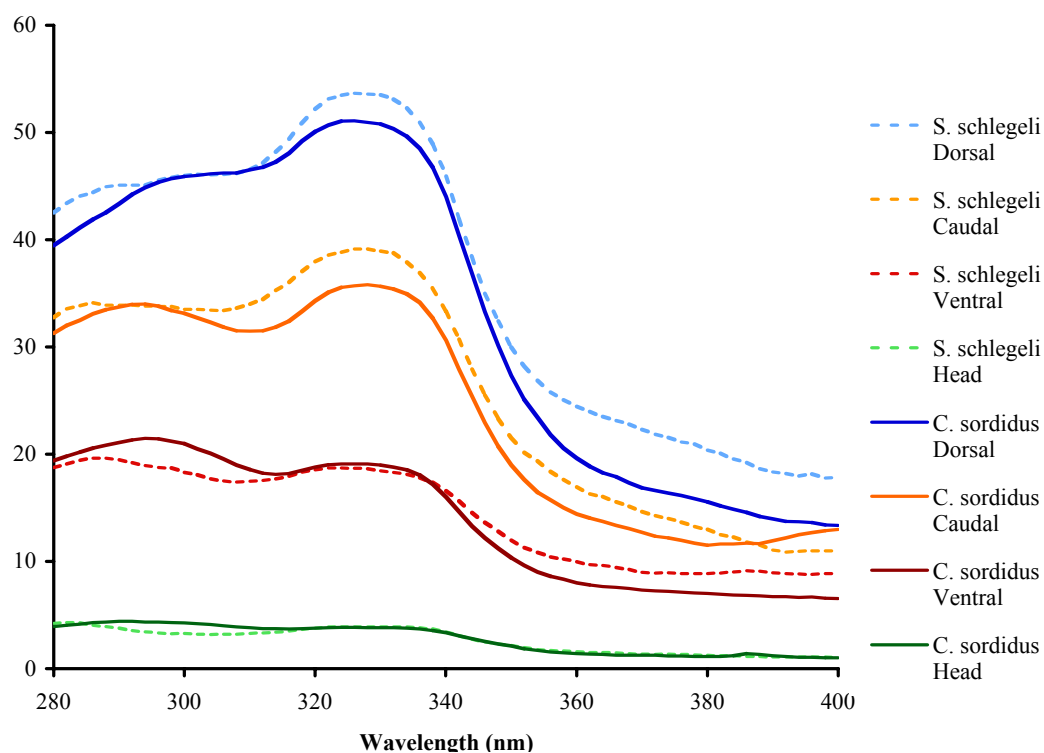


Figure 8. Average absorbance spectra of *C. sordidus* and *S. schlegeli*. Dotted line indicates *S. schlegeli* while solid line is *C. sordidus*. Peak around 330nm is likely asterina-330, a MAA. Peak around 290nm is likely a gadusol.

3.4 Possible MAA compounds in study fish

The primary MAA apparent in the *S. schlegeli* and *C. sordidus* from the UV spectral analysis is asterina-330, a common marine MAA (Dunlap et. al 1998). The peak absorption of asterina-330 is 330 nm, which is clearly a peak in the spectra of both fish species (fig. 8). There are several other MAAs that have maxima in the 320-330 nm absorption range, including palythine, which has been found in other reef fish (Zamzow et. al 2006). Palythine may also be present in the samples of mucus from *C. sordidus* and *S. schlegeli* but is being combined with asterina-330 peak. Though we were unable to analyze the absorption maximum of the mucus from each species, it appears to peak near 326 nm, suggesting a combination of palythine and asterina-330

(Zamzow et. al 2006). Gadusols are compounds similar to MAAs that have a maximum absorbance below 300nm (Dunlap et. al 2002), and a peak around 294 nm thus indicating its presence in the two species of Scaridae.

Another nearly ubiquitous MAA in marine ecosystems is mycosporine-glycine, which has an absorption maxima (peak) at 310 nm (Dunlap and Shick 1998). Based on the UV spectra of the two fish, there is no peak at 310 nm, indicating a lack of mycosporine-glycine, but further HPLC analysis would be needed to definitely confirm the compound's presence or absence. In addition, palythene ($\lambda_{\text{max}}=360\text{nm}$) is a MAA that has been found in Scaridae from the east coast of Australia (Eckes in press). It did not appear to be present in any of the Scaridae sampled from Western Australia.

3.6 Background UV results

UV data taken at three sites on the reef indicate that by far the most harmful and powerful rays of UV radiation come from directly above, confirming that the dorsal surface is the most exposed surface in the fish. While there was some reflected UV from sand and reef subsurface, it was negligible. UV reflectance off of sand and coral surfaces were also similar, indicating that there is no great difference in UV received based on microhabitat for the two species of parrotfish, though microhabitat can be important overall (Bellwood et. al 1990).

4.0 Discussion

4.1 Distribution of MAAs across body surface

Our research shows that fish may have the ability to not only differentially sequester MAAs (Zamzow and Losey 2002), but also differentially excrete MAAs on

the body surface of parrotfish. As hypothesized, the dorsal area of the fish receives the greatest amount of UV radiation, and thus the dorsal mucus had the highest concentration of MAA compounds. The ventral area received less UV radiation as the reflection of UV from the sea floor was negligible, and less absorbance indicates fewer MAAs. The caudal fin probably receives a level of UVR in-between that of the dorsal and ventral surfaces, and has an intermediate absorbance. Overall, this suggests a relationship to the amount of UV light and the amount of MAAs. This relationship has already been well established in whole fish; a study by Zamzow and Losey found that the wrasse *Thalassoma duperrey* changed MAA levels in mucus based on both natural UV light changes and experimental UV changes (2002), but not over different body surfaces.

The lowest concentration of MAAs was found in mucus covering the head of the fish. Mucus covering the head may lack many MAAs because important structures like the eyes already contain the compounds internally for protection (Mason 1998), and additional protection is less necessary. The head is also a relatively small area, and excluding the very top part of the head, it may receive intermediate amounts of radiation. In parrotfish, the glands that secrete mucus are located behind the head; it is believed that the opercular gland secretes the night mucus cocoon and the gill mucus gland secretes the daily mucus (Zamzow and Losey 2002, Rumney unpubl.). While the fish swims, the mucus is constantly secreted and distributed across the fish body surface. We hypothesize that the forward swimming motion of the fish makes it difficult to secrete a lot of mucus over the head. In addition, the small number of fish sampled for each area (and particularly for areas like the head), suggest that further research needs to be conducted to better detect the relationship between the mucus location and MAA concentration.

The increased UV absorbance and MAA concentration on the dorsal surface of the fish and the decreased MAA concentration in mucus over the ventral area is visually different on graphs, which could indicate that MAAs are ecologically expensive to sequester. In holothuroids and other marine organisms MAAs are found primarily in epidermal cells or in gonads, indicating that the UV-absorbing compounds are located preferentially in areas that are most sensitive to radiation and damage (Dunlap et. al 1998). It is believed that MAAs are not limited in most marine environments (Zamzow 2004), so there must be another ecological pressure that causes differential secretion. The differential secretion of MAAs could arise from the water-soluble nature of MAAs, causing a constant need for replacement. Differential secretion could also stem from the fact that MAAs require a certain amount of processing before secretion (Dunlap et. al 1998).

4.2 Size of fish and UV absorbance

Because there is an established relationship between size and the amount of UV-absorbing compounds in fish we categorized the experimental data into size classes. For *C. sordidus*, size did affect the integrated absorbance with fish of larger size having a high UV absorbance in their mucus on the dorsal surface. Why this occurs is not clear, but it could relate to the fact that MAAs are dietary-acquired compounds, so the size of the fish may indicate some dietary behavior that allows for more MAA ingestion because of a larger food intake. Large fish could either be more efficient at processing MAAs or better at eating foods that are rich in MAAs (Zamzow and Siebeck 2006). The relationship between size and integrated UV absorbance of fish mucus remains dubious because conflicting results have shown the correlation to be true in some fish species (Zamzow 2004) and not in others (Zamzow and Siebeck 2006). The data represented here for *S. schlegeli* did not have this

positive relationship between size and absorbance for the 21+ to 24 size class however for all the other size classes the relationship was positive for this species. Again, the variation was large, and no significance was found, but further data had yet to be analyzed in the experiment, increasing sample size.

4.3 Species of fish and MAA absorbance

Overall, the absorption spectra of *C. sordidus* and *S. schlegeli* suggest that the fish have similar concentrations of MAAs and similar combinations of MAAs that provide broad UV spectrum protection. Both species of fishes showed a differential secretion of MAAs across the body surface, which indicates that fish protect themselves more in areas that receive increased radiation exposure. The similarity in absorption spectra could result from a similar dietary regime; however because *C. sordidus* is an excavator and *S. schlegeli* is a scraper there must be some fundamental differences in diet. In addition, differences in microhabitats of the fish could cause absorbance differences because it has been shown that excavators and scrapers have different behaviors and feeding specificity (Bellwood et. al 1990). Any potential differences would best be resolved using HPLC analysis and a greater analysis of species.

4.4 Potential MAAs present

Though UV analysis is not the ideal method of determining MAAs, UV spectra showed the definite presence of asterina-330 and the possible presence of palythine. Gadusols, which are believed to be the evolutionary precursors of MAAs (Shick 2002) were also present. Fish often use a combination of MAAs in order to get a broad-spectrum range of protection (Jokiel 1980), and this appears to be the case in our study fish mucus as well. HPLC analysis will further elucidate MAAs present.

5.0 Conclusion

5.1 investigation of study aims

With regards to the primary study aim of determining the distribution of MAAs across the body surface of Scaridae, our data suggests that there is differential secretion of mucus based on UV radiation exposure, but that further research is needed. The data correlates with size class between two size classes of *C. sordidus*, but was not a factor in *S. schlegeli*. The difference in UB spectral absorbance between the two species was minimal, but HPLC analysis could determine farther differences. The only two UV-absorbing compounds that were definitively found in the mucus of both species were asterina-330 and a gadusol with a peak absorbance of 294 nm.

5.2 Future research

Beyond the research being done to see if there is a possible way to adapt mycosporine-like amino acids to be suitable for human use (Dunlap et. al 2000), there remains a great gap of knowledge on MAA compounds, particularly in fish. The relationship between marine biota and UV radiation deserves more intensive study. More research should be done on the distribution of MAAs across the body surface and whether fish can mediate what MAAs occur in mucus over different areas. Another exciting area of research is assessing the movement of MAAs in the ecosystems, particularly in Scaridae and the cleaner wrasses that sometimes “cheat” and consume the Scarid mucus (Grutter et. al 2003). Further research should be done investigating the ecological role or sequestering and secreting UV-absorbing compounds.

Additional research should seek to experimentally test if concentrations of MAAs are changed across the body surface if the source of UV light is located in a

different location. Putting a UV light source below the fish and seeing if the consequent response is an increase in MAA sunscreens on the ventral surface could achieve this.

5.3 Significance

As the ozone hole increases, as it is predicted to do for decades to come, organisms like *C. sordidus* and *S. schlegeli* must be able to prevent or fix UV damage or face population declines (Dunlap et. al 2000, Häder et. al 2005). Unlike DNA repair mechanisms, which repair damage and have a limited capacity to fix mutations, sunscreen compounds like MAAs prevent damage before it occurs. It is essential to understand how fish utilize these compounds to understand what sort of pressures they face as UV radiation increases.

The uptake and sequestration of MAAs is important to human populations as well. A sixth of the world's population relies on productivity of oceans to obtain 1/3 of their animal protein (Häder et. al 2005). If this productivity decreases because of UV it will have secondary effects for global populations. In addition, human populations rely heavily on fisheries and aquaculture where sunburn is a typical problem, which could be remedied by understanding how to provide fish with the means sequester and secrete MAAs (Zamzow 2004). Perhaps most importantly, as anthropogenic inputs to the atmosphere continue and temperatures rise, UV-induced damage will lower marine productivity and lessen the capability of global oceans to act as a CO₂ sink (Häder et. al 2005). MAAs have far-reaching impacts on marine ecosystems from Antarctica to California, and this consequently affects human activities and use of our global oceans.

6.0 References

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