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UV radiation influences covering behaviour in the urchin *Lytechinus variegatus*

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Many species of sea urchins cover their bodies with a variety of materials. One hypothesis for this behaviour is that the urchins are reducing their exposure to UV radiation. The effect of UV radiation on the covering behaviour of twelve sea urchins, *Lytechinus variegatus*, was recorded and the shells used as covering materials were quantified. During UV exposure, urchins used significantly greater numbers, areas and masses of shells than did urchins during non-UV exposure. Results suggest that not only were *L. variegatus* covering in response to UV exposure, but they were also making distinct choices regarding preferred covering items. Unlike in previous studies, the urchins did not simply choose familiar objects or even the lightest objects; rather, when exposed to UV radiation they selected intermediate-sized objects, perhaps demonstrating a trade-off between the energetic costs of carrying objects and the physiological costs of exposure to UV radiation. While there appear to be many reasons for why urchins cover, these results indicate that urchin covering is non-random and has important functional significance.

INTRODUCTION

Covering behaviour ('masking', Richner & Milinski, 2000) in sea urchins is defined as picking up items with the podia and spines, and placing them on the aboral surface. Covering behaviour has been noted for over 100 years (Verling et al., 2001) and has been explicitly studied in several species, including *Strongylocentrotus droebachiensis* (Adams, 2001), *Paracentrotus lividus* (Crook & Barnes, 2001) and *Toxopneustes roseus* (James, 2000).

Numerous hypotheses explaining this behaviour have been proposed, including protection against desiccation (*Echinus esculentus*; Orton, 1929), holding food (*Evechinus chloroticus*; Dix, 1970), a by-product of walking (*Centrostesphanus longispinus*; Dambach & Henschel, 1970), stability during wave action (*T. roseus*; James, 2000), defence against predation (*S. intermedius*; Agatsuma, 2001) and protection from UV radiation (*S. purpuratus*; Verling et al., 2004; for a more complete review, see Crook, 2003). Counter examples for each of these hypotheses can also be found. For example, many sea urchins cover, yet are located subtidally, excluding the explanation of covering to avoid desiccation, and experience little wave action, excluding the explanation of providing stability against wave action (Adams, 2001). If covering behaviour can be explained as the urchin simply holding food until it is consumed, urchins would not be commonly observed covering with inedible items (Crook et al., 1999; Adams, 2001; Barnes & Crook, 2001; Verling et al., 2001, 2004). Covering as a by-product of walking was ruled out for *S. droebachiensis*, since the amount of covering materials used differed between different light conditions (Adams, 2001). Predation was not found to be an important factor for covering by *P. lividus* (Crook & Barnes, 2001).

Covering behaviour is likely to have many explanations (Crook et al., 1999; Adams, 2001; Barnes & Crook, 2001; Crook, 2003; Verling et al., 2004). The hypothesis with the broadest support is covering as a defensive response to light. This hypothesis has been well supported; urchins may avoid light altogether by seeking out crevices during the day and only venturing out to graze at night (*Centrostephanus rodgersii*; Andrew, 1993; *Diadema antillarum*; Ogden & Carpenter, 1987). Those that are day-active cover more frequently during diurnal hours, during the summer months, and when they have migrated to the top of boulders near the water's surface (*P. lividus*; Barnes & Crook, 2001; Crook & Barnes, 2001). UVA and UVA+B radiation caused more urchins to cover than white light or no light (*P. lividus*, Verling et al., 2001), and more items were used to cover in treatments with photosynthetically active radiation (PAR)+UVA+UVB than in treatments excluding UVB and treatments excluding UV radiation altogether (*S. droebachiensis*; Adams, 2001). While studies have shown that sea urchins also will seek shade (Adams 2001), the ability to cover while foraging could further reduce absorbed UV radiation.

Although the effects of UV radiation on sea urchins are not entirely known, UVA and UVB are damaging to marine organisms. For example, UV radiation indirectly causes cellular damage by oxidizing proteins, DNA and membrane lipids (Adams & Shick, 2001), can kill fish eggs (Villafane et al., 2001), phytoplankton and zooplankton (Hader et al., 1998), and is thought to cause developmental problems in frogs (ibid). This evidence suggests that urchins could be

Table 1. *Covering materials (shells) available to urchins.*

Shell Size	Mean area (cm^2)	Area range (cm^2) low	high	Mean mass (g)	Area:mass (cm^2/g)	% of urchin's surface covered by 1 shell*
Small	1.51	1.47	1.55	0.315	4.80	4.50
Medium	2.88	2.76	3.00	0.693	4.16	8.58
Large	6.03	5.75	6.32	1.67	3.61	18.0
Extra Large	15.2	14.0	16.3	4.22	3.59	45.2

*Calculated as mean projected area for the shell size divided by the mean projected area for the 12 urchins (33.58 cm^2)×100.

covering to protect themselves from the damaging effects of UV radiation.

If covering behaviour serves, in part, to shield sea urchins from damaging UV radiation, then it is possible that sea urchins actively select covering materials. In previous studies, *P. lividus* selected shells and leaves, avoiding novelty items (Crook et al., 1999), and in a separate study, selected leaves rather than shells or stones (Crook, 2003). These results suggest that urchins select objects that are both familiar and light in weight, reducing the energetic costs of covering.

In the present study, we examined the effect of UV radiation on the covering behaviour of *L. variegates*, the ability of these urchins to choose covering materials based solely on the materials' area and mass (all materials were shells), and the time course of their covering behaviour through simulated day-night light cycles. Individuals were observed during four experimental conditions, light and dark periods of UV and non-UV radiation, to answer the following questions about covering behaviour in response to radiation: (i) do individual urchins differ in their covering behaviour? (ii) does radiation affect the numbers, area, or mass of shells used in covering? (iii) are urchins selective in their use of shells? The answers to these questions allowed us to evaluate the role of UV radiation in the covering behaviour of *L. variegates*, and assess whether these urchins actively select objects based on area, mass and UV-blocking properties.

MATERIALS AND METHODS

Subjects

Twelve *Lytechinus variegatus* (Lamarck, 1816) were purchased from Gulf Specimen Marine Laboratories (Panacea, Florida). Each urchin was marked with non-aluminium nail-polish for individual identification. Mean (±SEM) body diameter (excluding spines) was 6.77 ±0.19 cm, mean body height was 3.60 ±0.19 cm, mean body mass was 84.7 ±5.36 g (range 55.3–116.8 g) and mean projected area was 33.58 ±1.69 cm^2 (range 31.89–35.27 cm^2).

Apparatus

An experimental tank (120.8×30.5×43.4 cm deep) was divided with two screen partitions into two experimental sections, separated by a 22-cm gap. Water depth was 22.9 cm above the sediment. One lift tube with an airstone and one external mechanical and chemical filter were provided for each section. Each section contained an equal amount of substrate (crushed oyster shell) spread in an even layer. No hiding places were provided. Equal and plentiful numbers of shells of four size-classes (small, medium, large and extra large) were provided as covering materials (see Table 1).

Two lamps were placed 30.5 cm above the sediment in each section. In the UV section, two 15-watt ZooMed Repti Sun 5.0 light bulbs, which emitted 5% UVB (280–315 nm) and 20% UVA (315–400 nm) radiation, were used (Aas & Hojerslev, 2001). In the non-UV section, two 15-watt ZooMed Ocean Sun 10,000 K bulbs, which emitted all wavelengths of light except UVB rays and trace amounts of UVA rays, were used (see ZooMed specifications at www.zoomed.com). The room containing the experimental tank was lit with overhead fluorescent bulbs (no windows). Both the overhead lights and the tank lamps were connected to a simple household timer on a 12L:12D cycle (light 0900–2100 h). A small flashlight was used for observations during the dark period and shells were quantified in 6 minutes or less.

Tank water was prepared from Instant Ocean brand salts (Aquarium Systems, Inc., Mentor, Ohio, USA) mixed into reverse-osmosis filtered water. Water parameters were measured daily. Throughout the experiments, the temperature of the water was 21–22°C, the salinity was 33–34 ppt, the ammonia and nitrite levels were zero, the nitrate levels ranged from 15–20 ppm and the pH was 8.3.

Urchins were fed two grams of dried Ocean Nutrition brand red algae (Ocean Nutrition Canada, Dartmouth, Nova Scotia, Canada) every other day.

Procedure

Six urchins were placed in each experimental section. The urchins were given two days to acclimate to the tanks. The room's overhead lights, but not tank lamps, were on for 12 hours/day during the acclimation period. In the first one-week trial, UV lamps were turned on over one arbitrarily selected section of the experimental tank, and non-UV lamps were turned on over the other section. In the second one-week trial, which followed immediately thereafter, the UV lamps were exchanged with the non-UV lamps.

During each trial, urchins were observed twice per day at different times. Over the course of the week, the urchins were observed twice (on two different days) at the beginning (0930 h), middle (1200 h), and end (2030 h) of the light period, and twice (on two different days) at the beginning (2130 h), middle (0000 h), and end (0830 h) of the dark period. For every selected observation time, covering behaviour was quantified by counting the number of shells used of each shell size-class by each urchin. There was no overlap in the projected areas of the shells of different shell

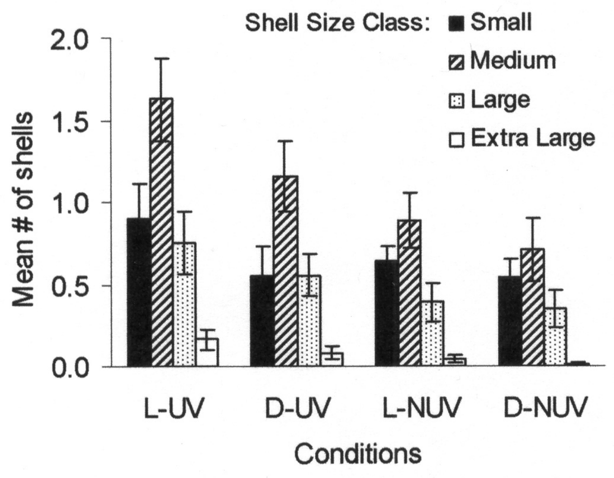

Figure 1. Mean (±SEM) number of shells used in each of the four experimental conditions. Urchins used significantly more shells of all size-classes combined in the light UV condition (L-UV) than in the non-UV conditions (L-NUV and D-NUV; Fr=17.3, N=12, k=4, P<0.0001). Urchins were selective in their use of shells, using medium shells significantly more often than shells in other size-classes ($F_{3,176}$=31.72, P<0.0001). There was no significant interaction between shell size and experimental condition (F=1.00, N=12, k=4, P>0.05).

size-classes (Table 1). Shell size-classes were distinguishable by eye. The means for each pair of same-time observations were calculated and used in all statistical analyses.

Data analysis

Observations were grouped into four conditions: light UV (L-UV), dark UV (D-UV), light non-UV (L-NUV) and dark non-UV (D-NUV).

To determine the mean projected area of shell coverage, a sample of shells of each size-class (small, medium, large and extra large) were processed using ImageJ (ImageJ analysis software available on the web; see Rasband, 2003). Shell outlines were traced, and projected areas of shells were computed. These same samples of shells were then weighed. The mean projected area and weight for each shell size-class was then calculated. Small shells offered the greatest projected area per unit mass, followed by medium shells, and then large and extra large shells (Table 1).

For individual urchins, covering area was computed by multiplying the mean number of shells of each size-class used in each experimental condition by the mean projected area for that shell size-class. Total shell covering area was computed by summing the shell areas for the four size-classes for each condition. The mean mass of shell coverage per urchin was calculated similarly.

Statistical analyses were performed using a chi-square test for r×k tables (individual variation), a Spearman rank order correlation (urchin mass in relation to total number of shells used), Friedman's non-parametric analysis of variance, ANOVA two-factor without replication, and ANOVA two-factor with replication (Siegel & Castellan, 1988; Zar, 1999). All urchins were exposed to all four treatments (L-UV, D-UV, L-NUV, D-NUV) to control for individual variation.

RESULTS

(i) Do individual urchins differ in their covering behaviour?

There were significant differences in the total number of shells used by different urchins during the course of the experiment (χ^2=68.6, df=11, P<0.001), with the urchin that used the most shells holding over four times as many shells over the course of the experiment as the urchin that used the least number of shells (median 27.75, range 11.0–52.5). No evidence was found for a relationship between urchin size (mass) and total number of shells used in the course of the experiment (r_s=0.27, df=11, P>0.25).

(ii) Does radiation affect the numbers, area, or mass of shells used in covering?

There was a significant difference in the total number of shells (all shell sizes combined) used per urchin between the four conditions (Fr=17.3, N=12, k=4, P<0.0001; Figure 1). Multiple comparison analyses revealed that urchins used significantly more shells in L-UV conditions than in the non-UV conditions (L-NUV, D-NUV). Shell use was not significantly different in D-UV from shell use in any of the other experimental conditions, and shell use in L-NUV and D-NUV were not significantly different from each other. Thus, urchins exposed to UV radiation dropped shells during the dark period (D-UV) such that the number of shells they used in D-UV was comparable to the shells they used in both L-NUV and D-NUV conditions.

There were also significant differences in both the mean projected area and the mean mass of coverage used by urchins under the different conditions (area: $F_{3,33}$=22.03, P<0.0001; mass: $F_{3,33}$=20.54, P<0.0001). Multiple comparison analyses revealed that both projected area and mass were significantly greater during L-UV conditions than all other conditions, significantly greater in D-UV than L-NUV and D-NUV, and not significantly different between L-NUV and D-NUV conditions.

(iii) Are urchins selective in their use of shells?

Urchins were selective in their use of shells ($F_{3,176}$=31.72, P<0.0001; Figure 1). Multiple comparison analyses showed that urchins used medium shells for covering significantly more often than other shell sizes. There was no significant difference between the number of small and large shells used, but both were used significantly more than extra large shells. There was no significant interaction between shell size and experimental condition ($F_{9,176}$=1.00, P>0.05).

In UV conditions, small shells were used significantly more often at 0930 h than at other times of the day (average 1.33 vs 0.61; Fr=20.12, N=12, k=6, P<0.01). The use of shells in other size-classes and in non-UV conditions did not differ between the different times of the day.

DISCUSSION

Individual urchins (*Lytechinus variegatus*) differed in the extent of covering behaviour they exhibited, supporting previous observations that significant variation exists between individuals (Crook et al., 1999; Crook, 2003). Unlike previous studies (*Paracentrotus lividus*; Crook et al., 1999), no evidence was found for a relationship between an individual's size and its covering behaviour.

Covering behaviour was not a response to light intensity. Light intensity information was obtained after the study was completed; non-UV bulbs provided greater light intensity (10,000K) than did UV bulbs (5500K).

UV radiation increased covering behaviour. Urchins used significantly more shells (number, area and mass) for covering when exposed to UV radiation (L-UV) than when exposed to non-UV radiation (L-NUV). Richner & Milinski (2000) questioned the importance of UV radiation to covering behaviour because UV radiation attenuates rapidly in water and urchins found in waters below the reach of UV radiation have been observed covering. UVA and UVB radiation can penetrate seawater at least to a depth of 8.3 m (Aas & Hojerslev, 2001) and from one to several metres in organically rich coastal seawater (Adams & Shick, 2001) where *L. variegates*, among other urchins, resides (Humann, 1992). Our study shows that where UV radiation penetrates, it is an important factor in urchin covering behaviour.

Urchins did not use shells randomly, supporting previous studies showing that urchins have the ability to choose items they use for covering (Crook et al., 1999; Crook, 2003). Crook (2003) and Crook et al. (1999) found that urchins chose leaves as covering material more often than shells, indicating that the urchins were able to choose lighter items that were less energetically costly to carry. In our study, when the urchins were initially exposed to UV radiation (0900 h), they rapidly increased their use of small shells. Since the small shells had the highest area to mass ratio (Table 1), rapid covering was achieved most efficiently. Further selectivity was demonstrated in their overall preference for medium shells, which were used more frequently at all times of day than any other shell size-class (Figure 1).

The urchins' preference for medium shells over small shells appears to be energetically inefficient. A possible function of this behaviour could be to improve coverage of the urchins' reproductive organs. Urchin gonads serve essential functions in both reproduction and nutrient storage (Russell, 1998). Richner & Milinski (2000) hypothesized that urchins' covering behaviour may be intended specifically to protect the reproductive and madreporite openings on the upper surface of the urchin. In our study, although using two small shells would be more energetically efficient than using one medium shell, the use of more than one shell could allow UV rays to penetrate between cracks separating the two shells. Medium shells could provide greater protection.

Urchins use shells to cover during D-UV and non-UV periods (L-NUV, D-NUV). Adams (2001) noted that because urchins (*Strongylocentrotus droebachiensis*) are known to cover in the aphotic zone, covering behaviour may be due to multiple factors. Our results support this conclusion; since urchins were using small amounts of covering materials when UV radiation was not present, UV radiation appears to be one of many contributing factors to urchin covering behaviour.

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