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Effects of ultraviolet radiation and visible light on the development of encapsulated molluscan embryos

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ABSTRACT: Benthic egg masses laid in intertidal habitats are exposed to numerous environmental stresses including potentially damaging ultraviolet radiation (UVR). We sought to determine the developmental effects of UVR and visible light on molluscan embryos within egg masses from habitats with differential UVR exposure. Capsular and gelatinous egg masses from 23 marine gastropod species were collected from 3 intertidal habitats: (1) full sun, (2) partial shade, and (3) full shade. Egg masses were then divided among 4 spectral treatments: full spectrum, no UV-B, no UV, and dark. An ANOVA confirmed that a significant interaction between original habitat and spectral treatment affected mortality. Egg masses from full shade habitats showed significant vulnerability to UVR and visible light and had a higher overall mortality than other egg masses. Egg masses that were originally partially shaded did not show any significant mortality differences among spectral treatments, but highest mortalities occurred in full spectrum treatments while lowest mortalities occurred in dark treatments. Egg masses from full sun habitats showed no significant mortality differences between spectral treatments, which is consistent with protection against the harmful effects of UVR. In addition, the encapsulation period of egg masses in the dark was longer than the other 3 light treatments irrespective of habitat.

KEY WORDS: Mollusc · Egg mass · Capsule · Ultraviolet radiation · Invertebrate reproduction

INTRODUCTION

Some intertidal organisms protect their offspring from the harsh conditions of their environment by enclosing them within benthic egg masses. Molluscs, in particular, often employ complex structures to contain their developing offspring. Gastropod encapsulating structures can be divided into 2 general categories: capsular and gelatinous. Capsular egg masses have discrete capsules with rigid external walls that encase embryos within an intracapsular fluid. These egg masses can be further subdivided into those of neogastropods, which encase their offspring in a leathery walled capsule, and those of neritids, which encase their embryos in a rigid calcareous capsule. In contrast, gelatinous egg masses are composed of an insoluble jelly matrix in which numerous eggs surrounded by vitelline capsules are embedded. Egg masses of some species may protect the embryos from various stresses (reviewed by Pechenik 1979 and Przeslawski 2004) such as predation (Rawlings 1990), bacterial infection (Benkendorff et al. 2001), desiccation (Strathmann & Hess 1999), temperature variations (Podolsky & Hoffmann 1998) and changes in salinity (Pechenik 1982). Molluscan embryos are also potentially vulnerable to ultraviolet radiation from sunlight, but research on the effects of ultraviolet radiation (UVR) on molluscan egg masses is currently limited to a small number of species (e.g. Biermann et al. 1992, Rawlings 1996) of which only *Aplysia dactylomela* shows evidence of UVR protection (Carefoot et al. 1998).

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UVR at the earth's surface comprises UV-A (320 to 400 nm) and UV-B (280 to 320 nm). UV-B is particularly deleterious and can negatively affect reproduction, development, and behavior in many organisms including marine invertebrates (reviewed by Haeder et al. 1998, Rodriguez et al. 2000, Kuffner 2001). Previous studies indicate that in some encapsulated molluscan embryos, UVR exposure can cause stunted development, deformities, and death (Biermann 1992, Rawlings 1996, Carefoot et al. 1998). Some species, however, may be less susceptible to the negative effects of UVR. A surge of recent interest in declining amphibian populations has revealed a striking species-specific difference in the vulnerability of eggs and embryos to the harmful effects of UVR (e.g. Blaustein, 1994). In fact, population-specific vulnerability has recently been documented in both terrestrial (Belden & Blaustein 2002) and subtidal marine organisms (Gleason & Wellington 1995) where populations in habitats with high levels of UVR showed decreased vulnerability than populations occurring in environments with low UVR. Our aim was to similarly examine the response of organisms that lay in differentially UVR-exposed habitats in the marine intertidal environment.

Biologically harmful UVR can penetrate more than 20 m below the ocean's surface (Karentz & Lutze 1990, Booth & Morrow 1997). Thus, even submerged egg masses in the intertidal and subtidal zones can be subjected to potentially harmful levels of UVR. Marine molluscs and other invertebrates may avoid exposure to UVR by depositing egg masses in shaded habitats. Many molluscs, particularly nudibranchs and neogastropods, always lay their egg masses in habitats shaded from light including UVR (Biermann et al. 1992, Benkendorff & Davis 2003) (Table 1). Other species, particularly many *Aplysia* spp. lay in both shaded habitats and areas exposed to sunlight (authors' pers. obs.). In turn, certain species consistently lay in areas exposed to full sunlight so their egg masses are barraged daily by UVR (Creese 1980, Benkendorff & Davis 2003). Given the thinning of the ozone layer and the potential negative effects of increased UVR, we sought to assess the vulnerability of those species laying in habitats with elevated UVR. Here, we use cut-off filters to modify the quality of the incident radiation and to examine effects on embryonic mortality and encapsulation period for 23 molluscan species drawn from habitats exposed to full sun, partial sun, and full shade.

Table 1. Egg masses used in this study with number of replicate egg masses (n) used in the analyses for mortality and encapsulation period. –: hatching analysis does not include that species, because hatching in all spectral treatments did not occur for any replicate egg mass. Refer to 'Introduction' for classification definitions for habitat and type

Superorder	Order/infraorder	Species	n (mortality)	n (hatching)	Habitat	Type
Neritopsina	Neritodoidea	*Nerita atramentosa*	3	n/a	Full sun	Capsular
Caenogastropoda	Naticidea	*Conuber* species[a]	7	7	Full sun	Gelatinous
		Naticid sand collar	5	5	Part shade	Gelatinous
	Littorinimorpha	*Bembicium nanum*	7	7	Full sun	Gelatinous
	Neogastropoda	*Agnewia tritoniformis*	5	–	Shade	Capsular
		Lepsiella reticulara	2	–	Shade	Capsular
		Mitra carbonaria	6	–	Shade	Capsular
Heterobranchia	Basommatophora	*Siphonaria denticulata*	7	7	Full sun	Gelatinous
		Siphonaria zelandica	6	6	Full sun	Gelatinous
	Cephalaspidea	*Bullina lineata*	5	5	Part shade	Gelatinous
		Hydatina physis	7	5	Part shade	Gelatinous
	Anaspidea	*Aplysia juliana*	3	2	Part shade	Gelatinous
		Aplysia sydneyensis	3	2	Part shade	Gelatinous
		Bursatella leachii[b]	5	5	Part shade	Gelatinous
		Dolabrifera brazieri	6	6	Shade	Gelatinous
		Stylocheilus striatus[b]	6	5	Part shade	Gelatinous
	Notaspidea	*Pleurobranchus* sp. 1	1	–	Shade	Gelatinous
		Pleurobranchus sp. 2	1	1	Shade	Gelatinous
	Sacoglossa	*Oxynoe viridis*	2	1	Part shade	Gelatinous
	Nudibranchia	*Austraeolis ornata*	3	2	Shade	Gelatinous
		Dendrodoris fumata	3	–	Shade	Gelatinous
		Hypselodoris obscura[b]	3	–	Shade	Gelatinous
		Platyodoris galbanni	3	–	Shade	Gelatinous

[a]Egg masses are from *Polinices sordidus*, *P. conicum*, and/or *P. melastomum* which are indistinguishable from each other
[b]Egg masses collected from adults held in aquaria

MATERIALS AND METHODS

Egg mass collection. Egg masses from 23 marine gastropod species were collected from intertidal habitats along the Illawarra coast, New South Wales, Australia, during the low spring tides of September 2001 to April 2002 (Table 1). All collection sites were rocky intertidal reefs with the exception of 1 estuarine mudflat. Most egg masses were identified according to previous research (Hurst 1967, Rose 1985, Smith et al. 1989), but with certain species, identification of a laying adult was the only way to identify the egg mass to species level. A few egg masses were obtained from adults that laid in laboratory aquaria within a week of captivity (Table 1). Many neogastropod and aplysiad species communally spawn, so individual egg masses can often be difficult to discern. In all cases, each collected egg mass was spawned from only 1 parent. In gelatinous egg masses like *Aplysia* species, this was attained by only collecting a single discrete thread from the tangled egg mass. In capsular masses, capsules belonging to a single female were often distinguished by a single continuous basal layer and uniform development compared to adjacent capsules from different females. Communal egg masses with indistinguishable regions were not collected.

Each species was classified according to the intertidal habitat in which they deposited egg masses (Table 1). Full sun species spawned only in areas consistently exposed to direct sunlight such as flat rock platforms. Shaded species laid eggs masses exclusively under boulders or in other areas completely blocked from sunlight. Partially shaded species deposited their spawn in habitats that varied in sunlight exposure such as algal beds or vertical rock faces. In addition, species such as *Aplysia juliana* that spawned in both full sun and shaded habitats were also classified as partially shaded species.

Experimental design. In our experimental design, species were nested within habitats, and replicate egg masses were nested within species. Rather than discard or limit data, the design was unbalanced at the species and egg mass level because abundances of egg masses varied among species over time, and a uniform number of egg masses collected for each species was unfeasible (Table 1). For example, it was relatively easy to find a large number of *Siphonaria* spp. egg masses, but the egg masses of many nudibranch species were found rarely, sometimes only once. Furthermore, the number of species within each habitat varied because relatively few species typically deposit egg masses in full sun rather than shaded or partially shaded habitats (Benkendorff & Davis 2003) (Table 1).

Each egg mass was divided equally among treatments in order to reduce potential variability due to different parental characteristics and developmental stages. Capsular masses were divided into 4 groups each containing at least 2 discrete capsules. Many capsules abruptly changed colour soon after collection. These capsules were not used because such colour change indicates stress and possible impending death (Pechenik 1982, authors' pers. obs.). Gelatinous masses were cut into 4 pieces, and weights were recorded after gentle blotting. Preliminary studies revealed that embryos at the edges of cut areas still developed normally. Each capsular group or gelatinous piece was then placed within one of four 25 × 18.5 cm shallow plastic containers representing each spectral treatment by using spectral cut-off filters as lids (Fig. 1): (1) full spectrum control using no filter, (2) UV-B cut-off filter using 0.175 mm thick mylar polyester film, (3) UV-A and UV-B cut-off filter using treated polyethylene window film, and (4) dark control using opaque plastic. A diode array spectrophotometer was used to confirm the spectral transmission properties of the cut-off filters (Fig. 1). Containers were each drilled with 46 holes with 0.5 cm radii to allow constant internal water circulation and submerged in an outdoor 300 l recirculating seawater system exposed to natural sunlight. Temperature and water flow were recorded in all containers at random times for the duration of the experiment, and no discernible difference between treatments was detected. Water flow was measured at 3.88 ± 0.694 cm s^{-1} using the speed of neutrally buoyant beads across the length of containers.

Examination of embryonic development. After collection, each egg mass was examined microscopically

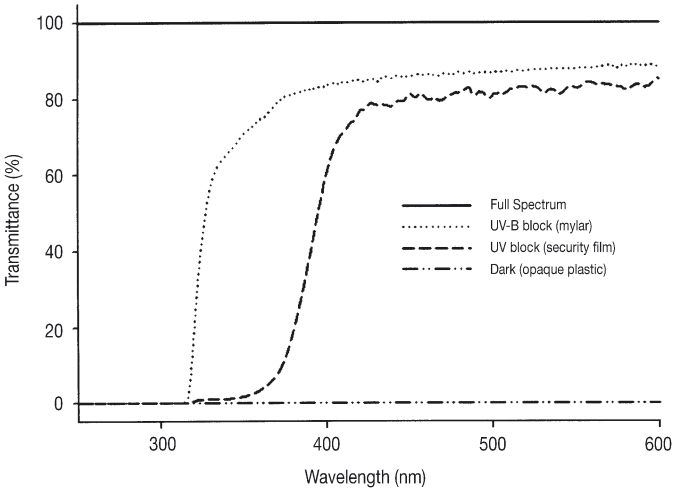

Fig. 1. Spectrophotometric reading of cut-off filters used in this study. UV-B blocking filter (polyester Mylar) blocked below 320 nm while UV blocking filter (acrylic window security film) blocked most wavelengths below 400 nm. Full spectrum treatments had 100% transmission while dark treatments under opaque plastic had 0%

to verify fertilization and developmental stage. Only masses with developing eggs or immobile, unshelled embryos were used; unfertilised or mature egg masses were discarded. For each gelatinous mass, average number of eggs mm^{-3} and wet weight of each mm^3 of egg mass was calculated. For each capsular mass, the number of eggs within each capsule was counted. In the case of *Mitra carbonaria*, the number of eggs was so high that 10 subsamples were taken.

The date of collection and initial examination was denoted Day 1. The egg masses were then placed in the experimental tank in their respective spectral treatments and inspected every 1 to 2 d. After each inspection, they were returned to the experimental tank so that their position and orientation to light were randomly changed every 1 to 2 d (e.g. Biermann et al. 1992). They were removed for examination when hatching was imminent. For gelatinous egg masses, this was determined when the gel began to soften and break apart. For capsular masses, this was determined by colour change associated with the development of shelled veligers or inviability as defined below. In addition, if capsular masses showed any obvious change in turgidity, they were removed for examination.

Upon removal, egg mass pieces and capsules were examined using a dissecting microscope (25× magnification). The egg mass pieces or capsules were returned to the treatment container if embryos were still encapsulated and viable and, in the case of gelatinous masses, if the gel was still fairly firm and intact. If hatching had occurred as described below, mortality was quantified.

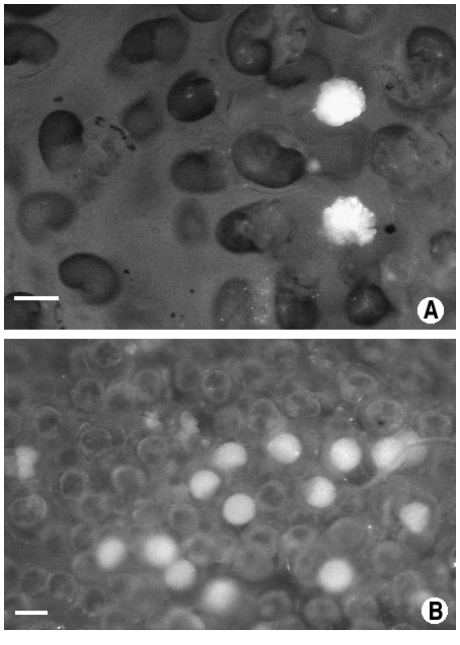

Fig. 2. Inviable eggs and embryos within molluscan egg masses. (A) Gelatinous egg mass of *Bembicium nanum*. Bright white eggs are very underdeveloped relative to the rest of embryos. (B) *Dolabrifera brazieri* embryos after exposure to UVR. All embryos have reached veliger stage, with the exception of the white eggs in the center which are relatively undeveloped or degenerating. Scale bars = 100 µm

Quantification of mortality. If the entire egg mass piece or capsule was obviously inviable, mortality was recorded as 100%. In capsular masses, complete mortality was often indicated by a dark black, brown, or purple colour change. In both capsular and gelatinous masses, total mortality was also determined when all embryos degenerated into free granules or fused into large undifferentiated masses while still encapsulated.

Due to different structures and hatching mechanisms among egg masses, it was necessary to develop different methods to quantify mortality of gelatinous egg masses, neogastropod capsules, and neritid capsules. Among gelatinous egg masses, each hatching or dissolving piece was first blotted and weighed. It was then placed in a small vial with 500 µl filtered seawater and agitated by hand for approximately 1 min until the egg mass was suspended in the seawater. This randomization ensured that embryos from the entire egg mass could be examined rather than only peripheral embryos, which may not be representative of the entire egg mass. From each suspended piece, ten 5 µl aliquots were examined microscopically. Dead embryos were counted within each sample, and the average was taken. This number was used to determine the estimated proportion of dead embryos (ß) within the 500 µl mixture. We considered embryos to be dead if they were degenerating and encapsulated or if they were relatively undeveloped (Fig. 2). Embryos without a protoconch or with other deformities were not considered dead since these malformations could have occurred during the homogenization procedure. Thus, mortality was conservatively estimated in this study. The following formula was used to quantify mortality proportion (M) within the entire gelatinous mass piece:

$$M = ß/[W_a(n/W_b)] \times 100\,\%$$

where ß = estimated number of dead embryos, W_a = blotted weight (mg) of the egg mass piece before homogenisation, W_b = average weight (mg) of mm^3 gel, and n = number of eggs mm^{-3}. A much simpler alternative would have been to score live embryos and dead embryos. However, this method was rejected because it would have likely resulted in inaccurately high mortality rates since many of the viable embryos are released from the disintegrating mass before the time of examination. These embryos would not have been scored as they would be drifting freely within the entire recirculating seawater system.

In all neogastropod capsules, mortality was calculated by dividing the number of dead embryos remaining in the capsule after hatching by the initial number of eggs in the capsule. As with gelatinous egg masses, mortality was thus conservatively estimated since a few dead embryos may have been released from capsules during the exodus of viable embryos. Preliminary developmental observations showed that none of the species examined in this study had nurse eggs, so they were not considered in the mortality calculation. The embryos of *Nerita atramentosa* are difficult to observe due to their opaque calcareous capsules. In order to observe development and maturity of this species, it was necessary to puncture the apical capsule so that it could be removed and the embryos within examined. Consequently, no initial count of eggs could be made as this would have destroyed the capsule and prevented further development. Replicate capsules collected at the same time were opened approximately every week until the majority of capsules within that treatment showed signs of hatching. As hatching approached, the apical shell became more flexible and split off easily from the basal membrane leaving the embryos exposed. At this time, the apical shell of the remaining intact egg capsules was removed, and the average proportion of dead to live embryos was determined.

Determination of encapsulation period. Length of encapsulation was the time taken to hatch for each egg mass or capsule under each spectral treatment. Hatching was obvious in caenogastropod capsular egg masses because the apical aperture opened easily during examination, and the viable embryos were released relatively quickly en masse. In gelatinous egg masses, hatching was often prolonged and less obvious. To standardize hatching time for gelatinous masses, hatching was recorded as the first day when more than 10% of the embryos had hatched or the gel dissolved. This value was chosen because preliminary observations revealed hatching to occur relatively quickly after this value had been reached. The hatching data from *Nerita atramentosa* was not used in the statistical analysis of hatching because determination of the length of encapsulation was too subjective and variable within this species. It was also likely to be affected by interference during examination. Furthermore, this species took up to 3 mo longer to hatch than many other species, and the resulting variance hindered statistical analysis.

Statistical analyses. Nested ANOVA tests were performed using restricted maximum likelihood (REML) technique in the statistical package JMP 4 with $\alpha = 0.05$ unless otherwise noted. A plot of the residuals of mortality versus the predicted values showed a binomial pattern. Because the variance was a function of the predicted values in the raw data, a standard angular transformation of arcsine($\sqrt{M}$) was used where M is the proportional data for embryonic mortality as defined previously (Zar 1998). Tukey's HSD multiple comparison tests were performed *a posteriori* on all data yielding significant effects.

Table 2. Effects of habitat, spectral treatment, species, and egg mass on marine gastropod embryonic mortality as determined by (a) nested ANOVA using restricted maximum likelihood with mortality arcsine transformed and random factors italicized, and (b) multiple comparisons of spectral treatments within and between each habitat using Tukey's Honest Significant Difference Test (HSD) ($\alpha = 0.05$). Mean mortalities are presented with the critical Q values in parentheses; lines connect treatments that are not significantly different. S: shaded habitats; PS: partial shade; FS: full sun

(a) ANOVA

Source	df	MS	*F*	p
Habitat	2	1.8133	34.7033	<0.0001
Treatment	3	1.2030	23.0443	<0.0001
Habitat × Treatment	6	0.5724	10.9645	<0.0001
Species [Habitat]	23	0.0921	2.0299	0.0153
Egg mass [Species, Habitat]	96	0.1236	3.1140	<0.0001
Residual	279	0.0522		
Corrected Total	383	0.2400		

(b) HSD

Within habitats	Full spectrum	UV-B block	UV block	Dark
Full sun (0.2000)	0.0619	0.0444	0.0419	0.0598
Partial shade (0.1821)	0.1826	0.1749	0.1051	0.0847
Shade (0.1904)	0.8184	0.6719	0.5589	0.3918

Between habitats	Shade	Partial sun	Full sun
Full spectrum	0.8184	0.1826	0.0619
UV-B block	0.6719	0.1749	0.0444
UV-block	0.5589	0.1051	0.0419
Dark	0.3918	0.0847	0.0598
	S × PS (0.1863)	PS × FS (0.1912)	FS × S (0.1953)

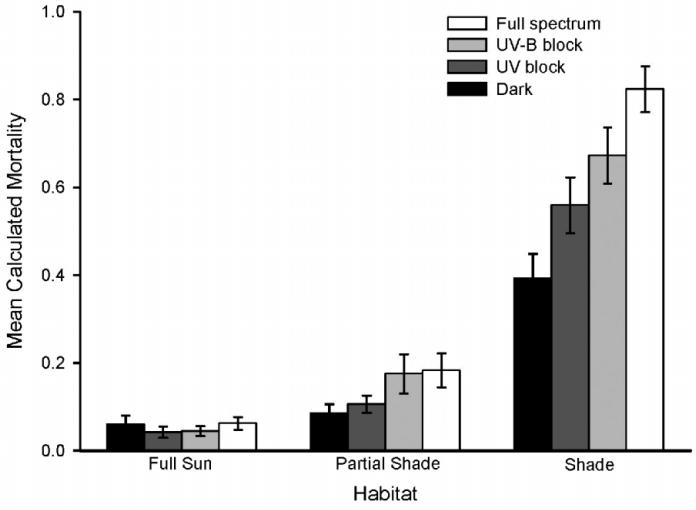

Fig. 3. Mean embryonic mortality of molluscan egg masses collected from full sun, partial sun, and full shade habitats under 4 different spectral treatments: (1) Full spectrum (no block); (2) UV-B block (mylar); (3) UV block (security film); and (4) Dark (opaque plastic). Error bars are SE of mean

RESULTS

Species that laid gelatinous egg masses were found more frequently than those that laid capsular egg masses (Table 1). More egg masses were found in shaded or partially shaded habitats than in areas exposed to full sunlight (Table 1).

Mortality and habitat

The effects of ultraviolet radiation and/or light on embryonic mortality depended upon the habitat in which the egg masses naturally occurred (Fig. 3). ANOVA revealed a highly significant interaction between habitat and treatment (F = 10.9645, $p < 0.0001$) (Table 2a). Shaded egg masses were most vulnerable to UVR, while egg masses from full sun habitats were the least vulnerable (Fig. 3). Tukey's HSD revealed that shaded egg masses had significantly higher embryonic mortality in open full spectrum treatments compared to UV-blocked and dark treatments. (Fig. 3, Table 2b). Egg masses naturally laid in full sunlight or partial shade had no significant differences in embryonic mortality between spectral treatments (Fig. 3, Table 2b).

Mortality of embryos within egg masses was low for those laid in full sun habitats relative to those laid in other habitats (Fig. 3, Table 2b). Tukey's HSD confirmed that egg masses from shaded habitats had significantly higher mortalities than the egg masses from other habitats at every spectral treatment (Table 2b). The embryonic mortality of partially shaded egg masses was intermediate between full sun and shaded egg masses (Fig. 3, Table 2b).

Mortality and egg mass type

Neogastropod capsular egg masses were found exclusively in fully shaded habitats (Table 1). Overall, embryos within these capsular egg masses exhibited a higher mortality than embryos within gelatinous masses (Fig. 4). For example, neogastropod capsules showed almost 100% mortality in full spectrum treatments while shaded gelatinous egg masses in the same light treatment showed an estimated mortality of 73.5%. A nested ANOVA confirmed a significant interaction between spectral treatments and egg mass type (F = 2.5951, $p = 0.0574$, $\alpha = 0.10$) (Table 3a). Tukey's HSD revealed a significant difference in mortality between the egg mass types in the UV-B treatments (Table 3b). Nevertheless, capsular and gelatinous egg masses showed similar patterns in their response to the different UV spectral treatments. For example, both types of egg mass had significantly lower mortality in UV block and dark than in full spectrum treatments (Table 3b).

Mortality and habitat (gelatinous egg masses only)

Due to the unequal distribution of egg mass types among habitats and the different embryonic mortalities, an ANOVA was performed using only gelatinous

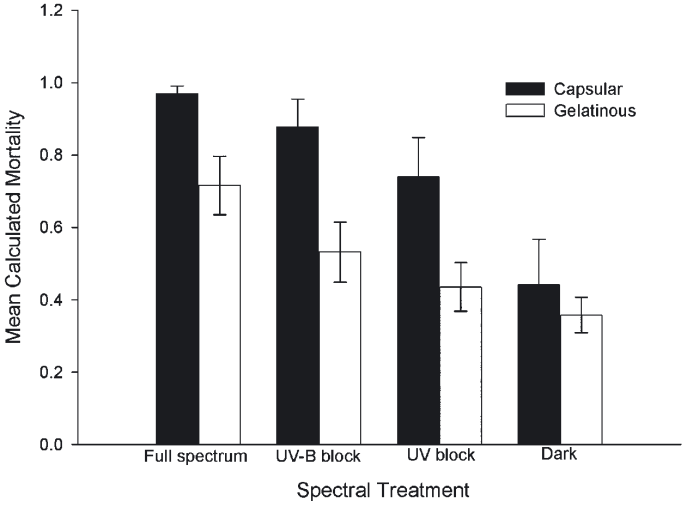

Fig. 4. Mean embryonic mortality of gelatinous and capsular molluscan egg masses collected from shaded habitats under 4 spectral treatments: (1) Full spectrum (no block), (2) UV-B block (mylar), (3) UV block (security film), and (4) Dark (opaque plastic). Error bars are SE of mean

Table 3. Effects of spectral treatment, egg mass type, species, and egg mass on marine gastropod embryonic mortality as determined by (a) nested ANOVA using restricted maximum likelihood with random factors italicized and (b) multiple comparisons using Tukey's HSD (α = 0.05). Mean mortalities of raw data are presented with the critical *Q* values in parentheses; lines connect treatments that are not significantly different

(a) ANOVA

Source	df	MS	*F*	p
Treatment	3	2.3521	21.4146	<0.0001
Type	1	0.4262	3.8801	0.0895
Type × Treatment	3	0.2850	2.5951	0.0574
Species [Type]	7	0.1182	1.0763	0.4096
Egg mass [Species, Type]	23	0.3126	2.8458	0.0002
Residual	90	0.1098		
Corrected total	127	0.2852		

(b) HSD

Within habitats	Full spectrum	UV-B block	UV block	Dark
Capsular (0.3715)	0.9686	0.8772	0.7398	0.4420
Gelatinous (0.3073)	0.7156	0.5314	0.4351	0.3575

Between habitats	Capsular	Gelatinous
Full spectrum	0.9686	0.7156
UV-B block	0.8772	0.5314
UV block	0.7398	0.4351
Dark	0.4420	0.3575

(0.3409)

egg masses to determine if UVR effects on shaded egg masses persisted without the inclusion of capsular masses. The results were consistent with the full data set, and our interpretation did not change. A significant interaction between habitat and spectral treatment was apparent (F = 8.0232, p < 0.0001), and Tukey's HSD revealed the same pattern as obtained for the full data set (data not shown).

Encapsulation period

Egg masses from all habitats hatched significantly slower in the dark than in the light (Fig. 5). Statistical analyses encompassed only gelatinous egg masses because no capsular egg masses hatched in full spectrum treatments (Table 1). ANOVA revealed no significant interaction between habitat and treatment on the length of the developmental period, but there was a significant difference between egg masses maintained in the dark and all 3 light treatments (F = 18.0211, p < 0.0001) (Table 4).

DISCUSSION

This study has shown that embryonic responses to UVR and visible light are strongly influenced by the habitat in which the eggs are naturally deposited. To date, there has been no previous study examining the effects of an environmental stress on molluscan egg masses drawn from such a broad range of taxa. Many studies have focused on 1 or 2 species and their responses to environmental variables (e.g. Scheltema 1967, Pechenik 1983, Biermann et al. 1992). Although representing valuable contributions to molluscan biology, research on single species is not sufficient to determine trends or to extrapolate to similar species or groups. This study has shown that a broad comparative approach for molluscan egg masses is feasible;

Table 4. Effects of habitat, spectral treatment, species, and egg mass on the embryonic length of encapsulation as determined by (a) nested ANOVA using restricted maximum likelihood with random factors italicized and (b) multiple comparisons using Tukey's HSD (α = 0.05). Mean mortalities of raw data are presented with critical *Q* values in parentheses; lines connect treatments that are not significantly different

(a) ANOVA

Source	df	MS	*F*	p
Habitat	2	1.4956	0.8569	0.4489
Treatment	3	41.0182	23.5034	<0.0001
Habitat × Treatment	6	1.8541	1.0624	0.3865
Species [Habitat]	12	5.8914	3.3758	0.0010
Egg mass [Species, Habitat]	55	39.2748	22.5044	<0.0001
Residual	201	1.7879		
Corrected total	279	18.9800		

(b) HSD

Treatment			
Full spectrum	UV-B block	UV block	Dark
13.5857	14.0000	13.8143	15.4143

(0.5889)

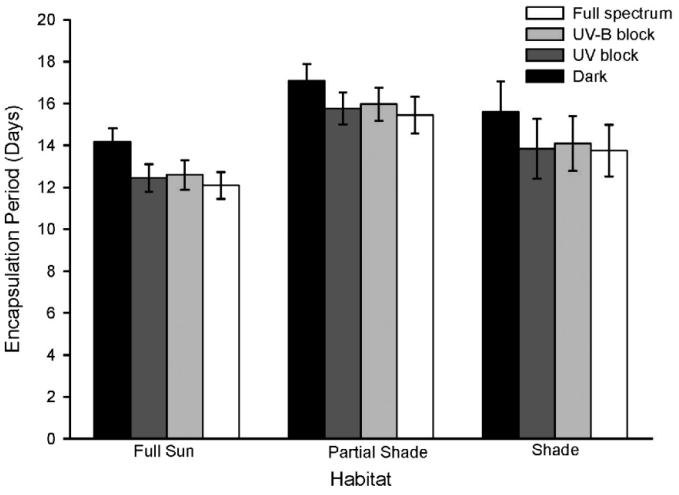

Fig. 5. Mean hatching day of molluscan egg masses collected from full sun, partial sun, and full shade habitats under 4 different spectral treatments: (1) Full spectrum (no block); (2) UV-B block (mylar); (3) UV block (security film); and (4) Dark (opaque plastic). Error bars are SE of mean

indeed, the relatively small error bars (Fig. 3) are remarkable considering the many species and egg mass structures examined as well as the conservative estimate of mortality. By using many species, we have been able to identify a strong pattern in the embryonic responses of molluscan egg masses to UVR based on their natural habitat.

The encapsulated embryos of species that lay exclusively in shaded habitats were not only more vulnerable to UVR but also had a higher overall mortality in this study (Fig. 4). However, unpublished observations during an ongoing survey of molluscan reproductive patterns indicate no difference in embryonic mortality between full sun and shaded egg masses in the field (R. Przeslawski pers. obs.). Thus, the higher overall mortality of shaded egg masses, particularly neogastropod capsules, was likely a result of experimental conditions. The uniform laboratory tank used in this study may not have reflected optimal conditions for the development all species. Indeed, optimal conditions for reproductive success are not known for the majority of species used in this study. Reasonably warm diurnal temperatures of 20.2 ± 1.0°C and water flow may have more closely represented conditions in open full sun habitats rather than the sheltered conditions under boulders. Despite the higher mortality observed among shaded egg masses, this study was primarily concerned with differences in mortality between spectral treatments within each egg mass habitat. Accordingly, shaded egg masses were clearly affected by UVR while full sun egg masses were not (Fig. 3).

Similarly, although embryos within caenogastropod capsules exhibited higher mortality, gelatinous egg masses collected from shaded habitats showed exactly the same pattern in response to the various UV treatments. This indicates that egg mass type was not a significant confounding factor in this study. Thus, molluscan egg masses collected from shaded intertidal habitats are more vulnerable to the effects of UVR irrespective of their phylogenetic origin. Stress endured during capsule removal may have resulted in increased mortality despite efforts to reduce effects of handling by only touching the capsule stalk or basal membrane during collection. Gelatinous egg masses are likely more resilient to the potential stress of removal from substrate as suggested by the normal development of embryos after the egg mass has been cut into small pieces (Rose 1986, authors' pers. obs.). The effects of removal from the substratum for both gelatinous and capsular egg masses warrant further investigation. Furthermore, the higher embryonic mortality in shaded capsular egg masses may be due to the embryos' lack of internal membranes. Since all embryos are freely integrated within a capsule, if 1 embryo becomes infected or dies, it could contaminate all the embryos in the capsule. Indeed, entire inviable capsules are quite common (Pechenik 1982). By comparison, molluscan embryos within gelatinous egg masses are separated individually or in small groups from the remaining embryos by a vitelline membrane.

Egg masses deposited in full sunlight are relatively resistant to UVR (Fig. 3). This study examined only embryonic mortality, however, and UVR may cause sublethal effects such as embryonic deformities or small embryo sizes within these egg masses (Biermann et al. 1992, Pahkala et al. 2002). These effects could be synergistic with other environmental stresses such as temperature (R. Przeslawski unpubl.). Laboratory experiments with controlled UVR intensity would clarify these issues.

Nevertheless, full sun egg masses most likely possess biochemical, structural, or cellular protection against potentially damaging UVR. In particular, it is likely that some of these egg masses contain UV-absorbing compounds such as mycosporine-like amino acids (MAAs). MAAs have been found in a variety of marine organisms including algae, cnidarians, echinoderms, and vertebrates; and they have been shown to protect against the damages of UVR (reviewed by Shick & Dunlap 2002). Carefoot et al. (1998) report that egg masses of some *Aplysia dactylomela* were rich in MAAs although their presence was strictly diet-dependent and not a direct response to UV-exposure. Several *Aplysia* spp. egg masses from partial shade habitats were used in this study (Table 1), and these did show some vulnerability to UV-B. If evolved biochemi-

cal protection via MAAs does occur in molluscan egg masses, it will most likely be found in gelatinous egg masses laid in full sunlight. Two previous studies have been unable to identify significant amounts of MAAs or any other UV-absorbing compounds within caenogastropod egg capsules collected from intertidal and subtidal habitats (Karentz et al. 1991, Rawlings 1996). Unfortunately, these studies do not specify whether the egg masses were collected from habitats exposed to UVR. Rawlings (1996) found that the outer capsule of a neogastropod egg mass absorbed UV-B, but he was unable to identify the means of absorption. Given the fact that embryos enclosed within these types of capsules are vulnerable to UVR (Fig. 4), the capsule wall may only be effective as a structural barrier against infrequent and low intensity UVR. In contrast, the egg capsules of *Nerita atramentosa* do occur in full sun habitats and appear to be resistant to the effects of UVR (Fig. 3) possibly due to the protection conferred by the opaque calcareous capsule. Research is currently in progress to identify and quantify potential MAAs in molluscan egg masses of many species including those used in this study. Preliminary spectrophotometric and HPLC analysis reveal the presence of MAAs or other UV absorbing compounds is likely (authors' unpubl. data).

The evolution of UV-resistant egg masses may provide several advantages to species that live on intertidal reefs. First of all, competition for an appropriate egg deposition site may be lower in full sun habitats due to the fact that many species are vulnerable to UVR (reviewed by Haeder et al. 1998). In addition, as shown in this study, egg masses laid in sunlight will hatch faster than those laid in the shade irrespective of temperature (Fig. 5). Therefore, direct sunlight might reduce the overall risks to embryos by minimizing the time spent in vulnerable developmental stages (Spight 1975, Strathmann et al. 2002). Finally, previous research has shown that ultraviolet radiation has been shown to decrease predation rates and harm marine bacteria (Jeffrey et al. 1996, Williamson et al. 1999). Current research on the effects of fouling on molluscan development has revealed that protist colonization is indeed higher in egg masses developing in the dark than in the light (authors' unpubl. data). Thus, egg masses laid in direct sunlight might be less prone to microbial infection or predation. Further research examining predation, competition for spawning habitat, and colonization by microrganisms may reveal reasons for the evolution of UVR-resistant egg masses.

The encapsulation period was shorter in egg masses that were exposed to light compared to those kept in the dark with hatching occurring on average 1 to 2 d earlier (Fig. 5). This result was unexpected for egg masses collected from shaded habitats because it implies that their selected habitat is a compromise to minimize mortality. The faster hatching in light could be a direct response to light or related to other confounding effects such as surface algal fouling. Various degrees of algal and protist fouling were noted on many egg masses in the experimental tank used in this study. Algal growth is dependent on light availability (e.g. Hernandez et al. 1997) and could alter the internal oxygen concentration of the egg mass during photosynthesis and cellular respiration. Oxygen availability has been shown to have a tremendous effect on the hatching rate within molluscan egg masses (Booth 1995, Cohen & Strathmann 1996).

In addition to the potential positive effects on embryonic developmental rates by photosynthetic surface fouling organisms, fouling might also have detrimental effects. For example, byproducts of certain algae may be detrimental to the health of embryos (Fogg 1983, Tang & Dam 2001). Algal fouling can also provide a foundation for protist colonization and infestation. Protists have recently been shown to lower the oxygen availability of embryos in certain egg masses to dangerous levels (Cancino et al. 2000).

Egg masses that were collected from shaded habitats showed significantly increased mortality in visible light treatments even when damaging UV wavelengths were blocked (Fig. 3). This may be due to a direct effect of intense levels of visible light. Although larvae of some marine invertebrates are photoresponsive (Svane & Dolmer 1995, Leys & Degnan 2001), no studies have examined if molluscan embryos are vulnerable to high intensities of visible light. Moreover, approximately 10% of UV-B and 25% of UV-A passed through the UV-blocked filter in visible light treatments (Fig. 1), and it is thus possible that these levels of UVR were significantly damaging to embryos from full shade egg masses. Another possibility is that the effect of visible light recorded here may be related to confounding factors such as surface fouling. Future research should consider the potential interaction among these variables in order to understand the potential synergistic effects of ultraviolet radiation and other factors such as algal fouling on encapsulated molluscan development.

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