Marine Biology (1998) 130: 389–396

T. H. Carefoot · M. Harris · B. E. Taylor
D. Donovan · D. Karentz

Mycosporine-like amino acids: possible UV protection in eggs of the sea hare *Aplysia dactylomela*

Received: 13 May 1997 / Accepted: 27 June 1997

Abstract We investigated mycosporine amino acid (MAA) involvement as protective sunscreens in spawn of the sea hare *Aplysia dactylomela* to determine if adult diet and ultraviolet (UV) exposure affected the UV sensitivity of developing embryos. Adults were fed a red alga rich in MAAs (*Acanthophora spicifera*) or a green alga poor in MAAs (*Ulva lactuca*). Adults on each diet were exposed for 2 wk to ambient solar irradiance with two types of acrylic filters; one allowed exposure to wavelengths >275 nm (designated UV) and one to wavelengths only >410 nm (designated NOUV). Spawn from each adult group was likewise treated with UV or NOUV and monitored during development for differences in mortality and metabolic rate (measured as oxygen consumption: $\dot{V}_{O_2}$). Also recorded were number of eggs or embryos per capsule, times to hatching, hatching success, size at hatching, and $\dot{V}_{O_2}$ of adults. Spawn from adults eating red algae was almost twice as rich in MAAs as spawn from adults eating green algae, suggesting that MAA content is diet-related. Although overall quantities of MAAs in the spawn reflected MAA contents of the adult diet, specific MAAs were differentially sequestered in the spawn. Thus, porphyra-334, found in high concentration in *Aplysia dactylomela*'s preferred red algal food, was present in only low concentration in the spawn. Conversely, mycosporine-glycine, in low concentration in red algal food, was the most abundant MAA in the spawn. UV treatment of adults had no effect on quantities of MAAs in the spawn. Adults exposed to UV had significantly higher $\dot{V}_{O_2}$s and spawned twice as often. The UV-treated adults produced spawn with significantly higher $\dot{V}_{O_2}$s and their embryos developed to hatching sooner. The only significant effect of UV exposure of the spawn was to reduce the percentage of veligers hatching from 71 to 50%. There was no significant effect on hatching time or size of the veligers at hatching, nor on number of eggs per capsule.

Communicated by M.F. Strathmann, Friday Harbor

T.H. Carefoot (✉) · M. Harris · B.E. Taylor · D. Donovan
Department of Zoology, University of British Columbia,
Vancouver, British Columbia V6T 1Z4,
Canada

D. Karentz
Department of Biology, University of San Francisco,
San Francisco, California 94117-1080,
USA

Introduction

Ultraviolet radiation in both the A (320 to 400 nm) and B (280 to 320 nm) portions of the spectrum has broad-ranging deleterious effects on marine organisms. Biological effects include inhibition of photosynthesis and motility of phytoplankton (Bühlmann et al. 1987; Häder and Häder 1991), reduced growth of kelp (Wood 1987), death of salmon (McArdle and Bullock 1987), melanin deposition in sharks (Lowe and Goodman-Lowe 1996), death of coral larvae (Gleason and Wellington 1995), reduced growth of corals (Jokiel 1980; Jokiel and York 1982; Gleason 1993), and "bleaching" of corals through expulsion of symbiotic zooxanthellae (Glynn et al. 1993). As well as adopting avoidance strategies to UV radiation (e.g. Biermann et al. 1992), soft-bodied marine organisms exposed to UV may have increased concentrations of photoadaptive enzymes, such as superoxide dismutase to inactivate O_2 radicals produced from UV-mediated reactions (Lesser and Shick 1989), and also UV-absorbing substances such as mycosporine-like amino acids (MAAs) which absorb UV radiation between 310 and 360 nm (Nakamura et al. 1982; Dunlap and Chalker 1986; Dunlap et al. 1986). MAAs have been identified in a broad range of marine organisms: phytoplankton (Carreto et al. 1990), seaweeds (Karentz et al. 1991), invertebrates (Nakamura et al. 1982; Chioccara et al. 1986; Dunlap and Chalker 1986; Dunlap et al. 1986, 1989; Shick et al. 1992; Karentz 1994a; Stochaj et al. 1994; Adams and Shick 1996), and fish (Dunlap et al. 1989). They are more prevalent in organisms inhabiting shallow ocean habitats (Dunlap et al. 1986;

Scelfo 1986; Karentz et al. 1991; Gleason 1993; Shick et al. 1995) and are abundant in red algae (Karentz et al. 1991; Karentz 1994b). There is some evidence that MAA concentration may be increased under exposure to UV radiation, but the results are not unequivocal (Scelfo 1986; Wood 1989; Shick et al. 1991, 1995; Gleason 1993). To date, over 20 MAAs have been identified; however, we know little of their biological roles in minimizing or preventing degradative action of UV radiation (but see Garcia-Pichel and Castenholz 1993; Garcia-Pichel et al. 1993; Adams and Shick 1996).

The sea hare *Aplysia dactylomela* is an ideal candidate for an investigation of the biological role of MAAs. It is a large marine opisthobranch gastropod which inhabits shallow tropical shoreline regions, eats red and green algae, lays large numbers of fertilized eggs in string-like gelatinous masses close to the sea surface, is hermaphroditic (hence, every individual is an egg-producer), and is nocturnally active but may be exposed to sunlight as it rests during the day (Carefoot 1987; Carefoot and Thomas 1993). Further, it incorporates pigments from its algal foods into its eggs. After 2 to 3 d (at 28 °C) a change of diet produces a corresponding and abrupt change in egg-strand colour (Carefoot 1987). This egg-strand coloration in *Aplysia* spp. was thought to be for adaptive camouflage of the spawn, deposited upon and amongst the sea hares' own seaweed foods, but it may be possible that algal-derived MAAs are selectively incorporated for UV protection. The precise disposition of the pigments in the egg is unknown, but appears to be in the yolk or as cytoplasmic inclusions. A greenish- or yellowish-coloured egg produced by an adult eating the green alga *Ulva* sp. will lead to a comparably coloured veliger at hatching. So distinctive and precise are these colorations, that an adult's dietary history can be surmised from the record of its spawn coloration (Carefoot 1987). The spawn is cylindrical (0.5 to 1.5 mm diam), and is laid in large festoons attached to seaweeds or rocks. The spawn string consists of capsules positioned more or less helically within a protective cylinder of mucopolysaccharides. Considerable variation exists in capsule spacing and in number of eggs per capsule in *A. dactylomela*, but typically a capsule bears 8 to 10 eggs, most of which hatch to viable veligers (Carefoot 1987).

This ideal model system allows a number of questions to be addressed relative to possible protective functions of MAA in the spawn of *Aplysia dactylomela*: (1) are MAAs incorporated from the food into the eggs and, if so, are certain MAAs selectively sequestered? (2) is there enhanced bioaccumulation in the eggs when the adults are exposed to light with UV present, as opposed to when UV is absent? (3) does a red- or green-algal diet affect the type and amount of MAAs incorporated into the eggs? and (4) are there differential effects on survival, growth, or metabolism of embryos and larvae depending on types and amounts of MAAs incorporated, and relative to whether or not UV light is present in their culture conditions?

Materials and methods

Collection and maintenance of specimens

Reproductively mature specimens of *Aplysia dactylomela* were collected from shallow inshore regions of Discovery Bay, Jamaica, in June 1995. This region hosts luxuriant growths of numerous red algal species, as well as moderate growths of the green alga *Ulva lactuca*. In the field, *A. dactylomela* preferentially eats red algae, particularly *Acanthophora spicifera* and *Centroceras clavulatum*, but in the laboratory will eat both red and green algae. Both promote good growth and spawn production (Carefoot 1987). Spawn produced on a diet of *A. spicifera* is mauve to purplish, while that on a diet of *Ulva lactuca* is green to greenish-yellow. On a given algal food, spawn colour is consistent for an individual sea hare, but differs slightly between individuals. Thus, spawn from individual adults can be determined by these variations in colour.

Sea hares of 400 to 600 g live mass were housed in floating plastic-mesh baskets (34 × 24 × 14 cm deep) in a 3000-liter outdoor tank supplied with a constant flow of fresh seawater (800 l h^{-1}). The sea hares were thus exposed to unshaded sunlight at a depth not exceeding 12 cm. Six sea hares were contained in each basket, and all baskets contained approximately equal initial live mass. Baskets were tethered to form two four-basket groups. Each group was covered with a 3 mm thick acrylic sheet which either blocked UV (Acrylite OP-3, designated NOUV: no wavelengths below 410 nm transmitted) or allowed its passage (Acrylite OP-4, designated UV: all wavelengths above 275 nm transmitted). The baskets received full sunlight from 07:00 to 17:00 hrs. The seawater varied from 28 to 29 °C and 24 to 28‰ S during the 2 wk study. Two baskets in each group were provided with red algae (*Acanthophora spicifera*) and two with green algae (*Ulva lactuca*). Algae were provided ad libitum each day at dusk, and uneaten remnants were removed at dawn. Thus, there were two replicates of six sea hares, each subjected to either UV or NOUV and fed either red or green algae.

Spawn collection and treatment

Spawn was collected for analysis after the green-alga treatment-groups began laying appropriately coloured spawn. This took ~3 d. Spawn was laid throughout the day and night. To standardize the start-time (Day 0) stage of development, only spawn in one- or two-cell stages of development was used for experiments.

Each day at 07:00 hrs, spawn masses were collected from each basket, blotted dry, weighed, and separated by colour to ensure single-adult origins. Replicate portions of each of these spawn masses (0.5 to 2.0 g) were set out in small floating mesh containers in the same outdoor tank as the adults, and were covered by UV or NOUV screens. The spawn-containers were plastic mesh (3 × 3 mm opening size) shallow cones sewn to 5 cm diam polystyrene ring-floats. In these shallow mesh-containers, the spawn floated just below the water's surface (2 cm depth), exposed fully to the sun. Daily examination of each spawn mass randomized its upward and downward orientation, thus maximizing exposure of the spawn to sunlight.

The design of the experiment allowed spawn from adults eating either red or green seaweed, and in either UV or NOUV conditions, to be cultured in either UV or NOUV conditions. We hypothesized that more protective UV-absorbing MAAs would be incorporated into the eggs by adults eating red algae under UV exposure, and thus would develop to larvae faster, and perhaps be of a larger size, with better survival and with less stress being exhibited (as reflected in the $\dot{V}_{O_2}$s) when cultured in UV conditions as compared with the other treatment combinations. Likewise, we hypothesized that spawn from all treatment combinations would do better when cultured in NOUV than in UV conditions. Application of ANOVA to various aspects of the data showed in all cases that there was no significant basket effect, so this was dropped as a potential factor.

Adult $\dot{V}_{O_2}$

To compare $\dot{V}_{O_2}$s of sea hares in the UV- and NOUV-treatment groups, individuals of known mass were enclosed individually in respirometers adapted from standard, glass, desiccator flasks (500 and 2000 ml vol). A Yellow Springs oxygen electrode was inserted into each respirometer through a rubber bung at the top, and P_{O_2} (partial pressure of oxygen) signals were amplified and recorded on a voltmeter (FLUKE Series II). A rotating stir bar under a plastic mesh screen at the bottom of the respirometer ensured complete mixing of the seawater within. A black plastic shroud over the respirometer screened the sea hare. When an individual was noted to move in the respirometer (usually signaled by a disturbance of the electrode) the data were discarded and the specimen was set aside until it became quiet. During a respirometry run, P_{O_2} was recorded at 2 min intervals until it reached 70% saturation level. Following this, the sea hare was removed from the respirometer, blotted dry, and weighed. All $\dot{V}_{O_2}$ measurements were carried out between 10:00 to 14:00 hrs (a time of normal daily quiescence for *Aplysia dactylomela*) and at 28 °C. UV and NOUV treatment groups were compared at Day 0 and Day 14 of captivity, using different specimens for each comparison. To eliminate size as a factor in the analysis, all oxygen-consumption rates were converted to $\dot{V}_{O_2}$ for equivalent 100 g live-mass individuals by application of the formula

$$\dot{V}_{O_{2(100g)}} = (100 \cdot \text{experimental live mass}^{-1})^{0.92} \cdot \dot{V}_{O_2\,(\text{measurement in experiment})},$$

whereby the exponent 0.92 was derived from the slope of a regression line relating $\dot{V}_{O_2}$ to log live-mass in an earlier study (Carefoot 1987).

Mycosporine-like amino-acids

Subsamples of Day 0 spawn (0.5 to 5 g live mass) were collected and frozen in 100% methanol at −20 °C at the same time as the spawn masses were divided into treatment groups. In addition, samples of freshly laid field-spawn were collected and frozen in methanol, as were samples of the seaweed foods employed in the study. All samples were later dried in a rotary evaporator, ground to a powder, and kept frozen at −20 °C until analysed for MAAs.

UV-absorption spectrograms over the range 190 to 420 nm were plotted for methanol extracts of all samples. MAAs were identified and quantified by high-performance liquid chromatography (HPLC) (see Dunlap and Chalker 1986; Karentz et al. 1991; Stochaj et al. 1994). Briefly, samples were sequentially extracted in 80% methanol and individual MAAs were separated by reverse-phase HPLC (RP-8 column with an aqueous mobile phase of 55% methanol containing 0.1% acetic acid). MAAs were identified by co-chromatography with prepared standards and by wavelength ratios of absorbance (313:340 nm; for details see Karentz et al. 1991).

Developmental rates, hatching percentages, and larval size at hatching

Spawn was examined daily for percentage survival of embryos and larvae, stage of development, and time and percentage hatching. As each spawn mass reached a level of ~50% hatching, 15 veligers were randomly collected and their largest diameters were measured by means of an ocular micrometer and compound microscope.

Spawn $\dot{V}_{O_2}$

At Day 0, Day 3, and commencement of hatching (Days 5 or 6, defined as when 10% of veligers were free of their capsules but still within the gelatinous strand), replicate 50 to 200 mg (live mass) portions were snipped from a single spawn mass and placed in respirometers. These respirometers were made of blown glass, and consisted of a 2 ml central animal chamber surrounded by an insulating water jacket. A Yellow Springs oxygen electrode was inserted into the chamber via a molded glass tunnel. Water within the respirometer was mixed by a small magnetic stir-rod rotated beneath a mesh screen in the chamber. P_{O_2} was recorded at 2 min intervals until it reached a 70% saturation level. Terminal oxygen depletion was usually reached within 10 to 20 min from the start of a run, depending on the developmental stage of the embryos and, hence, their activity. A typical portion of spawn used in respirometry experiments contained ~15 000 individuals. The number of capsules was counted in a 1 cm weighed portion of the same spawn strand used in the respirometry experiments, and the mean number of eggs, developing embryos, or veligers within each capsule was determined from counts of 20 capsules at random locations along this strand. This allowed $\dot{V}_{O_2}$ to be expressed per individual developmental stage.

Results

Adult $\dot{V}_{O_2}$

Aplysia dactylomela subjected to UV exhibited significantly higher $\dot{V}_{O_2}$s than sea hares subjected to NOUV (5.8 vs 4.6 ml O_2 h^{-1} for 100 g live-mass individuals, respectively; $F_{1,29} = 7.91$, $p = 0.009$; ANOVA), suggesting stress effects of UV exposure. There was no significant effect of time in captivity on $\dot{V}_{O_2}$ (5.7 vs 5.1 ml O_2 100 g^{-1} h^{-1} for Days 0 and 14, respectively; $F_{1,29} = 2.50$, $p = 0.12$), nor was there an effect of diet on $\dot{V}_{O_2}$ (5.2 vs 5.1 ml O_2 100 g^{-1} h^{-1} for red- and green-algae diet groups, respectively; $F_{1,29} = 0.08$, $p = 0.78$).

Spawn production

Table 1 provides information on spawn mass and number of eggs produced by the four adult treatment groups. Most spawn appeared to be produced by adults eating red algae and subjected to UV radiation; however, significance could not be shown ($F = 1.28$, $p = 0.32$; ANOVA). Total number of spawnings by UV-treated adults was almost twice as great as that by NOUV-treated individuals (24 and 13 spawnings, respectively). No significant pattern was discerned in number of egg capsules per unit mass of spawn (14 to 19 capsules mg^{-1} live spawn: Table 1), in number of eggs per capsule (10 to 11 eggs: Table 1), nor in total number of eggs produced per day (46 000 to 64 500 eggs: Table 1).

Mycosporine-like amino acids

Fig. 1 shows representative absorption spectrograms for methanol extractions of algal foods and of spawn sampled from adult sea hares feeding on either red or green algae. Two features are notable: first, spawn from adults eating *Acanthophora spicifera* is richer in substances absorbing at ≥310 nm than is spawn from adults eating *Ulva lactuca*; second, there is no obvious difference between UV-treated and NOUV-treated adults with

Table 1 *Aplysia dactylomela*. Daily spawn production (live g $d^{-1}100\ g^{-1}$ live-mass adults; range in parentheses) under conditions of UV or NOUV and eating red (*Acanthophora spicifera*) or green (*Ulva lactuca*) alga. Each treatment group (*N*) comprised six sea hares in each of two baskets. Values for spawn production normalized to 100 g adult mass to account for differences in total mass among the eight baskets during 2 wk period during which spawn was collected (*UV* exposed to ultraviolet irradiation in sunlight; *NOUV* ultraviolet wavelengths screened-out)

Adult treatment, diet	(*N*)	Total spawnings (2 wk period)	Daily spawn production	No. egg capsules mg^{-1} live spawn	No. eggs per capsule	Total no. eggs d^{-1} 100 g^{-1} live-mass adults
UV						
red	(2)	13	0.43 (0.29–0.57)	15	10	64 500
green	(2)	11	0.29 (0.24–0.34)	18	11	57 400
NOUV						
red	(2)	6	0.22 (0.16–0.28)	19	11	46 000
green	(2)	7	0.30 (0.26–0.34)	14	11	46 000

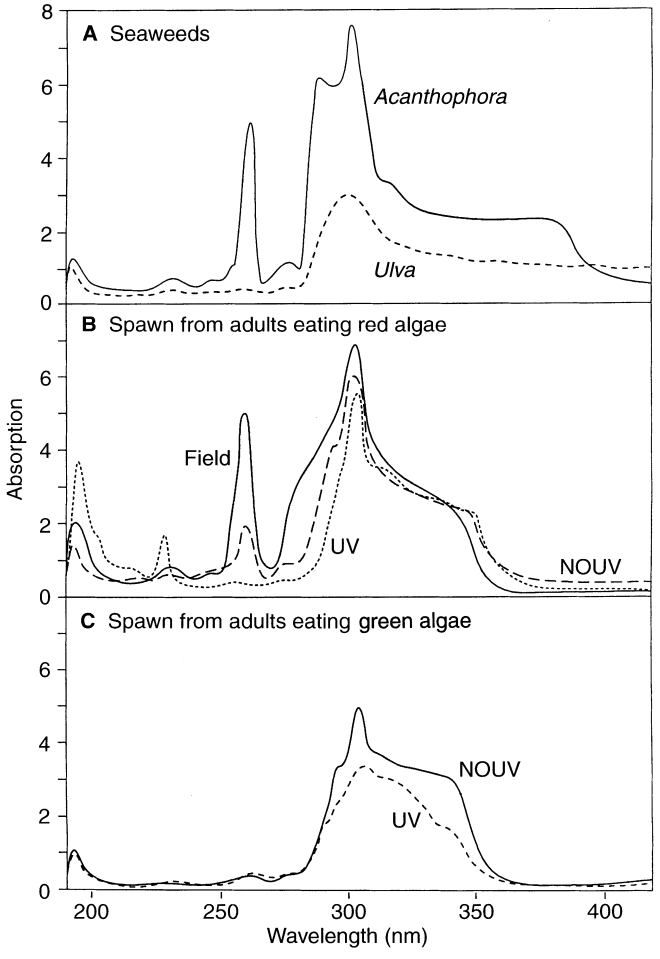


Fig. 1 Absorption spectograms for methanol extracts of *Acanthophora spicifera* and *Ulva lactuca* (**A**), and of spawn produced by adult *Aplysia dactylomela* eating these seaweeds (**B**, **C**). Spawn from field specimens is included as a comparison. Absorption data are based on normalized fresh mass of sample. Field diet includes a variety of red alga (*UV* exposed to ultraviolet radiation in sunlight; *NOUV* ultraviolet wavelengths screened-out)

respect to amounts of UV-absorbing substances in their spawn. Only peaks registering at 310 nm and above are MAAs. The chemical nature of those below 310 nm as, for example, the predominant peak at 260 nm for *A. spicifera*, is unknown.

Table 2 *Aplysia dactylomela*. Content of specific mycosporine amino acids (*MAA*) in spawn (data from all diet, UV, and field samples combined). $N = 40$ for each MAA

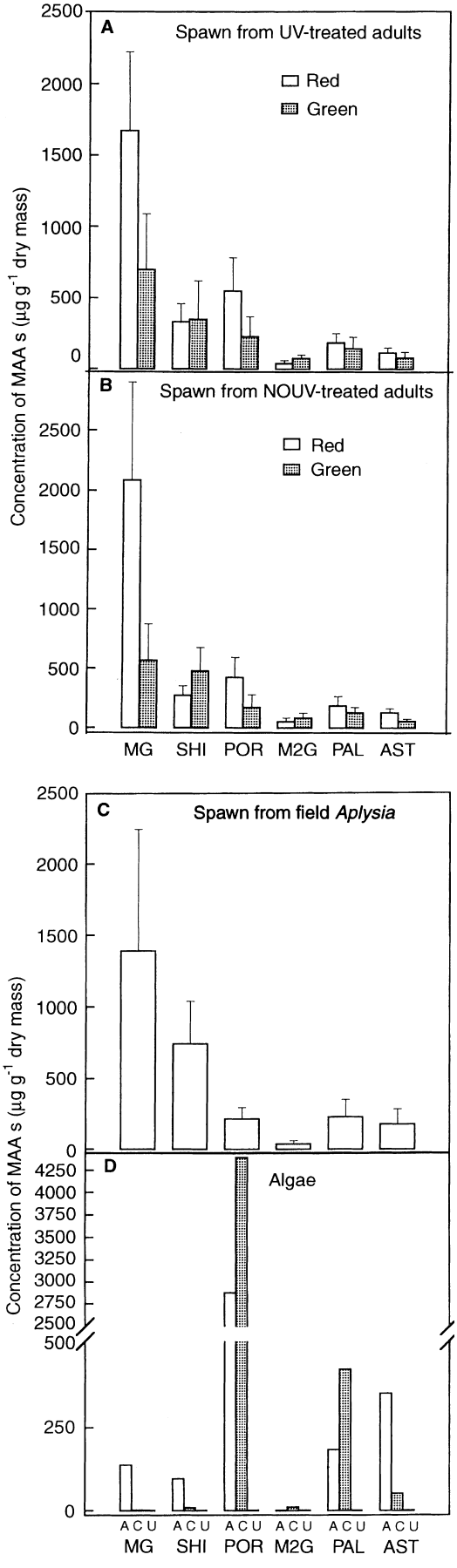
MAA	Mean amount (μg g^{-1} dry mass ± SD)
Mycosporine glycine	1304 ± 814
Shinorine	427 ± 266
Porphyra-334	332 ± 217
Mycosporine-2-glycine	54 ± 34
Palythine	175 ± 87
Asterina-334	112 ± 68

Table 3 *Aplysia dactylomela*. Total content of MAAs in Day 0 spawn. Adult treatment and diet designations as in Table 1, except that red for field treatment signifies diet of mixed red algae

Adult treatment, diet	(*N*)	Mean total content (mg g^{-1} dry mass ± SD)
UV		
red	(10)	2.88 ± 0.74
green	(9)	1.57 ± 0.81
NOUV		
red	(7)	3.13 ± 1.11
green	(6)	1.47 ± 0.58
Field		
red	(8)	2.75 ± 1.26

Six MAAs were identified in the spawn (Table 2). Amounts of MAAs differed significantly ($F_{5,239} = 109.4$, $p \ll 0.001$; ANOVA), with mycosporine glycine present in highest concentration, shinorine and porphyra-334 next highest, and mycosporine-2-glycine, palythine, and asterina-334 in lowest concentrations.

The lack of effect of UV exposure of adults on the MAA content of the eggs is demonstrated more clearly in Table 3, which gives the total mass of MAAs, regardless of type, in the spawn of each of the four treatment groups plus field individuals. Statistical analysis revealed a highly significant difference in amounts of MAAs in the spawn ($F_{4,39} = 5.31$, $p < 0.001$; ANOVA), with the data segregating into two statistically homogenous subgroups, one represented by the *Acanthophora spicifera*-eating UV and NOUV groups and the



field adults [2.75 to 3.13 mg g^{-1} dry mass; $p < 0.05$; Newmann–Keuls multiple-comparison tests: (N–K test], the other by the *Ulva lactuca*-eating UV and NOUV groups (1.47 to 1.57 mg^{-1} g dry mass). These results show that UV-exposed adults did not significantly increase the content of MAAs in their spawn. Rather, diet had the main effect, with *U. lactuca*-eating adults incorporating only about half the amount of MAAs in their eggs as compared with *A. spicifera*-eating adults. Field individuals eat almost entirely red algae, and this is reflected in the high level of MAAs in their spawn.

Fig 2A, B illustrates the effect of UV and NOUV exposure and diet treatments on allocation of specific MAAs in the spawn. Analysis disclosed a significant difference in disposition ($F_{20,239} = 7.18$, $p < 0.001$; ANOVA), with the main differences revolving around the most abundant MAA, mycosporine glycine (see Table 2). Significantly higher concentrations of this MAA were present in the spawn of sea hares eating *Acanthophora spicifera* and the spawn of field specimens (Fig. 2C) than in spawn of individuals eating *Ulva lactuca* ($p < 0.05$; N–K tests). While mycosporine glycine was prevalent in the spawn, it was scarce in the red seaweeds tested (A. *spicifera* and *Centroceras clavulatum,* the latter also a common field diet of *Aplysia dactylomela* in Jamaica), while the reverse was true for porphyra-334. No MAAs were present in *U. lactuca*.

Developmental rates, hatching percentages, and larval size at hatching

There was a significant difference in hatching time for the UV and NOUV treatments of the adults. UV-treated adults produced eggs which hatched in 6.1 d, versus 6.9 d for NOUV-treated adults ($F_{1,59} = 16.17$, $p < 0.001$; ANOVA; $N = 30$ for each). There was no significant effect of diet (6.3 and 6.5 d for red and green algal diet groups, respectively $F_{1,59} = 0.22$, $p = 0.64$; ANOVA), for baskets (6.6 and 6.2 d for each of the replicated baskets, respectively: $F_{1,59} = 1.88$, $p = 0.18$; ANOVA), for UV and NOUV treatments of the eggs (6.4 and 6.4 d, respectively: $F_{1,59} = 0.08$, $p = 0.77$, ANOVA), nor for any interactions of these effects on hatching time.

The percentage of veligers hatching was significantly lower in spawn subjected to UV irradiation compared with NOUV (50 and 71%, respectively: $F_{1,59} = 10.19$, $p = 0.002$, ANOVA; $N = 30$ for each). Further, the interaction of UV and NOUV treatments of adults with diet had a significant effect on percentage hatching ($F_{1,59} = 8.46$, $p = 0.005$; ANOVA), with the data re-

◀

Fig. 2 *Aplysia dactylomela.* Mycosporine-like amino acid concentrations in spawn from UV- and NOUV-treated specimens, and from field specimens and algal foods (*MG* mycosporine glycine; *SHI* shinorine; *POR* porphyra-334; *M2G* mycosporine-2-glycine; *PAL* palythine; *AST* asterine; *A Acanthophora spicifera*; *C Centroceras clavulatum*; *U Ulva lactuca*) $N = 6$ to 10 for each data set in **A**, **B** and **C**; in **D**, $N = 1$ for each of *A* and *C*, and 3 for *U*

solving into two statistically homogenous subgroups. One subgroup, represented by adults exposed to UV and eating green algae, produced spawn with only 43% hatching; the other, represented by the remaining three combinations (UV- and NOUV- adults eating red algae, and NOUV adults eating green algae), produced spawn with 55 to 72% hatching ($p < 0.05$, N–K test). Our overall prediction that UV or NOUV exposure of the adults would interact with diet to produce eggs whose survival would be differentially affected by exposure to UV during development was thus not supported ($F_{1,59} = 0.007$, $p = 0.93$; ANOVA).

Finally, there was no significant effect of UV and NOUV treatment of the spawn on size of veligers at hatching. The mean diameter of shells of veligers exposed to UV was 134 μm ± 4 SD compared with 133 ± 1.0 μm for those exposed to NOUV (Student's $t = 0.16$, $p = 0.88$, $N = 30$ for each). Adults eating red algae produced 134 ± 1 μm-sized veligers while adults eating green algae produced 132 ± 1 μm-sized ones.

Spawn $\dot{V}_{O_2}$

Table 4 gives the $\dot{V}_{O_2}$s of *Aplysia dactylomela* spawn for all treatment groups. Because of variability in development rates within a single spawn mass and the difficulty in distinguishing between different developmental stages, especially when capsule densities were high, $\dot{V}_{O_2}$s were calculated as daily means for a generalized individual embryo rather than attempting to differentiate between specific developmental stages. These ranged around 20 nl O_2 individual^{-1} d^{-1} for all treatments and all stages.

ANOVA tests on the data in Table 4 revealed that UV-treated adults produced spawn with a significantly higher $\dot{V}_{O_2}$ than spawn from NOUV-treated adults (22 ± 6 SD vs 19 ± 6 nl O_2 individual^{-1} d^{-1}; $F_{1,53} = 4.24$, $p = 0.04$). An interaction of UV and NOUV treatments of the adults and diet was also identified ($F_{1,53} = 7.46$, $p = 0.009$; ANOVA) which segregated into one statistically homogenous subgroup represented by the spawn of NOUV red algae-eating adults (15.5 nl O_2 individual^{-1} d^{-1}) and another subgroup represented by the remaining three combinations (20 to 23 nl O_2 individual^{-1} d^{-1}; $p < 0.05$, N–K test). UV and NOUV treatments of the eggs did not significantly effect their $\dot{V}_{O_2}$ (20 vs 21 nl O_2 individual^{-1} d^{-1}, respectively; $F_{1,53} = 0.40$, $p = 0.53$; ANOVA), nor did diet ($F_{1,53} = 0.52$, $p = 0.48$; ANOVA).

Table 4 *Aplysia dactylomela*. $\dot{V}_{O_2}$ (mean nl O_2 individual^{-1} d^{-1} ± SD) during embryonic development at 28 °C during UV and NOUV treatments of adults, red- or green-alga diet of adults, and UV and NOUV treatments of spawn (*red* diet of red alga, *Acanthophora spicifera*; *green* diet of green alga, *Ulva lactuca*)

Adult treatment, diet	Spawn treatment	(*N*)	$\dot{V}_{O_2}$
UV			
red	UV	(10)	22 ± 4
	NOUV	(11)	24 ± 5
green	UV	(4)	20 ± 10
	NOUV	(6)	21 ± 8
NOUV			
red	UV	(5)	15 ± 3
	NOUV	(6)	16 ± 4
green	UV	(6)	23 ± 6
	NOUV	(6)	21 ± 8

Discussion

UV effects on spawn of *Aplysia dactylomela* were demonstrated in several parts of this study. First, adults exposed to UV exhibited significantly higher $\dot{V}_{O_2}$s than those subjected to NOUV, a likely response to stress (Adams 1990). It is also known that sea hares similarly exposed to natural levels of UV irradiance in shallow tropical waters have elevated levels of blood glucose (Carefoot and Thomas 1993). Blood-glucose level has been shown to be a sensitive indicator of metabolic response to a variety of natural stressors in *A. dactylomela*, including air exposure, elevated temperature, and low salinity (Carefoot 1994). This stress may also explain the nearly two-fold greater number of spawnings by sea hares exposed to UV compared with NOUV groups. Spawning is a general response of freshly collected sea hares and other opisthobranchs brought into the laboratory (Carefoot 1987). It can result from temperature and salinity stresses, as well as from food deprivation.

A second effect of UV exposure of the adults was their production of eggs that hatched faster than eggs from NOUV-exposed adults. This has obvious ecological benefits, but was surprising in view of the fact that UV exposure of the eggs themselves had no deleterious effect on hatching times. Associated with this faster developmental time – and possibly a cause of it – was the significantly higher metabolic rate ($\dot{V}_{O_2}$) recorded for spawn from UV-treated adults compared with NOUV-treated adults.

Exposure of the eggs to UV irradiance markedly reduced hatching percentages. Moreover, adults exposed to UV and fed green algae produced eggs with the lowest hatching percentages of all. Both observations suggest that exposure to UV would be detrimental to the eggs and that a green algae diet would lead to less protection being provided to the eggs. However, UV exposure of the adults did not interact with diet to yield eggs whose survival was differentially affected by exposure to UV during development. In a similar experiment to test the potential role of MAAs as sunscreens in eggs of the sea urchin *Stronglyocentrotus droebachiensis*, Adams and Shick (1996) showed that adults eating red algae incorporated over six times more MAAs (mostly shinorine) into their eggs than adults eating brown algae. The authors further showed that early development in the MAA-rich embryos was delayed significantly less following exposure to UV compared to MAA-poor em-

bryos, thus clearly demonstrating a photoprotective role for MAAs.

MAAs were not differentially distributed in the spawn of *Aplysia dactylomela* in response to UV exposure of the adults. Similar results to these were obtained by Scelfo (1985), who showed no significant difference in the levels of UV-B-absorbing compounds in the zoanthid *Zoanthus pacificus* exposed to natural levels of sunlight for 56 d with and without UV. Treatment times would be expected to be critical in such studies of UV or NOUV exposure, yet results to date are equivocal. For example, Shick et al. (1991) reported only small (but significant) alteration in the concentration of asterina-330 in the octocoral *Clavularia* sp. after a 208 d treatment with and without UV, while Scelfo (1986) recorded significantly higher concentrations of UV-absorbing compounds in the coral *Montipora verrucosa* after only a 13 d exposure to UV. No significant effect of a 28 d exposure to UV on MAA concentrations in the sea anemone *Anthopleura elegantissima* was found by Stochaj et al. (1994), nor of increasing daylength over a 70 d period on the MAA content of the Antarctic sea urchin *Sterechinus neumayeri* during 1991 springtime ozone depletion by Karentz et al. (1997).

Diet strongly influenced amounts of MAAs incorporated into the spawn in *Aplysia dactylomela*, a finding consistent with previous observations on octocorals and sea urchins (Shick et al. 1991; Adams and Shick 1996; Carroll and Shick 1996). Since MAAs are probably diet-derived, we presume that the lesser amounts in spawn from the *Ulva lactuca* diet-groups arose from the absence of MAAs in this alga. A similar finding was recorded for the sea urchin *Strongylocentrotus droebachiensis* by Adams and Shick, who reported that adults eating brown algae produced eggs with substantially less MAAs than those eating red algae. Any MAAs present in the spawn of *U. lactuca*-fed adult *A. dactylomela* could be explained by a latency period during which the MAAs were mobilized from bodily stores, presumably the digestive gland, and distributed to the eggs (see also Adams and Shick 1996). The MAA store would in this case have originated from red algae comprising the field diet of the sea hare prior to the commencement of these studies. How long this store would last and the effects of its depletion on egg vitality are questions for future research. We could discern no obvious pattern of depletion over time for any MAA, but the 2 wk study period might have been too short to reveal this.

Another feature of the MAA data was a disproportionately high concentration of mycosporine glycine in the spawn compared with amounts in the preferred field algal foods, *Acanthophora spicifera* and *Centroceras clavulatum*. The opposite was true for porphyra-334. Such preferential sequestration may be a common feature of invertebrates, with possibly different MAAs being involved in different species. For example, Shick et al. (1992) found highest concentrations of mycosporine-glycine in the gonads of several coral reef-inhabiting holothuroids and, in a later study on sea urchins, Adams and Shick (1996) found shinorine to be the most abundant MAA incorporated into the eggs. The pattern for mycosporine-glycine in *Aplysia dactylomela* could suggest either sequestration from their seaweed food or biochemical transamination from another MAA or other amino acid (Shick et al. 1992; Stochaj et al. 1994). With regard to the first possibility, even though mycosporine glycine is present at a relatively low concentration in *Acanthopora spicifera* (Fig. 2D), it is easy to calculate that there is ample for all spawn produced. Production of 0.43 live g spawn d^{-1} by a standard-sized individual of 100 g live mass (highest value in Table 1) would require provision of 56 μg mycosporine glycine (Table 2, using 0.1 conversion from live to dry mass of spawn). Based on the data in Fig. 2D, this amount would be present in 0.41 g dry wt, or ~4 g fresh wt, of *A. spicifera*. An *Aplysia dactylomela* of 100 g live mass would be expected to eat ~40 g fresh *Acanthophora spicifera* per day (Carefoot and Thomas 1993), more than enough to provide the required mycosporine glycine even if absorption were as low as 10%. A second line of evidence suggesting that mycosporine glycine is diet-derived is the much lower concentration of this MAA in spawn from adult sea hares eating *Ulva lactuca*. This would be expected if bodily stores of MAAs were being exhausted in the absence of a dietary supply of MAAs. While algae-derived sources of MAAs have also been proposed for cnidarians (Dunlap and Chalker 1986; Shick et al. 1991), holothuroids (Dunlap et al. 1991; Shick et al. 1992), and sea urchins (Adams and Shick 1996; Carroll and Shick 1996), the possibility of biosynthetic origins (cf. Stochaj et al. 1994) has not been closely examined. An argument for a dietary origin of MAAs in *Aplysia dactylomela* could be strengthened by monitoring feces for MAAs to show their selective and/or preferential absorption, and monitoring the digestive gland or other tissues and spawn of *U. lactuca*-eating adults over time to reveal eventual exhaustion of MAAs from possible body stores.

Are MAAs involved in UV protection in the eggs of *Aplysia dactylomela*? Our data on hatching percentages show that natural levels of UV can potentially exert strong selective effects on larval survival, and that diet, in this case the green alga *Ulva lactuca*, can further exacerbate these effects. It is apparent from the colour of the spawn that algal pigments contribute in some way to the development and survival of larval sea hares, but at this time we are unable to say what this contribution might be, nor can we state with confidence that MAAs are involved.

Acknowledgements We thank M. Haley, Director of the Discovery Bay Marine Laboratory, University of West Indies, Jamaica, and his staff for providing research facilities for our work. Financial support was provided to D. Donovan and M. Harris through University Graduate Fellowships from UBC to T. Carefoot and B. Taylor through grants from the Natural Sciences and Engineering Research Council of Canada, and to D. Karentz through a research grant, DPP-9017664, from the National Science Foundation of the U.S. We thank CYRO Canada Inc. for generously providing, without charge, the acrylic sheets used in the study.

References

Adams NL, Shick JM (1996) Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin *Strongylocentrotus droebachiensis*. Photochem Photobiol 64: 149–158

Adams SM (1990) Status and use of biological indicators for evaluating the effects of stress on fish. Am Fish Soc Symp 8: 183–191

Biermann CH, Schinner GO, Strathmann RR (1992) Influence of solar radiation, microalgal fouling, and current on deposition site and survival of embryos of a dorid nudibranch gastropod. Mar Ecol Prog Ser 86: 205–215

Bühlmann B, Bossard P, Uehlinger U (1987) The influence of longwave ultraviolet radiation (u.v.-A) on the photosynthetic activity (^{14}C-assimilation) of phytoplankton. J Plankton Res 5: 935–943

Carefoot TH (1987) *Aplysia*: its biology and ecology. Oceanogr mar Biol A Rev 25: 167–284

Carefoot TH (1994) Effects of environmental stressors on blood-glucose levels in sea hares, *Aplysia dactylomela*. Mar Biol 118: 579–583

Carefoot TH, Thomas E (1993) Nocturnalism in sea hares: has UV light played a role in its evolution? Proc 7th int coral Reef Symp 2: 821–826 [Richmond RH (ed) University of Guam Press, Mangilao, Guam]

Carreto JI, Carignan MO, Daleo G, De Marco SG (1990) Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? J Plankton Res 12: 909–921

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

Chioccara F, Zeuli L, Novellino E (1986) Occurrence of mycosporine related compounds in sea urchin eggs. Comp Biochem Physiol 85B: 459–461

Dunlap WC, Banaszak AT, Rosenzweig TT, Shick JM (1991) Ultraviolet light-absorbing compounds in coral reef holothurians: organ distribution and possible sources. In: Yanagisawa T, Yasumasu I, Oguro C, Suzuki N, Motokawa T (eds) Biology of Echinodermata. Proceedings of the Seventh International Echinoderm Conference, Atami, 9–14 September 1990. AA Balkema, Rotterdam, p 560

Dunlap WC, Chalker BE (1986) Identification and quantitation of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. Coral Reefs 5: 155–159

Dunlap WC, Chalker BE, Oliver, JK (1986) Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. J exp mar Biol Ecol 104: 239–248

Dunlap WC, Williams DM, Chalker B, Banaszak A (1989) Biochemical photoadaptation in vision: UV-absorbing pigments in fish eye tissues. Comp Biochem Physiol 93B: 601–607

Garcia-Pichel F, Castenholz RW (1993) Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl envirl Microbiol 59: 163–169

Garcia-Pichel F, Wingard CE, Castenholz RW (1993) Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gleocapsa* sp. Appl envirl Microbiol 59: 170–176

Gleason DF (1993) Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astreoides*. Limnol Oceanogr 38: 1452–1463

Gleason DF, Wellington GM (1995) Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient. Mar Biol 123: 693–703

Glynn PW, Imai R, Sakai K, Nakano Y, Yamazato K (1993) Experimental responses of Okinawan (Ryukyu Islands, Japan) reef corals to high sea temperature and UV radiation. Proc 7th int coral Reef Symp 1: 27–37) [Richmond RH (ed) University of Guam Press, Mangilao, Guam]

Häder D-P, Häder M (1991) Effects of solar and artificial U.V. radiation on motility and pigmentation in the marine *Cryptomonas maculata*. Environ expl Bot 31: 33–41

Jokiel PL (1980) Solar ultraviolet radiation and coral reef epifauna. Science, NY 207: 1069–1071

Jokiel PL, York RH Jr (1982) Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. Bull mar Sci 32: 301–315

Karentz D (1994a) Prevention of ultraviolet radiation damage in Antarctic marine invertebrates. NATO ASI Ser (I: Stratosph Ozone Depl) 18: 175–180

Karentz D (1994b) Ultraviolet tolerance mechanisms in Antarctic marine organisms. Antarctic Res Ser 62: 93–110

Karentz D, Dunlap WC, Bosch I (1997) Temporal and spatial occurrence of UV-absorbing mycosporine-like amino acids in tissues of the antarctic sea urchin *Sterechinus neumayeri* during springtime ozone-depletion. Mar Biol 129: 343–353

Karentz D, McEuen FS, Land MC, Dunlap WC (1991) Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108: 157–166

Lesser MP, Shick JM (1989) Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida*: primary production, photoinhibition, and enzymic defenses against oxygen toxicity. Mar Biol 102: 243–255

Lowe C, Goodman-Lowe G (1996) Suntanning in hammerhead sharks. Nature, Lond 383: p 677

McArdle J, Bullock AM (1987) Solar ultraviolet radiation as a causal factor of 'summer syndrome' in cage-reared Atlantic salmon, *Salmo salar L*: a clinical and histopathological study. J Fish Dis 10: 255–264

Nakamura H, Kobayashi J, Hirata Y (1982) Separation of mycosporine-like amino acids in marine organisms using reversed-phase high-performance liquid chromatography. J Chromat 250: 113–118

Scelfo G (1985) The effects of visible and ultraviolet solar radiation on a UV-absorbing compound and chlorophyll A in a Hawaiian zoanthid. Proc 5th int coral Reef Congr 6: 107–112 [Gabrie C, Harmelin VM (eds) Antenne Museum-EPHE, Moorea, French Polynesia]

Scelfo G (1986) Relationship between solar radiation and pigmentation of the coral *Mortipora verucosa* and its zooxanthellae. In: Jokiel PL, Richmond RH, Rogers RA (eds) Coral reef population biology. Tech Rep Hawaii Inst mar Biol Univ Hawaii, Honolulu 37: 440–451

Shick JM, Dunlap WC, Chalker BE, Banaszak AT, Rosenzweig TK (1992) Survey of ultraviolet radiation-absorbing mycosporine-like amino acids in organs of coral reef holothuroids. Mar Ecol Prog Ser 90: 139–148

Shick JM, Lesser MP, Dunlap WC, Stochaj WR, Chalker BE, Wu Won J (1995) Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooanthellate coral *Acropora microphthalma*. Mar Biol 122: 41–51

Shick JM, Lesser MP, Stochaj WP (1991) Ultraviolet radiation and photooxidative stress in zooxanthellate Anthozoa: the sea anemone *Phyllodiscus semoni* and the octocoral *Clavularia* sp. Symbiosis 10: 145–173

Stochaj WR, Dunlap WC, Shick JM (1994) Two new UV-absorbing mycosporine-like amino acids from the sea anemone *Anthopleura elegantissima* and the effects of zooxanthellae and spectral irradiance on chemical composition and content. Mar Biol 118: 149–156

Wood WF (1987) Effect of solar ultra-violet radiation on the kelp *Ecklonia radiata*. Mar Biol 96: 143–150

Wood WF (1989) Photoadaptive responses of the tropical red alga *Eucheuma striatum* Schmitz (Gigartinales) to ultra-violet radiation. Aquat Bot 33: 41–51