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Differential meiospore size and tolerance of ultraviolet light stress within and among kelp species along a depth gradient

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Abstract Kelp are differentially stratified along a gradient of UV exposure (as a function of water depth). The role of ultraviolet light in seaweed zonation has not been fully explored. This study found a significant meiospore size difference within and among the kelp species examined: *Pterygophora californica* Ruprecht (high to mid-subtidal), *Macrocystis integrifolia* Bory (high to mid-subtidal), *Laminaria groenlandica* Rosenvinge (high-subtidal), *Alaria marginata* Postels and Ruprecht (low-intertidal) and *Hedophyllum sessile* Setchell (mid-intertidal). This size difference was correlated with the depth distribution of adult plants, with the largest meiospores originating from shallow-dwelling adult kelp exposed to high UV light. Under UV stress in the laboratory, meiospores from adults growing in high-UV environments displayed greater germination and survival rates than the progeny of adult kelp occupying lower-UV environments. This suggested that in Barkley Sound, British Columbia, Canada, differential tolerance to UV (possibly determined by meiospore size) may limit the upper settlement position of kelp species and individuals. Tolerance to UV may be an important determinant of kelp zonation on rocky shores.

Introduction

The causes of seaweed zonation along coastal shores has been the subject of much debate (Druehl and Green 1982; Norton 1986; Lüning 1990; Lobban and Harrison 1994; Davison and Pearson 1996). Biotic and abiotic mechanisms, often in combination, have been proposed as important factors determining zonation. One such mechanism is light exposure, specifically photosynthetically active radiation (PAR) (~400 to 700 nm) (Kain 1966; Druehl and Hsiao 1977; Gerard 1988; Graham 1996). UV light has also been suggested as a major environmental stress on aquatic phototrophic organisms (Franklin and Forster 1997). Increases in surface-level ultraviolet exposures caused by anthropogenic air pollutants inducing stratospheric ozone depletion (Kerr and McElroy 1993), have focused attention on the effects of the solar UV spectrum (UV-A: 399 to 321 nm; UV-B: 320 to 290 nm) on algae species and on algae communities (Wood 1987; Larkum and Wood 1993; Post and Larkum 1993; Clendennen et al. 1996; Dring et al. 1996; Franklin and Forster 1997).

In marine and aquatic habitats, UV light can be attenuated dramatically along vertical depth gradients (Flamarique and Hawryshyn 1993; Holm-Hansen et al. 1993; Franklin and Forster 1997); and in the dynamic, coastal, light environment, differential tolerance to UV stress among and within algal species may be partially responsible for structuring communities. Typical of most nearshore coastal waters (Franklin and Forster 1997), summer seawater penetration of UV radiation (UVR) is low in Barkley Sound, British Columbia, Canada with 96% of UV light attenuated by 3 m depth (Flamarique and Hawryshyn 1993). Average extreme UV (Diffey-weighted) in southern British Columbia (1996 to 1998) ranges from a summer high of 227 mW m^{-2} to a winter low of 2.5 mW m^{-2} (Taylor 1998). Weekly monitoring of PAR light in Barkley Sound (1979 to 1981) showed surface-level PAR and seawater extinction coefficients (k) ranging from 1600 μmol m^{-2} s^{-1} and 0.5 m in summer to 30 μmol m^{-2} s^{-1} and 0.09 m in winter (Druehl 1981).

Among phytoplankton species, UV radiation has been shown to be responsible for a decrease in species diversity and a shift in community structure to larger-

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celled species more tolerant of UV exposure (Worrest et al. 1978; Karentz et al. 1991a; Bothwell et al. 1993). Karentz et al. (1991a) observed fewer UV-induced photoproducts in larger-celled species of phytoplankton following periods of UV stress. Many physiological processes are likely to contribute to the overall UV tolerance of organisms. In single-celled organisms, however, a larger cell size or a low surface area:volume ratio can reduce nuclear DNA damage, the primary target of lethal UV exposure (Rozema et al. 1997), by creating a greater depth of UV-absorbent cytoplasm and thus impairing the transmission of UV photons (Karentz et al. 1991a; Mitchell and Karentz 1993).

Kelp also possess a single-celled stage in their life cycle, the meiospore. Haploid meiospores develop within unilocular sporangia found either within sporophylls or in soral patches along the blade (Scagel et al. 1982). Upon maturation, pyriform or bean-shaped meiospores disperse via flagella and water motion until their settlement on substratum; they then retract their flagella and secrete a cell wall. Although spores can be found kilometers away from the source of origin (Reed et al. 1988), 95% of effective recruitment occurs centimeters from parental plants (Kendrick and Walker 1991). Settlement is followed by germination and differentiation into haploid male and female filamentous gametophytes (Scagel et al. 1982). Following fertilization by a motile sperm, syngamy, zygote formation and the development of a diploid embryonic sporophyte occur on the female gametophyte.

Recently, Dring et al. (1996) demonstrated the higher sensitivity of meiospores and gametophytes of *Laminaria* sp. to UV exposure compared to juvenile and mature sporophyte stages. The haploid nature of meiospores and gametophytes are probably responsible for this observed vulnerability; however, changes in surface area to volume ratios of cells at the various stages of a kelp's life cycle may also be responsible. Our study tested four hypotheses to ascertain the potential role of kelp meiospore size and UV light as determinants of kelp zonation on rocky shores: (1) meiospore cell size varies as a function of kelp depth-distributions, (2) interspecific or (3) intraspecific differences in the ability of a kelp meiospore to survive UV stress exist within populations, and (4) meiospore cell-size determines its ability to survive UV-light stress. These hypotheses were tested by observing the natural distribution of several kelp species and determining their meiospore sizes and tolerances to artificial UV-light stress.

Materials and methods

Kelp collections and spore release

Collections were made from Scott's Bay and Brady's Beach, Barkley Sound, Bamfield, British Columbia, Canada (48°50′N; 125°08′W) from February to November 1997. Kelp species were selected based on their representative position within the water column. Ten individuals of each species sharing similar tidal positions and the presence of mature sori were selected. Tidal height was measured from the lowest predicted tidal height (LLW).

To achieve spore release, soral patches were vigorously wiped with a paper towel and agitated in 0.07% iodine (Aspen Veterinary Resources Ltd., Kansas City, Missouri) seawater solution three times for 10 s each time. Sori were then rinsed with sterile Millipore™-filtered (0.22 μm) seawater, blotted dry, and left for 12 h in plastic bags at 10 °C. The sorus from each individual was then placed into sterile glassware containing filtered seawater and kept under bright light for 1 to 3 h at 10 °C. After spore release, excess mucilage was removed by straining through cheesecloth.

Meiospore size

To test the hypothesis that kelp meiospore size was related to the water depth of the parental plant, soral tissues were removed from individuals of five kelp species collected on 12 November 1997 [deep *Pterygophora californica* (−10.5 m) and shallow *P. californica* Ruprecht (−1.5 m)], on 29 July 1997 [deep *Macrocystis integrifolia* (4.5 m) and shallow *M. integrifolia* (0 m)], and on 5 February 1997 [deep *M. integrifolia* Bory(−4.5 m), *Laminaria groenlandica* Rosenvinge (−1 m), *Alaria marginata* Postels et Ruprecht (+0.25 m), and *Hedophyllum sessile* Setchell (+1.25 m)]. Meiospore release was induced using the methods described in the foregoing subsection, and densities from each individual were measured with a haemocytometer. Conspecific spores from the same tidal heights were combined in equal numbers. The resulting mixture was then diluted to 5000 actively-swimming meiospores per 100 μl. Prior to size measurements, meiospore mixtures were kept under 38 μmol m^{-2} s^{-1} PAR (1 h light:3 h dark:1 h light) at 10 °C to allow settlement of contaminating diatoms/debris and to provide effective controls for the experiment described in a later subsection "Meiospore size and UV-B survival". To determine meiospore size for each representative species, meiospores (Fig. 1) were examined microscopically on an ice-chilled haemocytometer to prevent heat damage. To eliminate confounding data from immature or abnormal meiospores, only actively swimming meiospores were measured. Meiospore images were captured using a bifocal microscope fitted with a JVC KY-555BU color video camera and PC-compatible "Snappy Video Snapshot" image-capture software (Play Inc., Rancho Cordova, California). Images of meiospores were measured using SigmaScan Pro 4.0 image-analysis software

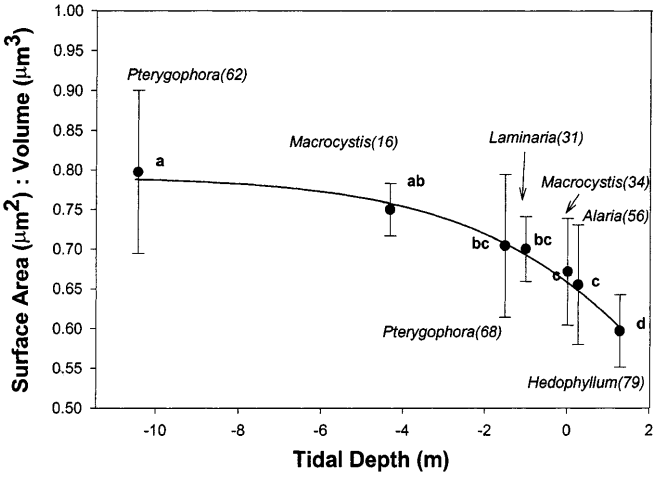

Fig. 1 Relationship between surface area (μm²) to volume (μm³) ratio of swimming kelp meiospores and depth origin of parent plant [*Vertical bars* standard deviations; *numbers in parentheses* sample sizes; *letter groups* significant difference ($p < 0.05$; Dunn's method)] Line was fit by sigmoidal regression analysis; surface area:vol = $0.7949 \div [1 + \exp(-(\text{depth}-4.63) \div -2.904))]$; $r^2 = 0.981$, $F = 108.1007$, $df = 2$, $p = 0.0003$

(Jandel Corp., San Rafael, California) and known size-standards. Resolution of images with this system could detect a 0.012 μm difference in size. Measurements of pyriform-shaped meiospores were converted to the surface area [perimeter × ($2\pi r \div 2$)] and volume [surface area × ($2\pi r \div 2$)] for each spore, with r equal to the minimal radius. The combined surface area:volume ratios of all test groups were examined for significant differences using a one-way ANOVA (Sigma Stat for Windows 2.0, Jandel Corporation San Rafael, California; Zar 1984). A pairwise multiple-comparison procedure (Dunn's method) was used to test for significant differences among the groups (Sigma Stat 2.0; Zar 1984). Regression analysis was used to illustrate any trends with respect to surface area:volume ratios and tidal position of the parent plant.

Interspecific UV effects on meiospore germination

To test the hypothesis that interspecific differences in the ability to tolerate UV exposure occur among kelp species, meiospores of deep *Macrocystis integrifolia*, *Laminaria groenlandica*, *Alaria marginata* and *Hedophyllum sessile* from the meiospore size experiment were used. On 5 February 1997, meiospores were distributed (5000 per well) into five single-species tissue-culture wells (4 treatment times + 1 control) on three separate multiwell tissue-culture plates (3 light treatments). The wells were then filled with chilled Millipore-filtered seawater to a depth of 1 cm. Three multiwell plates were then covered by either 0.08 mm cellulose diacetate sheets (light transmission >290 nm), clear 0.08 mm polyester (light transmission >320 nm, Cadillac Plastics Ltd., London, Ontario), or 3 mm clear Lexan™ (light transmission >400 nm, General Electric Canada). Plates were transferred to a 10 °C refrigerated incubator, and the meiospores were exposed to either 30, 60, 120 or 240 min light treatment by sliding UV-blocking Lexan™ sheets over the treatment wells. Incubators were fixed with one UV-B fluorescent tube (Light Sources FS20T12 UV-B, Kelsun Distributors, Bellingham, Washington; 25 mW m^{-2}) (Fig. 2) and two cool-white fluorescent tubes (F20T12/CW 20 W, Phillips Canada) (~31 to 38 μmol m^{-2} s^{-1} PAR). PAR levels were measured with a Li-1000 datalogger (LiCor Instruments, Lincoln, Nebraska) fitted with a Li-193SA Spherical Quantum Sensor (LiCor Instruments, Lincoln, Nebraska). UV-B was measured with a Bluewave BW-10 ultraviolet monitor (Vital Technologies, Bolton, Ontario). UV-A was not measured, but, was known to be present in the spectra of the UV-B tube.

After treatments had been completed, the UV-B tube was removed and the meiospores, under Lexan sheets, were allowed to settle and develop (10 °C, 12 h light:12 h dark) after an initial 12 h dark cycle. Settled meiospores within each well were assessed for germination microscopically at 24, 48 and 96 h post light-treatment. All multiwell plates were assessed under the microscope in a 10 °C coldroom to avoid mechanical injury, dislodgement, or heat stress. Fifty haphazardly encountered meiospores within each well were scored for the presence of germ tubes, indicating germination, at 24, 48 and 96 h post-treatment. The 96 h post-treatment assessment of meiospore germination was selected for statistical comparisons, as all controls had reached maximum observed germination levels (Table 1) and previously noted deformations and lysis of germinated and non-germinated meiospores were no longer observed. Regression analysis and 95% confidence intervals were used to compare treatment groups (Zar 1984). UV-B exposure corresponding to 37% germination/survival of meiospores was calculated from regression lines and used to determine the D_{37} values for each species (Table 2). D_{37} is a standard calculation used in radiobiology for survival-curve analysis and species comparison (Jagger 1976; Karentz et al. 1991a), and D_{37} values represent the UV exposure (J m^{-2}) that causes the same level of mortality in each species and is the equivalent to the average exposure that would causes one lethal event per cell. The smaller the D_{37} value, the greater the sensitivity of the cell.

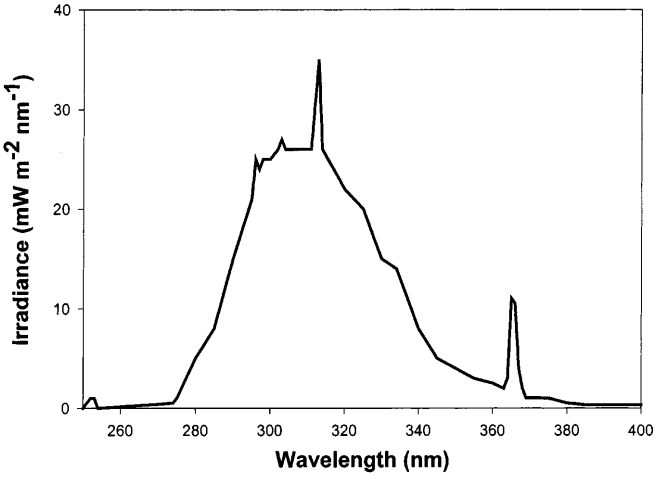

Fig. 2 Spectral output of UV-B fluorescent tube (Light Sources FS20T12 UV-B)

Intraspecific effect of UV-B on meiospore germination

To test the hypothesis that intraspecific differences in the ability to tolerate UV exposure exist among kelp populations and that tolerance is related to the depth of the parental plant, ten *Pterygophora californica* (−1.5 and −10.5 m; 12 November 1997) and ten *Macrocystis integrifolia* (0 and −4.5 m; 25 July 1997) were collected by SCUBA from both the shallowest and deepest locations of vertically continuous populations. Meiospores were released from each individual and pooled in equal numbers with conspecifics from the same tidal height. Meiospores were distributed (5000/well) in 96-well, multiwell plates, 1 cm deep. Using the same light incubator, replicates (n = 10) covered by 0.08 mm cellulose diacetate sheets (light transmission >290 nm), were subjected to light treatments of 30, 60, 120 or 240 min of UV-B + UV-A + PAR light exposure (UV-B: 25 mW m^{-2}, PAR: 38 μmol m^{-2} s^{-1}). Controls were covered by UV-blocking Lexan™ (light transmission >400 nm; PAR: 38 μmol m^{-2} s^{-1}). After 96 h treatment, using the methods described in the preceding subsection, 50 haphazardly encountered meiospores were scored for germination. Groups were compared using a one-way ANOVA, and differences among groups were determined with the Student–Newman–Keuls (SNK) multiple-comparison method (Sigma Stat 2.0, Jandel Corp., San Raphael, California; Zar 1984). D_{37} values of each test group were determined from predicted regression lines for interspecific and intraspecific comparisons (Table 2).

Meiospore size and UV-B survival

To test the hypothesis that meiospores with small surface area to volume ratios (sa:vol) would survive UV exposure better than meiospores with a large sa:vol, *Macrocystis integrifolia* (−4.5 m), *Alaria marginata* (+0.25 m) and *Hedophyllum sessile* (+1.25 m) meiospore releases from the earlier experiment (subsection "Meiospore size") were inoculated into single-species petri dishes with filtered seawater (10 °C) to a depth of 1 cm, under low PAR light (38 μmol m^{-2} s^{-1}). Using premarked locations in each species' dish, we made periodic microscopic examinations to assess meiospore settlement and meiospore activity. Settlement and activity were assessed under the microscope in a 10 °C coldroom. After 1 h, all settling meiospores had been observed to be active (alive) prior to experimentation. Unsettled meiospores were poured off and replaced with filtered seawater to a depth of 1 cm. Settled meiospores were then exposed to 1 h PAR (38 μmol m^{-2} s^{-1}) + UV-A + UV-B (25 mW m^{-2}), followed by 3 h darkness and 1 h PAR (38 μmol m^{-2} s^{-1}). Cells were then incubated in an 0.01% solution of Evans Blue vital stain (Fisher Scientific Company, Ottawa, Ontario). After 30 min, the stain was washed away and settled meiospores were scored microscopically (400×) for survival,

Table 1 Germination (%) of 50 haphazardly encountered kelp meiospores exposed to UV-B radiation (25 mW m^{-2}) 24, 48 and 96 h after light treatment [(+) represents each species, maximum germination rate for each UV-B exposure-time treatment (0, 30, 60, 120, 240 min) among all post-treatment assessments (24 h, 48 h, 96 h)]

Post-treatment assessment	UV-B exposure time (min)	*Macrocystis integrifolia* (−4.5 m)	*Laminaria groenlandica* (−1 m)	*Alaria marginata* (+0.25 m)	*Hedophyllum sessile* (+1.25 m)	Average germination rate (%)
24 h	0	62	30	30	68	47.5
	30	6	18	20	32	19
	60	2	6	12	6	6.5
	120	0	0	8	0	2
	240	0	0	0	0	0
48 h	0	70	40	48	72	57.5
	30	26 (+)	22	18	34	25
	60	16 (+)	22 (+)	26 (+)	30 (+)	23.5 (+)
	120	0	8 (+)	16 (+)	24 (+)	12 (+)
	240	0	0	4 (+)	26 (+)	7.5 (+)
96 h	0	72 (+)	56 (+)	54 (+)	86 (+)	67 (+)
	30	14	26 (+)	28 (+)	52 (+)	30 (+)
	60	0	2	12	22	9
	120	0	0	4	12	4
	240	0	0	0	2	0.5

as indicated by the absence of stain within the cytoplasm (Saga et al. 1989). Since this level of PAR exposure had not been observed to be damaging to meiospores in the previous experiment (subsection "interspecific UV effects on meiospore germination") nor in previous experiments (Graham 1996) and all meiospores were alive prior to experimentation, it was assumed that UV light stress was responsible for observed meiospore mortality. Surface area and volumes of observed live and dead meiospores were recorded (SigmaScan Pro 4.0; Jandel Corporation San Raphael, California) and compared statistically to each other and to control, non-UV-treated meiospores (population means) of the experiment on meiospore size using a one-way ANOVA and Tukey multiple-comparisons (Sigma Stat 2.0, Jandel Corporation; Zar 1984).

Results

The surface area to volume ratio of swimming kelp meiospores in relation to the depth of the parental plant followed a sigmoidal relationship: surface area:volume = $0.7949 \div [1 + \exp(-(\text{depth}-4.63) \div -2.904))]$ (Fig. 1). The regression line of group means ($r^2 = 0.981$, $n = 7$) was significant (one-way ANOVA; $F = 108.1007$, $df = 2$, $p = 0.0003$). Heteroscedasticity of groups could not be corrected through geometric transformation, therefore a non-parametric test was employed (Kruskal–Wallis one-way analysis of variance on ranks) which revealed significant differences among the tested species ($H = 166.184$, $df = 6$, $p < 0.001$). Pairwise multiple-comparison tests (Dunn's method) indicated that significant ($p < 0.05$) interspecific differences existed and that *Hedophyllum sessile* possessed the largest meiospores and deep *Pterygophora californica* possessed the smallest meiospores (Fig. 1). Significant intraspecific difference in meiospore sizing was also observed between shallow and deep stands of *Macrocystis integrifolia* and *P. californica*. Specifically, deep *M. integrifolia* and *P. californica* had significantly smaller meiospores than shallower conspecifics.

Observations of meiospore germination under PAR, UV-A + PAR, and UV-B + UV-A + PAR light-treatments demonstrated several trends. Based on comparisons of 95% confidence intervals of predicted regression lines of the 96 h post-treatment observations (Fig. 3), *Hedophyllum sessile* meiospores were less inhibited by increasing UV-B exposure than were those of deep *Macrocystis integrifolia* and *Laminaria groenlandica* at all time intervals, as well as A*laria marginata* meiospores at exposures under 2 h (Fig. 3). *A. marginata*

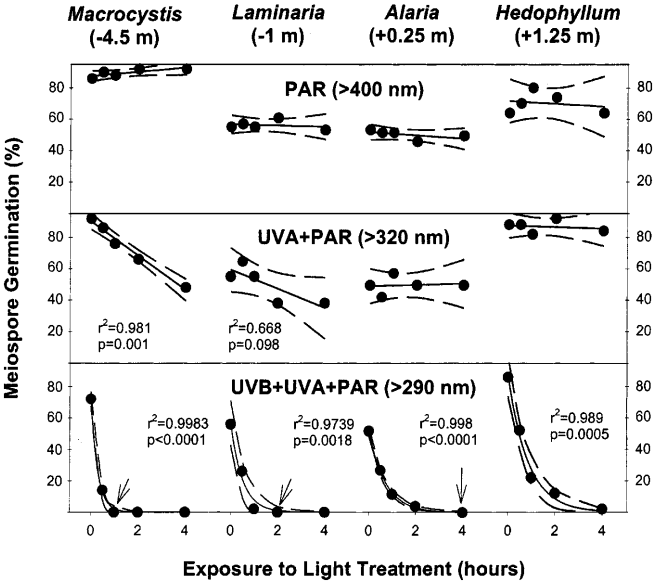

Fig. 3 Relationship between increasing light exposure and germination of kelp meiospores from different species and tidal depths (*Data points* germination of 50 meiospores scored 96 h post-treatment; *arrows* zero germination treatments) Lines were fit by linear and polynomial regression analysis (*dashed lines* 95% confidence intervals) UV-B exposure = 25 mW m^{-2}, PAR = 38 μmol m^{-2} s^{-1}

meiospores were less inhibited by increasing UV-B than were all deep *M. integrifolia* meiospore treatments and *L. groenlandica* meiospores exposed to UV-B under 2 h. Treatment groups exposed to UV-B light possessed regressions of exponential decay that were all significant at $p < 0.001$; deep *M. integrifolia* ($r^2 = 0.9983$, $F = 1773$, $df = 1$, $p < 0.0001$), *L. groenlandica* ($r^2 = 0.9739$, $F = 111.97$, $df = 1$, $p = 0.0018$), *A. marginata* ($r^2 = 0.998$, $F = 1603.72$, $df = 1$, $p < 0.0001$), *H. sessile* ($r^2 = 0.989$, $F = 271.42$, $df = 1$, $p = 0.0005$). A relationship existed between inhibition of meiospore germination and the depth of the kelp species. *M. integrifolia* meiospores from −4.5 m no longer germinated after 1 h UV-B exposure, whereas *H. sessile* meiospores at +1.25 m depth continued to germinate even after 4 h UV-B exposure. Deep *M. integrifolia* meiospores were also inhibited by increasing UV-A light ($r^2 = 0.981$, $F = 154.29$, $df = 1$, $p = 0.001$). No other species were inhibited by UV-A or by the PAR treatments. Observations of 24, 48 and 96 h germinations indicated that meiospore germination was suppressed by exposure to UV-B (Table 1).

Varying inhibition of meiospore germination under increasing UV-B exposure was also observed between shallow and deep populations of *Pterygophora californica* and *Macrocystis integrifolia* (Fig. 4). Equal variance of means could not be achieved through geometric transformation; therefore, a non-parametric test (Kruskal–Wallis one-way ANOVA on ranks) was used to determine significance among groups. Germination was not observed in any of the 120 or 240 min treatments, and was therefore not included in statistical comparisons. Statistical tests revealed significant differences in germination among species (Kruskal–Wallis *H*-statistic = 106.134, $df = 11$, $p < 0.001$). Pairwise multiple-comparison tests revealed significant differences among the groups ($p < 0.05$). Shallow *M. integrifolia* meiospores exposed to 30 and 60 min UV-B were less inhibited by increasing UV-B exposure than similarly treated conspecifics from the deeper location. Germination of *M. integrifolia* meiospores ceased after 30 min UV-B exposure for deep plants, but only after 60 min exposure for the shallow plants. Multiple-comparison tests revealed significant differences ($p < 0.05$) in germination rates of deep and shallow *P. californica* plants exposed to 30 min UV-B, but not in those exposed to 60 min UV-B. Shallow *P. californica* plants also continued to germinate after 1 h UV-B exposure, whereas the deep plants ceased to germinate after 30 min. The results of *P. californica* germinations, however, should be viewed cautiously, since shallow and deep control groups were not statistically equal. Overall, shallow *M. integrifolia* meiospores were found to be least inhibited by UV-B exposure and deep *P. californica* meiospores most inhibited by UV-B exposure.

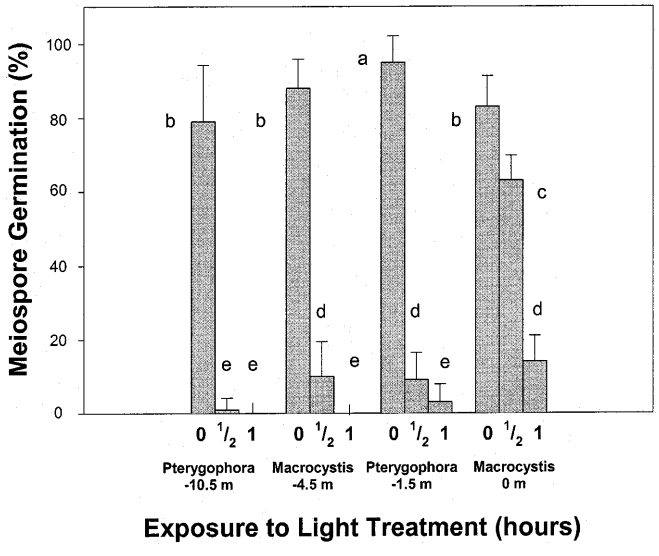

Fig. 4 Germination of kelp meiospores 96 h after light-treatment [*Histograms* mean germination of 10 replicate groups of 50 meiospores each; *error bars* standard deviations; *letter groups* significant difference (SNK multiple-comparison test, $p < 0.05$)] UV-B exposure = 25 mW m^{-2}, PAR = 38 μmol m^{-2} s^{-1}

Among UV-B exposed meiospores, adjusted D_{37} values (normalized to 100% germination for controls) ranged from a high of 77 J m^{-2} for *Hedophyllum sessile* to a low of 11 J m^{-2} for deep *Pterygophora californica* (Table 2). Adjusted D_{37} values were positively correlated with the depth of the parental plant ($r = 0.811$, $p < 0.05$, $n = 7$).

The use of a vital-cell stain (Evans Blue) demonstrated that meiospore size (sa:vol) was related to survival to UV-B exposure. In all tested species (deep *Macrocystis integrifolia* ($df = 2$, $F = 25.4$, $p < 0.001$), *Alaria marginata* ($df = 2$, $F = 29.97$, $p < 0.001$) and *Hedophyllum sessile* ($df = 2$, $F = 30.71$, $p < 0.001$)), dead meiospores possessed significantly larger sa:vol ratios than live meiospores and non-UV-treated control/population mean meiospores ($p < 0.05$; Tukey multiple-comparison) (Fig. 5). The mean sa:vol of control/population meiospores was not significantly different than the sa:vol of live meiospores. Although variation in size of meiospores within a single individual was not grossly apparent to the authors (personal observation), the results should be viewed cautiously, as possible variation within an individual (not measured in this study) may have biased the findings based on small sample sizes.

Discussion

Our results suggested that the ability of a kelp meiospore to germinate and survive under periods of UV-B stress is related to its size. Kelp meiospore size, germination/survival rates and D_{37} values were all related to the tidal depth and/or UV exposure of the parent plant. These results are similar to studies of phytoplankton species (Worrest et al. 1978; Karentz et al. 1991a; Bothwell et al. 1993), in which larger single-celled organisms were

Table 2 Average cell dimensions of kelp meiospores and relative sensitivity to UV-B exposure (D_{37}), in order of decreasing depth. Controls normalized to 100% germination for adjusted D_{37} ($AdjD_{37}$). Depth of parental plants was measured from lowest predicted tidal height (lower low water)

Species	Depth (m)	sa:vol	D_{37} (J m^{-2})	Adj D_{37} (J m^{-2})	Surface area (μm^2)	Vol (μm^3)	Length (μm)	
							max.	min.
Pterygophora californica	−10.5	0.798	8	11	84.29	110.06	7.55	2.42
Macrocystis integrifolia	−4.5	0.749	17/17[a]	24/21[b]	157.43	210.63	6.54	5.10
Pterygophora californica	−1.5	0.704	18	20	118.09	173.16	7.97	3.20
Laminaria groenlandica	−1	0.700	20	47	178.93	257.75	7.54	5.26
Macrocystis integrifolia	0	0.671	62	72	199.96	306.11	7.76	5.58
Alaria marginata	0.25	0.655	24	63	213.18	339.00	8.25	5.67
Hedophyllum sessile	1.25	0.597	66	77	251.95	428.65	9.11	6.15

[a,b] 5 February 1997 and 29 July 1997 *Macrocystis integrifolia* collections, respectively

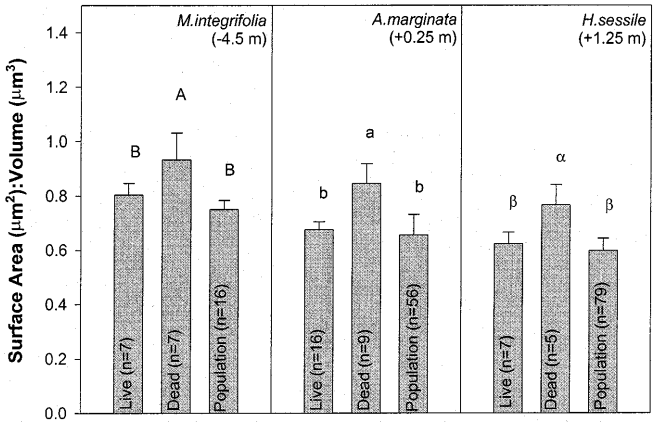

Fig. 5 Comparison of surface area (μm^2) to volume (μm^3) ratio of meiospores from live, dead and control-population kelp following 1 h PAR (38 μmol m^{-2} s^{-1}) plus UV-B (25 mW m^{-2}), 3 h dark and 1 h PAR (38 μmol m^{-2} s^{-1}), as determined by Evans Blue vital-cell stain [*Histograms* mean surface area:volume; *error bars* standard deviations; *letters* significant difference (Tukey multiple-comparison test; $p < 0.05$)]

found to be more tolerant of single- and long-term exposure to UV. The present study also concurs with the suggestion that sa:vol ratios of single-celled organisms are an indicator of UV sensitivity (Karentz et al. 1991a). Kelp meiospore sa:vol ratios and parental settlement depth followed a sigmoidal relationship, with the smallest meiospores (large sa:vol) found in deep locations and the largest meiospores (small sa:vol) in shallow locations. The asymptotic nature of meiospore size at −3 to −5 m indicates a possible depth limit of significant UV light stress on Barkley Sound kelp species. UV levels measured previously at these depths in Barkley Sound have been 4 to 0.6% of levels just below surface waters at noon in midsummer (Flamarique and Hawryshyn 1993). Changes in water pressure may also partially determine meiospore size; however, the linear nature of pressure change with depth and the sigmoidal nature of our data are more consistent with non-linear attenuation of UV in seawater.

To our knowledge, this is the first study to illustrate any species-specific size differences among kelp meiospores, and the first to implicate UV-B and meiospore size as possible mechanisms limiting the upper settlement positions of kelp species and kelp populations. Although not intended to simulate normal solar exposures, laboratory exposures of PAR (38 μmol m^{-2} s^{-1}) and UV-B (25 mW m^{-2}) were, respectively, approximately equal and 10 times higher than the observed low levels in surface sunlight encountered during winter (Druehl 1981; Taylor 1998). Laboratory light levels of PAR and UV-B were 1/200 and 1/10, respectively, of high sunlight extremes encountered during summer. However, due to the timing of tides, the seasonality of UVR and the light-attenuating qualities of nearshore coastal waters, benthic seaweed species are likely to encounter quite different light regimes than those encountered at the sea surface. Previously reported lethal solar exposures of kelp gametophytes (which has included UVR) have ranged from 850 (Graham 1996) to 1000 (Lüning and Neushul 1978) μmol m^{-2} s^{-1} PAR. UVR exposure may be more damaging to macroalgae when combined with excessive PAR irradiation (Lüning 1980; Dring et al. 1996). Although not examined in the present study, UVR radiation in combination with excessive solar PAR light may ultimately determine and give rise to the observed differences in meiospore size and UVR tolerance found within natural kelp populations. Field studies utilizing natural solar exposures and UV-A and UV-B selective filters as well as continuous in situ measurements of light exposures are needed to help resolve these issues. Nonetheless, this study demonstrates that kelp species under low PAR light conditions are differentially vulnerable to UV-A and UV-B exposure.

Recently, Dring et al. (1996) recorded the germination rates of three *Laminaria* species following periods of UV stress. They could not correlate the general settlement patterns of the kelp species with tolerance to UV. Several factors, including differences among the species tested, the amplitude of tidal depth tested, local variations in UV or experimental protocols, may explain this result. Our comparison of germination rates at 96 h compared to Dring et al. 's 24 h post-treatment assessment of meiospore germinations was a likely source of this disparity. The 96 h assessment was used because

control groups had reached maximal germination levels and previously observed meiospore lysis and deformation of germ tubes were no longer observed within experimental cultures. Graham (1996), using natural sunlight and epifluorescence microscopy, noted that pigmentation of germinated meiospores of *Macrocystis pyrifera* Agardh decreased 36 h after first exposure to natural UV, indicating a delayed cell stress or degeneration independent of normal germ-tube formation. Delayed cell-lysis following periods of UV exposure may explain the temporarily high germination rates observed during the present study and, in part, the disparity between our findings and that of Dring et al. Future studies utilizing germination as a means of assessing kelp cell survival should consider delayed effects of environmental stresses. Meiospore germinations were suppressed following UV-B stress, similar to observations on higher plants, in which seed germination and emergence have been altered by periods of UV exposure (Bornman and Teramura 1993; Barnes et al. 1995; Rozema et al. 1997). These observed intraspecific differences in germination rates and meiospore sizes indicate that natural populations may be pre-adapted genetically or phenotypically to the UV conditions of the parent plant; thus, tidal depths of all plant materials should be closely monitored.

The reduction of UV-induced photoproducts in larger celled organisms, as observed by Karentz et al. (1991a), as well as the increased germination and survival of larger kelp meiospores following periods of UV-B stress, suggests that increasing cell volume or cytoplasm depth has a filtering effect on UV-B which might otherwise impact DNA, the primary chromophore, or other UV-vulnerable cellular constituents. Possible components of the cytoplasm that may decrease UV-B impact through cytoplasmic absorption include RNA, proteins, dense cellular organelles, or organelles containing pigments (Karentz et al. 1991a) such as UV-absorbing mycosporine-like-amino acids (Karentz et al. 1991b). Any species-specific differences in the quantity or quality of these compounds may alter susceptibility to UV-induced DNA damage.

The prevalence of larger, more UV-tolerant meiospores originating from species or populations from sites exposed to high UV light (high tidal height) suggests that kelp meiospores are pre-adapted to the UV conditions of the parent plant. Whether this ontogeny reflects genetic adaptation or is another example of kelp phenotypic plasticity could not be ascertained with the design of this experiment. Nonetheless, genetic adaptations to PAR light have been previously demonstrated within the Laminariales (Gerard 1988), and may also exist for potent environmental stresses such as UV. Future work should consider the use of common garden experiments to assess whether kelp meiospore-size represents a genetic or phenotypic adaptation to ambient UV conditions.

Most kelp gametophytes are sexually dimorphic, with the males having smaller cell widths than female conspecifics (Fritsch 1959; Druehl et al. 1989). The ratio of male to female gametophytes is 1:1 (Schreiber 1930). Sakanishi and Saga (1991) observed female *Macrocystis pyrifera* gametophytes, which were considered to be larger than males, to be nearly twice as tolerant to UV exposure. This finding suggests that, like the meiospore, the UV vulnerability of a gametophyte cell is related to size. If the size of a cell is indicative of vulnerability to UV (Karentz et al. 1991a), another effect of increased UV light on nearshore communities might be to disrupt normal sex ratios, favoring female gametophytes and reducing successful fertilization. Whether this is presently a natural feature of current gametophyte assemblages is unknown, as little direct information is available regarding kelp gametophytes and sex ratios in the natural environment.

Because latitude and water clarity both affect UV and PAR light exposure (Holm-Hansen et al. 1993), global seaweed zonation patterns may reflect the combined effect of these two variables on local seaweed communities. How currently observed increases in UV-B exposure (Kerr and McElroy 1993) are affecting seaweed zonation patterns is also a matter of speculation. The variation in intraspecific meiospore size and UV tolerance within populations of *Pterygophora californica* and *Macrocystis integrifolia*, however, suggest that a potential for adaptation already exists. Alternatively, meiospore establishment within the upper tidal range of a kelp species may become increasingly difficult with increasing UV-B levels, limiting meiospore development to deeper or more turbid locations. In the subtidal zone of Barkley Sound, herbivore pressure by sea urchins (Pace 1981) and light competition with established perennial or canopy-forming kelp species may preclude re-establishment of vulnerable kelp species to lower-UV sites, resulting in their local extirpation. The greater resistance of kelp sporophytes to UVR compared to conspecific gametophytes or meiospores (Dring et al. 1996) suggests that perennial kelp species and species capable of vegetative propagation (Druehl and Kemp 1982; Graham 1996) may resist UVR better than annual kelp species or species with obligate sexual processes. Whether the UV-inducible phlorotannin exudate of kelp, which has been shown to reduce UVR seawater penetration and UV-B damage to developing kelp meiospores (Swanson and Druehl unpublished data), also mitigates UVR damage to meiospores within established kelp beds remains to be tested. Undoubtedly biotic and other abiotic factors, such as excessive PAR irradiation, also contribute to the successful settlement patterns of kelp species. However, our experiments suggest that differential tolerance to UV, possibly determined by meiospore size, may operate to limit the upper tidal positions of kelp species and individuals within kelp populations.

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