

Variation in sunscreen compounds (mycosporine-like amino acids) for marine species along a gradient of ultraviolet radiation transmission within Doubtful Sound, New Zealand

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Abstract We examined the response of four species of New Zealand marine algae (*Ecklonia radiata*, *Apophlaea lyallii*, *Rhodymenia* spp., *Ulva lactuca*) and a sea urchin (*Evechinus chloroticus*) to spatial variation in ultraviolet radiation (UV-R) by examining the concentration of UV-R absorbing compounds known as mycosporine-like amino acids (MAAs). The purpose was to understand how, and the degree to which, local marine species could potentially respond to any future increases in incident UV-R in the New Zealand marine environment. The research was undertaken in Doubtful Sound, where we observed a gradient of water column UV-R transmission along the 40 km length of the fiord. We examined spatial differences in MAAs along the UV-B gradient in the macrophytes and temporal changes in MAAs in sea urchin gonads. Among the algae, thallus MAA concentrations (nmol mg⁻¹ protein) ranged from 12.5 to 87.8 in *E. radiata*, from 433.1 to 1446.4 in *A. lyallii*, 12.7 to 103.4 in

Rhodymenia spp., but were not detected in *U. lactuca*. For *E. chloroticus*, gonadal MAA concentrations ranged from 83.9 to 224.3 nmol mg⁻¹ protein spatially, and over the year from 1.85 to 14.12 nmol mg⁻¹ dry weight (DW) depending on site and gametogenic cycle. Laboratory manipulations indicated that concentrations of MAAs in *E. chloroticus* gonads and eggs are influenced by diet. MAA concentration could be correlated with UV-B intensities in two of the algal species. *E. chloroticus* MAA concentrations could also be correlated with UV-B transmission, which we concluded was a reflection of the greater ingestion and accumulation of MAA-rich macrophytes at those sites where higher ambient UV-R induced greater MAA concentrations to occur in the algae. Given this, we suggest that one response of marine species to increases in UV-B would be an increase in the synthesis and/or accumulation of MAAs for photoautotrophs and a dietary accumulation of those MAAs in *E. chloroticus*, an important herbivore in this system.

Keywords mycosporine-like amino acids; Doubtful Sound; *Evechinus chloroticus*; ultraviolet radiation; sunscreen

INTRODUCTION

An increase in ultraviolet-B radiation (UV-B, 290–320 nm) as a result of stratospheric ozone depletion is a serious environmental concern (Madronich et al. 1998), and the problem is predicted to persist to at least the year 2050 (Soloman 1999). Although ozone depletion (and concurrent increase in UV-B) is most pronounced at the Earth's polar regions, lower latitudes have also experienced increases in incident UV-B. For example, ozone depletion over temperate latitudes in the Northern Hemisphere (30–50°N) and equatorial regions (Kerr & McElroy 1993) will result in elevated UV-B irradiance.

Marine organisms counteract the effects of ultraviolet radiation (UV-R) through a number of behavioural and physiological strategies, including

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negative-phototaxis (Pennington & Emllet 1986; Adams 2003), sunscreens (Shick & Dunlap 2002), non-enzymatic antioxidants (Dunlap et al. 2000), antioxidant enzymes (Lesser & Farrell 2004), DNA repair (Mitchell & Hartman 1990; Kim & Sancar 1993; Malloy et al. 1997), and expression of cell-cycle genes (Lesser et al. 2001, 2003). Despite these mitigating strategies, UV-R can have detrimental influences on the marine environment at all trophic levels (see review by de Mora et al. 2000). Examples include the detrimental effects of UV-B on phytoplankton productivity (Smith et al. 1991; Neale et al. 1998a,b), marine macroalgae (van de Poll et al. 2001; Kuhlenskamp et al. 2001; Michler et al. 2002; Apprill & Lesser 2003), zooplankton mortality (Keller et al. 1997), and larval fish mortality (Lesser et al. 2001).

Research in New Zealand indicated a 12% increase in incident UV-R recorded at the Lauder Observatory (169°68'E, 45°35'S) during the decade preceding 1999 (McKenzie et al. 1999). More recent analysis of the New Zealand environment has suggested that the decrease in column ozone concentrations and increase in UV-B have not been sustained in recent years (McKenzie et al. 2004). Nevertheless, in comparison to the Northern Hemisphere, New Zealand (45°S) typically receives around 50% more UV-R in summer than Southern Germany (45°N) (Seckmeyer & McKenzie 1991; McKenzie et al. 1999). Unfortunately, very little is known about the present-day influences of UV-R on the New Zealand marine environment, making predictions on the effects of future changes in UV-B difficult. In particular, understanding how, and the degree to which, organisms will react to and mitigate the effects of increased UV-B in the future is a key question.

To address this question, our approach here is to examine present-day spatial differences in the effects of UV-R on a group of prominent marine species, and how those species react to present-day differences in ambient UV-R. Spatial differences are then used as a proxy for understanding the effects of temporal changes in UV-B, and how marine species are likely to react to future increases in UV-B.

This approach was possible in Doubtful Sound, Fiordland, where the unique oceanographic conditions that occur in the fiord results in a persistent horizontal gradient of UV-R penetration along the 40 km length of the fiord. The key feature of this fiord is the presence of a freshwater lens containing high concentrations of UV-R absorbing dissolved organics (Davis-Colley 1992). This freshwater lens increases in thickness with increasing distance into

the fiord (Gibbs et al. 2000; Gibbs 2001; Wing et al. 2001). We examined how organisms occurring along this gradient react to the difference in ambient UV-R levels. Specifically, we examine the concentrations of sunscreen compounds in the sea urchin, *Evechinus chloroticus*, and in four species of marine macroalgae throughout Doubtful Sound. *E. chloroticus* is ubiquitous throughout the fiord, whereas algal densities tend to decrease with increasing distance into Doubtful Sound.

Previous research has shown that macroalgae and corals can adapt to UV-R by producing sunscreen compounds such as mycosporine-like amino acids (MAAs) which absorb strongly in the UV-R wavelengths (Dunlap et al. 1986; Karsten et al. 1998a,b; Franklin et al. 1999). These MAAs can also be passed up to higher trophic levels through the processes of grazing and predation (Carefoot et al. 1998; Carroll & Shick 1996; Adams & Shick 2001; Adams et al. 2001). In this way, a community can potentially acquire protection against higher ambient UV-R conditions through the production and accumulation of sunscreen compounds.

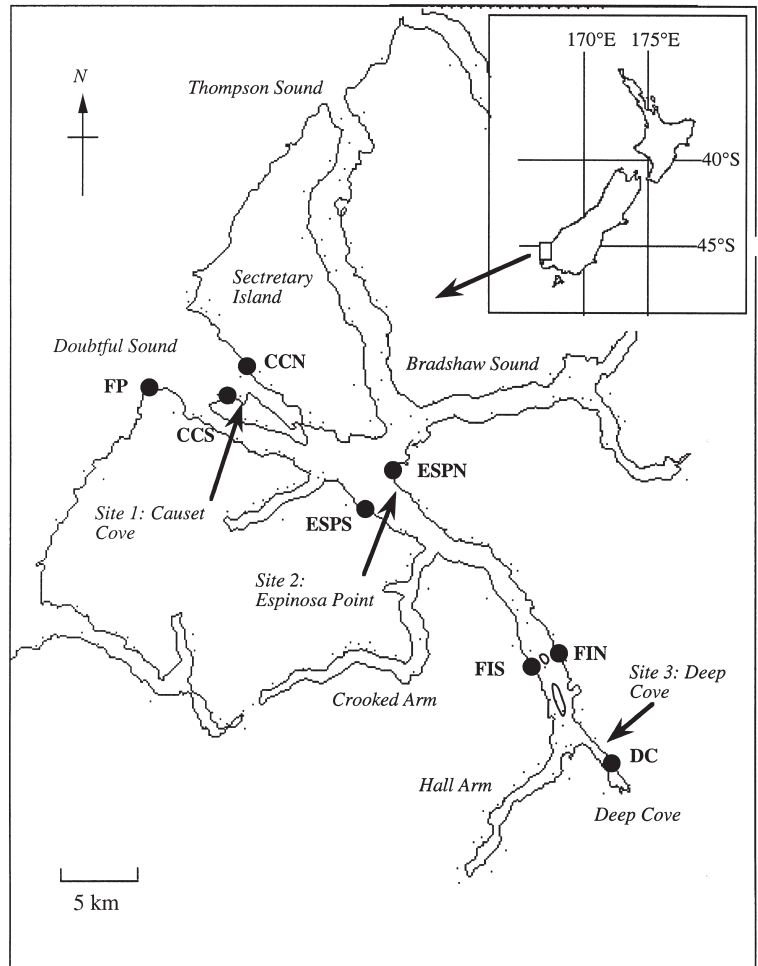
We are interested in the plasticity of the acclimatisation response to increased ambient UV-R that can occur in the New Zealand marine environment. Specifically, are there increases in sunscreen compounds in photoautotrophs that are then passed through higher trophic levels in response to increased ambient UV-R? To understand this we quantified: (1) spatial differences in ambient UV-R levels; (2) spatial differences in the concentrations of MAAs in four species of macroalgae (*Ulva lactuca*, *Apophlaea lyallii*, *Ecklonia radiata*, *Rhodomenia* spp.); (3) spatial differences in the gonadal concentrations of MAAs in the sea urchin, *Evechinus chloroticus*; and (4) the degree to which MAA concentration in *E. chloroticus* gonadal tissue is influenced by algal diet. Our findings are discussed in terms of potential responses of the marine environment to future increases in UV-R in New Zealand seas.

MATERIALS AND METHODS

Sites and collections

Sample sites were located within the Doubtful-Thompson Sound complex, a long and narrow (100 km long and on average 1 km wide) glacial fiord located on the south-western coast of New Zealand (Fig. 1). We sampled at eight sites so that they extended from the fiord head to the fiord entrance along a 40 km transect (Fig. 1). Six of the

Fig. 1 Map of Doubtful Sound, New Zealand showing the location of sampling sites. Sites where biological samples were collected between 23 and 25 October 2001 are indicated (●). These were also the sites where the oceanographic and light readings were made. (FP, Febrero Point; CCN, Causet Cove North; CCS, Causet Cove South; ESPS, Espinosa Point South; ESPN, Espinosa Point North; FIS, Fergussen Island North; FIS, Fergussen Island South; DC, Deep Cove.) Sites where the longer-term *Evechinus chloroticus* samples were collected (April 2002 – March 2003) are indicated by arrows (→, Site 1, Causet Cove; Site 2, Espinosa Point; Site 3, Deep Cove.)



sites were paired (Causet Cove North, Causet Cove South, Espinosa North, Espinosa South, Fergussen North, Fergussen South) to represent northern and southern aspect sites. The substratum at each site was hard bedrock and steeply sloped (between 60° and 90°).

Biological collection

Biological sampling at eight sites within Doubtful Sound was made using SCUBA divers over a 3-day period between 23 and 25 October 2001. We collected replicate samples of the sea urchin *E. chloroticus* (Echinodea, subtidal) and four species of macrophytes, *Rhodomenia* spp. (Rhodophyta, shallow subtidal), *Ulva lactuca* (Chlorophyta, shallow subtidal), *Ecklonia radiata* (Phaeophyta, subtidal), and *Apophlaea lyallii* (Rhodophyta, intertidal). The latter two species were restricted to

the outer fiord and therefore were only collected from the outer three sites (Febrero Point, Causet Cove North, Causet Cove South). Samples were taken from between 0 m and 5 m depth and varied between species (Table 1). Within each species however, we attempted to collect from the same depth range at each site (Table 1). The exception was for the sea urchin, *Evechinus*, which is mobile and had a greater depth and patchy distribution within the shallow subtidal.

Replication of samples involved taking three samples from three individuals of each species at each site. Each sample was placed in collection bags under water. Samples lifted to the surface were kept in cool, dark containers for transportation back to the field laboratory, where they were initially processed within 6–10 h of collection. For *E. chloroticus*, a longer-term sampling programme was carried out

between 16 April 2002 and 17 March 2003. During this time, samples of five individual urchins were taken from 3 sites (Causet Cove, Espinosa Point, Deep Cove) within Doubtful Sound approximately every 30 days (Fig. 1).

For these monthly samples the reproductive cycle of *E. chloroticus* was also examined from the change in the gonad index over the year, calculated monthly as:

$$\text{Gonad index (\%)} = \frac{\text{Total gonad wet weight (g)}}{\text{Total drained wet weight (g)}} \times 100$$

Drained wet weight is the total weight of the sea urchin after draining the perivisceral fluid from the animal.

Sample processing and MAA extraction

Following collection, samples were processed in two ways. Algal samples collected between 23 and 25 October were washed, cleaned of epiphytes, and placed in 5 ml of 100% HPLC grade MeOH and stored at -20°C before analysis by high pressure liquid chromatography (HPLC). Sea urchin samples collected on 23–25 October 2001 were dissected and c. 1 g of gonadal tissue was placed in 5 ml of 100% HPLC grade MeOH and stored at -20°C until analysed.

Alternatively, sea urchin samples collected over the 1-year period were immediately frozen, later lyophilised and crushed into a powder before extraction. MAAs were extracted by placing 100 mg of each sample in 5 ml 100% MeOH, sonicated for 5 min, and left overnight in the dark at 4°C . Before HPLC analysis, extracts were centrifuged at 5000 rpm for 1 min.

Chromatography

Analysis was made on a Shimadzu SPD-6AV instrument fitted with a Phenomenex C8 Octyl guard column (4×3 mm) and a Phenosphere C8 analytical column (250×4.6 mm). Separation of extracts was made isocratically in an aqueous mobile phase of 75% MeOH and 0.1% glacial acetic acid, at a flow rate of 0.3 ml min^{-1} (pressure 90 psi). For each sample, 25 μl was injected and the absorbance of the extracts monitored at 334 nm. To confirm the identity of MAAs, samples were co-chromatographed with known standards and run at 310 nm, and the absorbance ratio of 310:334 nm examined.

Quantification of individual peaks was made using response factors calculated from primary and secondary MAA standards (as described by Apprill & Lesser 2003) using the Shimadzu VPClass v5.032 software. All MAA concentrations were either expressed as nmol MAA mg^{-1} dry weight (DW) of sample for samples lyophilised, or standardised to soluble protein concentration (i.e., nmol MAA mg^{-1} soluble protein). Soluble protein was quantified using a BIO-RAD protein kit that utilises Coomassie brilliant blue and a bovine serum albumin standard.

Oceanography

At each site, a conductivity temperature pressure sensor (Seabird SBE-19) was deployed to a depth of 15 m, from which depth profiles of salinity (psu) were collected.

Underwater irradiances

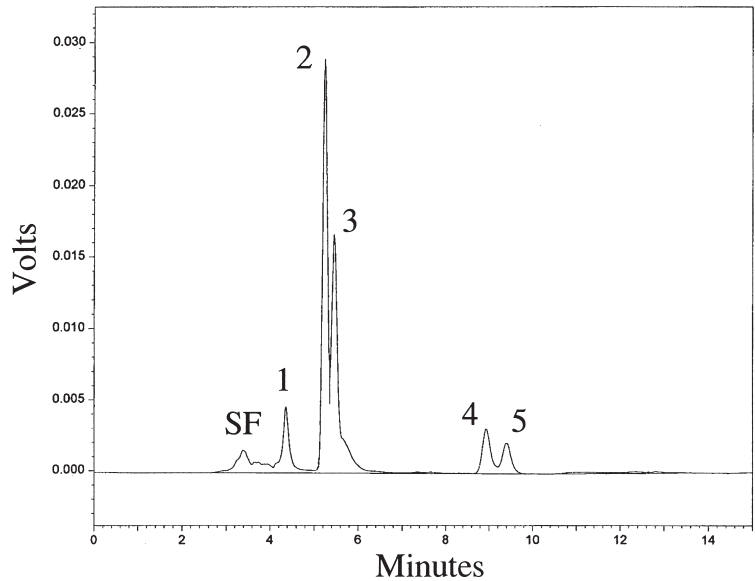
For each site, spectral irradiance was measured at the surface and three depths (1, 5, and 10 m) using a LiCor Li-1800UW spectroradiometer. Scans of

Table 1 Details of sampling, including observed depth distribution of each species throughout Doubtful Sound, New Zealand, depth range samples taken, number of sites sampled, and number of individuals sampled.

Species	Depth distribution	Depth range of sampling	No. of sites sampled	No. of individuals sampled*
<i>Evechinus chloroticus</i>	2–10 m	3–5 m	3 or 8*	3 or 5*
<i>Rhodymenia</i> spp.	2–6 m	3–4 m	8	3
<i>Ulva lactuca</i>	2–4 m	2–3 m	8	3
<i>Ecklonia radiata</i>	4–15 m	4–5 m	3	3
<i>Apophlalea lyalli</i>	0+ m	0+ m	3	3

*For *Evechinus chloroticus* the number of sites and individuals sampled varied depending on analysis (see text for details).

Fig. 2 Representative high performance liquid chromatography (HPLC) chromatograph of mycosporine-like amino acid (MAA) standards. Peaks observed and known concentrations in the chromatographs are: (1) Mycosporine-glycine, 178 nmol; (2) Shinorine, 1656.6 nmol; (3) P-334, 726.8 nmol; (4) Palythine, 91.16 nmol; (5) Palythiol, 116.41 nmol. Solvent front is indicated (SF).



spectral irradiance ($\text{W m}^{-2} \text{nm}^{-1}$) were made between 300 and 700 nm at 1 nm intervals, with three consecutive replicate scans made for each depth (replicates averaged). All scans were made on 24 October between 1200 and 1500 h, during which time the sky was cloudless and the sea calm.

Optical properties of the water column were quantified by calculation of bulk spectral attenuation coefficients ($K_d \text{m}^{-1}$) at each wavelength using the equation:

$$I_D(\lambda) = I_0(\lambda) \exp^{-kd}$$

where $I_D(\lambda)$ = irradiance at wavelength (λ) at depth (d), $I_0(\lambda)$ = irradiance at wavelength (λ) at the surface, k = light extinction coefficient, and d = depth (m). For each scan, UV-B (300–320 nm, W m^{-2}), UV-A (320–400 nm, W m^{-2}), and photosynthetically active radiation (PAR, 400–700 nm, $\mu\text{mol quanta m}^{-2}$) were also calculated. The attenuation of total UV-B, UV-A, and PAR was then calculated using the above equation.

In vitro dietary manipulation of MAA concentrations in *Evechinus* eggs

Forty-five *E. chloroticus* were collected from Doubtful Sound in January 2002, and transported to the Portobello Marine Laboratory where they were placed in three large plastic tanks ($700 \times 900 \times 300$ mm) that were supplied with a constant supply of fresh sea water ($8 \text{ litres min}^{-1}$). Each tank (containing 15 sea urchins) was fed *ad libitum* one of three macroalgal diet treatments, either

Gracillaria secundata (Rhodophyta), *Ulva lactuca* (Chlorophyta), or *Macrocystis pyrifera* (Phaeophyta). Food was replaced approximately weekly, at which time the tanks were drained and cleaned. Water temperatures over the year of feeding ranged from 9°C to 17°C . After 1 year, we spawned females using an inter-coelomic injection of $0.5M$ KCl. The concentration of MAAs in unfertilised eggs was quantified for three females from each algal treatment group using the analytical methods described above.

Statistical analysis

Spatial and temporal differences in MAA concentration, where appropriate, were analysed using ANOVA. To homogenise variances, all data were transformed by $\ln(x+1)$ before ANOVA and back transformed for graphical presentation.

RESULTS

HPLC chromatography

Chromatographs indicated the presence of UV-R absorbing compounds in the biological material collected from Doubtful Sound. We were able to quantify a total of five MAAs (Mycosporine-glycine, Shinorine, Porphyra-334, Palythine, and Palythiol) using our standards (Fig. 2, Tables 2 and 3). There were also a number of smaller unknown peaks.

MAA concentrations

MAA composition and total concentration of identified MAAs differed between species for samples taken between 23 and 25 October 2001 (Tables 2 and 3). No MAAs were detected in *U. lactuca*, consistent with previous observations for this species (Carefoot et al. 2000). For the remaining algae (Table 2), concentrations ranged from 12.5 to 1446.4 nmol mg⁻¹ protein and tended to be higher in the intertidal *A. lyallii* (mean = 840.7 nmol mg⁻¹ protein) compared with *Rhododymenia* spp. (mean = 49.8 nmol mg⁻¹ protein) and *E. radiata* (mean = 37.9

nmol mg⁻¹ protein). Intermediate concentrations of MAAs were recorded in *E. chloroticus* (Table 3), ranging from 83.9 to 224.3 nmol mg⁻¹ protein (mean = 172.5 nmol mg⁻¹ protein). In terms of MAA composition, P-334, palythine and palythanol were present in the algae but not detected in *E. chloroticus* tissue.

The presence of MAAs in samples of *E. radiata* is unusual because these compounds are not frequently found in brown algae (Karsten et al. 1998). It is possible that the MAAs that were detected in *E. radiata* samples were epiphytic diatoms that remained on the textured blades of *E.*

Table 2 Average mycosporine-like amino acid (MAA) ($n = 3$) concentrations (nmol mg⁻¹ protein) in thallus tissue of *Rhododymenia* spp., *Apophlaea lyallii*, and *Ecklonia radiata* collected at sites in Doubtful Sound, New Zealand between 23 and 25 October 2001.

Site	MAA concentration (nmol mg ⁻¹ protein)					Total (\pm SE)
	Mycosporine-glycine	Shinorine	P-334	Palythine	Palythanol	
<i>Rhododymenia</i> spp.						
Febrero Point	20.1	25.7	0.00004	0.00015	0	45.7 (103.6)
Causet South	7.8	50.3	0.00039	0.0034	0	58.2 (22.1)
Causet North	4.3	8.3	0.000041	0.00011	0	12.7 (3.9)
Espinosa South	22.4	81.4	0	0.00039	0	103.4 (39.5)
Espinosa North	15.1	1.3	0	0.00005	0	16.4 (1.2)
Fergusson South	11.2	39.6	0	0.00026	0	50.8 (21.4)
Fergusson North	6.9	82.1	0	0.00033	0	89.1 (48.3)
Deep Cove	16.3	6.2	0.000037	0	0.00016	22.5 (2.8)
<i>Apophlaea lyallii</i>						
Febrero Point	1446.4	0	0	0	0	1446.4 (424.1)
Causet South	433.1	0	0	0	0	433.1 (16.7)
Causet North	642.8	0	0	0	0	642.8 (230.7)
<i>Ecklonia radiata</i>						
Febrero Point	87.8	0	0	0.000016	0	87.8 (64.4)
Causet South	13.6	0	0	0.000001	0.000026	13.6 (3.7)
Causet North	12.5	0	0	0.000013	0	12.5 (6.6)

Table 3 Average mycosporine-like amino acid (MAA) ($n = 3$) concentrations (nmol mg⁻¹ protein) in gonadal tissue from *Evechinus chloroticus* collected at eight sites in Doubtful Sound between 23 and 25 October 2001.

Site	MAA concentration (nmol mg ⁻¹ protein)					Total (\pm SE)
	Mycosporine-glycine	Shinorine	P-334	Palythine	Palythanol	
Febrero Point	146.6	77.6	0	0	0	224.3 (112.1)
Causet South	428.3	69.1	0	0	0	497.4 (301.4)
Causet North	124.7	69.9	0	0	0	194.6 (82.1)
Espinosa South	166.2	75.5	0	0	0	241.7 (81.5)
Espinosa North	97.2	53.6	0	0	0	150.9 (64.8)
Fergusson South	43.0	40.9	0	0	0	83.9 (33.4)
Fergusson North	113.1	85.7	0	0	0	198.9 (46.1)
Deep Cove	65.4	52.5	0	0	0	118.0 (23.7)

radiata samples despite our efforts to remove them. Although diatoms generally contain low concentrations of MAAs (Hannach & Sigleo 1998), they can contain high concentrations of porphyra-334 (Karentz et al. 1991).

Using two-way ANOVA, statistical differences in the total concentration of MAAs were tested among species at the three sites where all species were present (Febrero Point, Causet Cove North, and Causet Cove South). The analysis (Table 4) indicated that there were significant differences in MAA concentrations between species ($P = 0.024$) and site ($P < 0.001$). Tukeys post-hoc test for differences among sites (Table 4) indicated that MAA concentrations were significantly higher ($P = 0.007$) at Febrero Point compared with Causet Cove North. Among species, post-hoc tests (Table 4) indicated that MAA concentrations were significantly higher ($P < 0.001$) in *Evechinus* compared with *Ecklonia* and *Rhododymenia*, but significantly lower compared with *Apophlaea*. Among the algae, *Apophlaea lyallii* had significantly ($P < 0.001$) higher MAA concentrations than the remaining species, with no differences between *Ecklonia* and *Rhododymenia*.

Spatial variation in the total MAA concentration along the entire fiord gradient was examined in *E. chloroticus* and *Rhododymenia* spp. (the two species that occurred at all eight sites, Tables 2 and 3). A one-way ANOVA indicated no significant differences existed among sites for either *E. chloroticus* ($F_{(7,16)} = 0.640$, $P = 0.717$) or *Rhododymenia* spp. ($F_{(7,16)} = 0.969$, $P = 0.485$).

The effect of site aspect (north versus south) on total MAA concentration was tested in *E. chloroticus* and *Rhododymenia* spp. among those sites where paired samples (North and South) were taken (Causet Cove, Espinosa Point, Fergussen Island). One-way ANOVA indicated that there were no significant differences based on aspect for either *Evechinus* ($F_{(1,16)} = 0.077$, $P = 0.785$) or *Rhododymenia* spp. ($F_{(1,16)} = 1.999$, $P = 0.177$).

Temporal changes in total MAA concentration in gonad tissue as a function of annual reproductive cycle was examined in three *Evechinus* populations (Causet Cove, Espinosa Point, and Deep Cove, Fig. 3). MAA concentrations were variable during the year, ranging from 1.85 to 10.33 nmol mg⁻¹ DW at Causet Cove, 2.29 to 10.29 nmol mg⁻¹ DW at

Table 4 Two-way analysis of variance of mycosporine-like amino acid (MAA) concentration among four species (*Evechinus chloroticus*, *Rhododymenia* spp., *Ecklonia radiata*, and *Apophlaea lyallii*) among three sites in Doubtful Sound, New Zealand. $N = 3$ for each species at each site. MAA concentrations were $\ln(x+1)$ transformed for analysis and back-transformed for presentation. Post-hoc comparisons among sites and species are given. Significant differences ($\alpha < 0.05$) are given in bold.

Two-way ANOVA of MAA concentrations among sites and species					
Analysis of variance					
Source	SS	d.f.	MS	F ratio	p
Species	75.918	3	25.306	34.861	<0.001
Site	6.311	2	3.155	4.347	0.024
Site*Species	4.996	6	0.833	1.147	0.366
Error	17.422	24	0.726		
Post-hoc comparisons of MAA concentrations among sites					
Site	Febrero			Causet South	
Febrero		*			
Causet South	0.109			*	
Causet North	0.007			0.215	
Post-hoc comparisons of MAA concentrations among species					
Species	<i>Evechinus</i>	<i>Ecklonia</i>		<i>Apophlaea</i>	
<i>Evechinus</i>	*				
<i>Ecklonia</i>	< 0.001	*			
<i>Apophlaea</i>	0.001	< 0.001		*	
<i>Rhododymenia</i>	< 0.001	0.171		< 0.001	

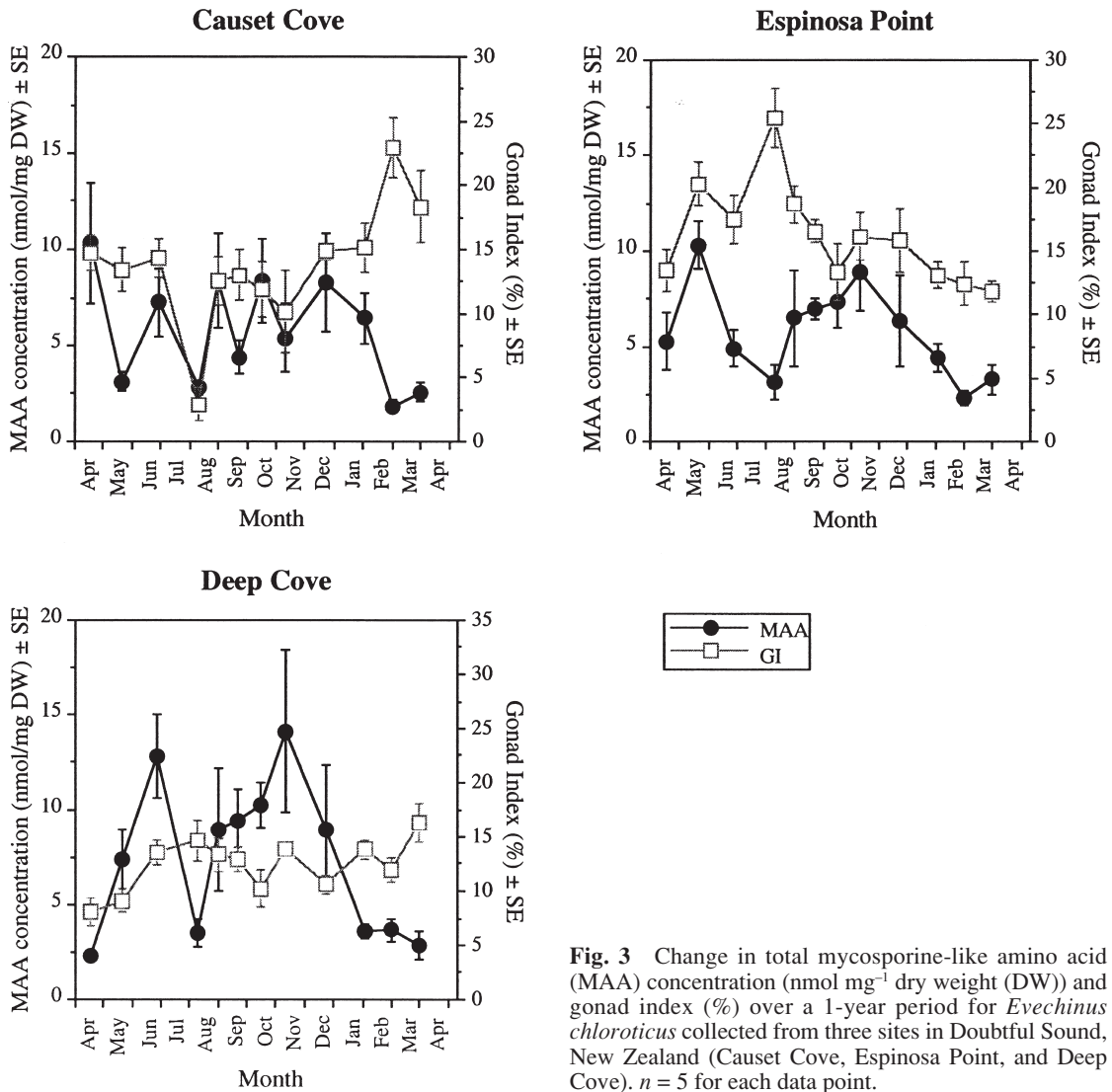


Fig. 3 Change in total mycosporine-like amino acid (MAA) concentration (nmol mg⁻¹ dry weight (DW)) and gonad index (%) over a 1-year period for *Evecchinus chloroticus* collected from three sites in Doubtful Sound, New Zealand (Causet Cove, Espinosa Point, and Deep Cove). *n* = 5 for each data point.

Table 5 Mycosporine-like amino acid (MAA) concentration (±SE) in unfertilised *Evecchinus chloroticus* eggs from females fed one of three algal diets (*Ulva lactuca*, *Gracillaria secundata*, *Macrocystis pyrifera*). (*N* = 3 for each diet treatment; DW, dry weight.)

Diet	MAA concentration (nmol mg ⁻¹ DW) ± SE				
	Mycosporine-glycine	Shinorine	P-334	Palythine	Total
<i>Ulva</i>	5.09 (0.88)	3.42 (0.19)	0	0.02 (0.01)	8.54 (0.84)
<i>Gracillaria</i>	5.02 (1.45)	91.10 (8.34)	0	0.52 (0.51)	96.65 (8.42)
<i>Macrocystis</i>	11.22 (1.96)	2.47 (0.36)	2.71 (1.51)	0.11 (0.11)	16.53 (0.79)

Espinosa Point, and 2.31 to 14.12 nmol mg⁻¹ DW at Deep Cove. At all three sites there was a general decrease in MAA concentrations from November/December through to March/April, with concentration decreasing in the order of 70–80%. Gonad indices ranged from 2.9% to 15.4%, and varied during the year. At Causet Cove and Deep Cove, indices tended to rise during the 1-year sampling period, whereas at Espinosa Point indices peaked in August and decreased during the remainder of the sampling period.

***In vitro* dietary manipulation of MAA concentrations in *Evechinus* eggs**

The concentrations of MAAs in unfertilised eggs are given in Table 5. Total MAA concentrations were significantly different among all three treatments ($F_{(2,6)} = 251.389$, $P < 0.001$), ranging from 8.54 nmol mg⁻¹ DW in the *U. lactuca* treatment, 16.53 nmol mg⁻¹ DW in the *M. pyrifera* treatment, to 96.65 nmol mg⁻¹ DW in the *G. secundata* treatment. The high MAA concentration found in eggs from the *Gracillaria* treatment was a result of the accumulation of large amounts of Shinorine (91.10 ± 8.34 nmol mg⁻¹ DW), whereas the *Macrocystis* treatment had higher concentrations of Mycosporine-glycine (11.22 ± 1.96 nmol mg⁻¹ DW).

Oceanography and solar irradiances

The transmission of light through the water column of Doubtful Sound is complicated because of the presence of a low salinity surface layer (LSSL) overlying the full salinity sea water (FSL). This presence of a LSSL was evident on 24 October 2001 (Fig. 4), with the depth of the LSSL (defined here as salinities <30 psu) ranging from 1.4 m (Causet Cove North) to 2.4 m (Deep Cove) (Table 6). The depth of the LSSL generally increased with increasing distance into the fiord (Table 6). Surface salinities ranged from 30.99 psu (Causet Cove North) to 4.90 psu (Deep Cove), and correlated with the depth of the LSSL (Table 6).

The LSSL contained high concentrations of humic substances (Doubtful Sound galvin absorption coefficient at 340 nm (g_{340}) = 1.87 to 6.93 m⁻¹, and at 440 nm (g_{440}) = 0.457 to 1.53 m⁻¹, Davies-Colley 1992) that altered its optical properties and increased the attenuation of UV-R and visible wavelengths compared with the underlying FSL.

Given the differences in the optics, the wavelength specific attenuation of UV-R and visible radiation in the LSSL (0–1 m) and FSL (5–10 m)

were examined separately at each site (Fig. 4). At all sites, the attenuation of light was higher in the LSSL waters compared with the FSL. The bulk attenuation coefficient (K_d m⁻¹) was wavelength-dependent, being highest in the shorter and longer wavelength. In the LSSL (Fig. 4), attenuation coefficients were highest at Deep Cove (K_d 1–7 m⁻¹) and lowest at the outer fiord sites such as Causet Cove and Febrero Point (K_d 0.5–2 m⁻¹). In the LSSL, there was a marked increase in the attenuation of light in the shorter wavelengths, especially in the UV portion of the spectrum (<400 nm), whereas maximum transmission of visible radiation occurred in the blue and green portions of the spectrum (450–600 nm).

In the FSL (Fig. 4), attenuation coefficients between 5 and 10 m were similar between sites, indicating that they shared similar inherent optical properties (Fig. 4). Attenuation was highest in the UV-R wavelengths (<400 nm) and in the longer red wavelengths (>600 nm). Overall, the attenuation coefficients observed were low (K_d 0.1–0.8 m⁻¹) and comparable to those previously described for coastal waters off the west coast of New Zealand (Howard-Williams et al. 1995).

As a result of differences in optics between the LSSL and the FSL, and the difference in the depth of the LSSL, the percentage of surface irradiance at 1 m depth varied between sites and between UV-B, UV-A, and PAR. For UV-A, this ranged from 35.3% (Febrero Point) to 4.3% (Deep Cove), whereas for UV-B, the percentage varied from 20.3% to 0.16%. The percentage of PAR at 1 m was highest at Espinosa Point South (68.5%) and lowest at Deep Cove (32.0%). The attenuation of UV-B, UV-A, and PAR in the water column was site-specific and could be correlated with the depth of the LSSL (Fig. 5). This highlights the affect of the relatively opaque LSSL water on light penetration in Doubtful Sound, and its predictable decrease with increasing distance along the fiord.

In addition to the optical properties of the water column, the fiord walls are typically steep (>45°) and lofty (1000–1400 m), which result in substantial shading of the south-facing shores and lower UV-R irradiances (Table 6). For example, the total amount of incident UV-R at the northern aspect sites (Causet Cove South, Espinosa South, Fergussen South) was between 7% and 93% higher than the corresponding south facing sites (Causet Cove North, Espinosa North, Fergussen North).

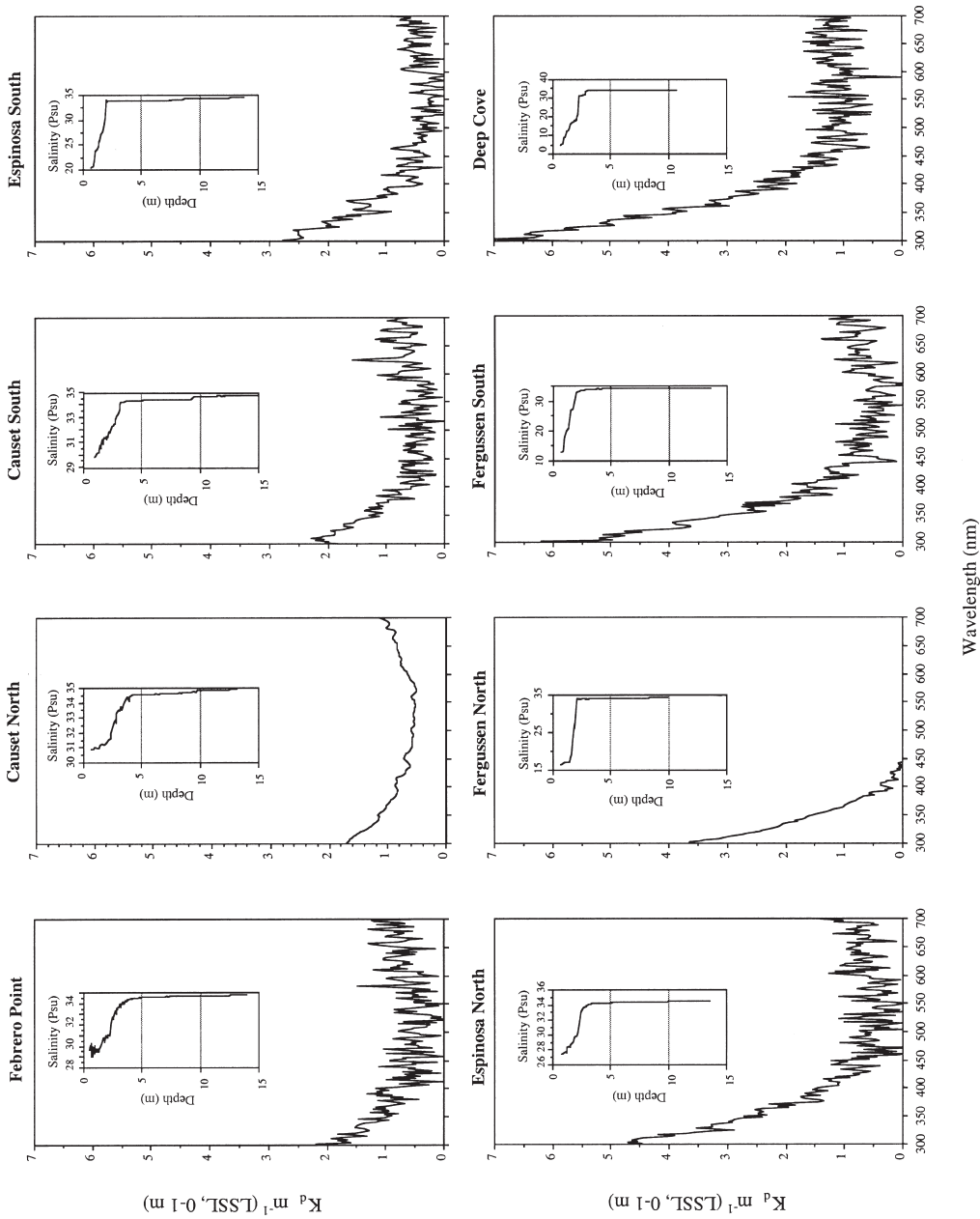


Fig. 4 (and opposite) Bulk attenuation coefficients (K_d , m^{-1}) versus wavelength within the low salinity surface layer (LSSL) (0–1 m) and the full salinity layer (FSL) (5–10 m) for each site. Inset for each site is the salinity profiles (psu) through the water column (0–15 m). Depth of the LSSL (salinities <30 psu) is given in Table 6.

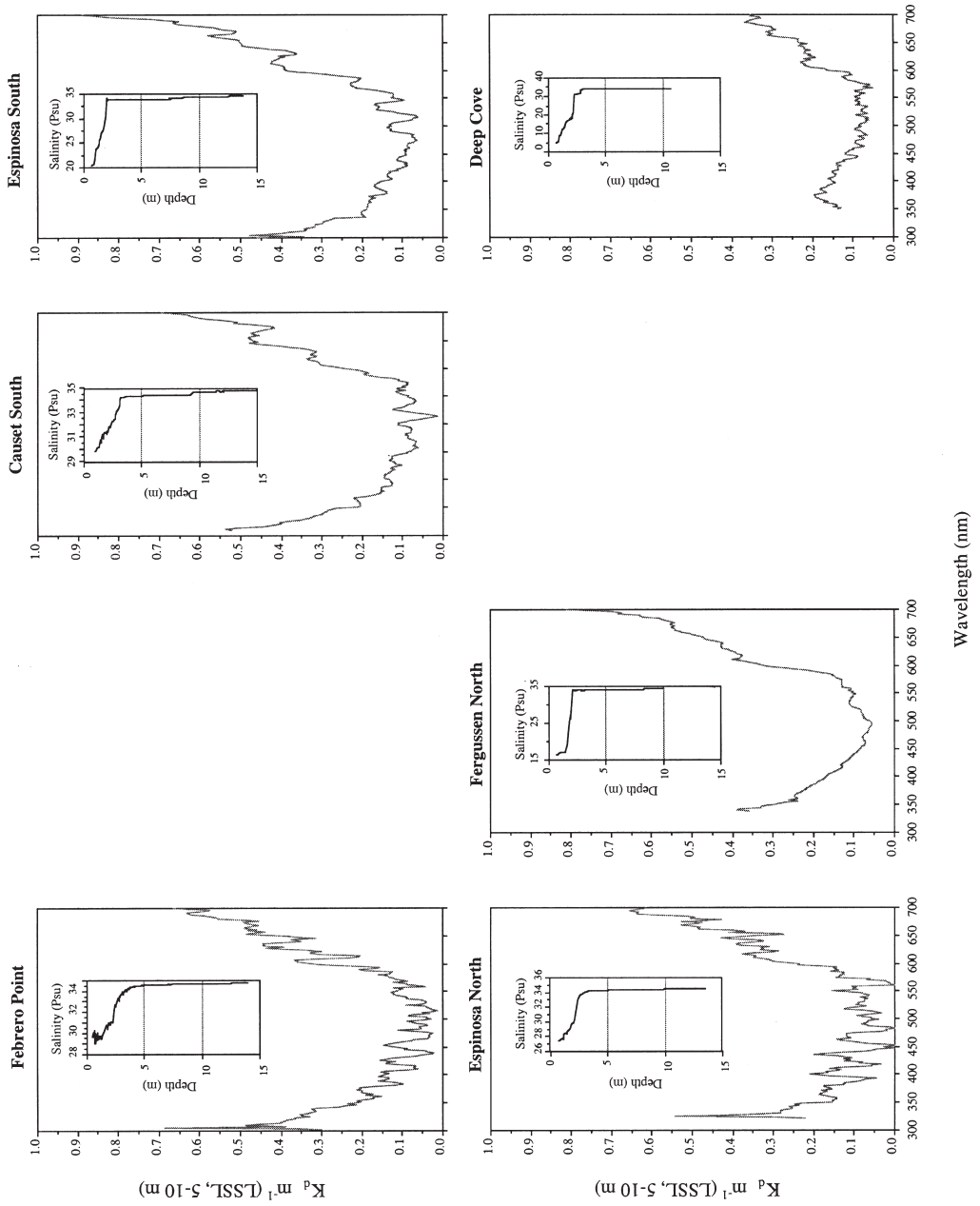


Fig. 4 (continued)

Table 6 Surface irradiances, % of surface irradiances at 1 m, attenuation coefficient (K_d m^{-1}) of UV-B, UV-A, and photosynthetically active radiation (PAR) for eight sites in Doubtful Sound, New Zealand. Surface salinity and depth of the low salinity surface layer (LSSL, defined as surface salinities <30 psu) is given for each site.

	Febrero Pt.	Causet N	Causet S	Espinosa N	Espinosa S	Fergusson N	Fergusson S	Deep Cove
UV-B (300–320 nm)								
Surface irradiance ($W m^{-2}$)	1.58	0.51	1.66	1.56	1.66	0.17	1.48	1.85
% of surface irradiance at 1 m	19.88%	20.35%	13.04%	1.47%	8.18%	5.29%	0.74%	0.16%
K_d m^{-1} (\pm SE)	1.62 (\pm 0.019)	1.59 (\pm 0.022)	2.03 (\pm 0.019)	4.21 (\pm 0.001)	2.50 (\pm 0.004)	2.93 (\pm 0.001)	4.89 (\pm 0.001)	6.42 (\pm 0.001)
Surface salinity (psu)	28.99	30.99	29.86	27.41	20.34	16.42	12.91	4.90
Depth of the LSSL (m)	1.8	1.4	1.5	2.2	1.8	1.9	2.0	2.4
UV-A (320–400 nm)								
Surface irradiance ($W m^{-2}$)	40.68	7.72	41.50	39.86	42.26	2.74	38.90	51.28
% of surface irradiance at 1 m	35.37	33.95	32.47	13.02	27.37	32.16	11.43	4.37
K_d m^{-1} (\pm SE)	1.01 (\pm 0.161)	1.07 (\pm 0.148)	1.11 (\pm 0.142)	2.03 (\pm 0.095)	1.29 (\pm 0.061)	1.12 (\pm 0.061)	2.16 (\pm 0.023)	3.12 (\pm 0.048)
Surface salinity (psu)	28.99	30.99	29.86	27.41	20.34	16.42	12.91	4.90
Depth of the LSSL (m)	1.8	1.4	1.5	2.2	1.8	1.9	2.0	2.4
PAR (400–700 nm)								
Surface irradiance (μ mol quanta m^{-2})	1627.57	81.65	1674.06	1748.43	1703.51	32.15	1709.63	2114.28
% of surface irradiance at 1 m	57.88	51.32	59.36	56.39	68.58	53.42	53.42	32.06
K_d m^{-1} (\pm SE)	0.24 (\pm 0.088)	0.176 (\pm 0.126)	0.249 (\pm 0.082)	0.346 (\pm 0.129)	0.247 (\pm 0.04)	0.094 (\pm 0.038)	0.62 (\pm 0.125)	1.109 (\pm 0.311)
Surface salinity (psu)	28.99	30.99	29.86	27.41	20.34	16.42	12.91	4.90
Depth of the LSSL (m)	1.8	1.4	1.5	2.2	1.8	1.9	2.0	2.4

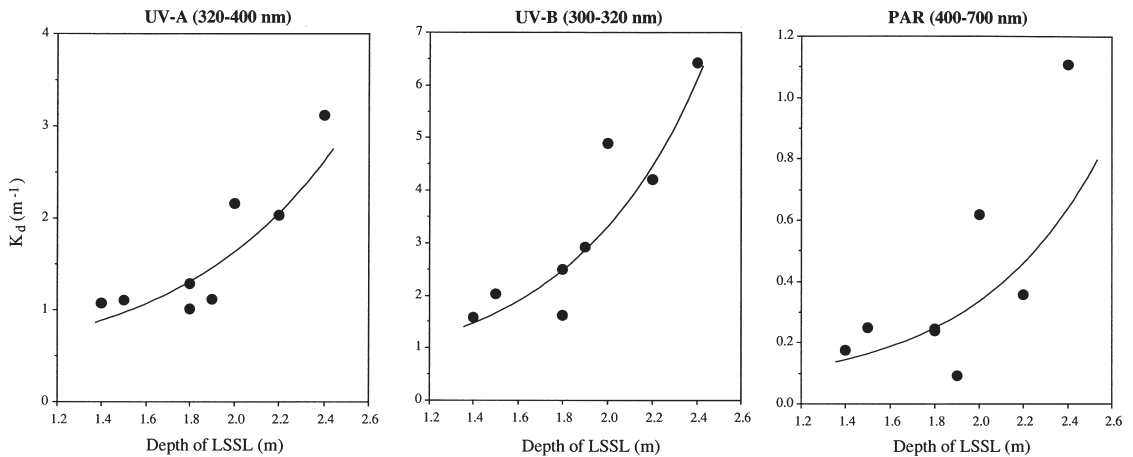


Fig. 5 Relationship between low salinity surface layer (LSSL) depth (m) and attenuation (K_d m^{-1}) of ultraviolet-B radiation (UV-B) (300–320 nm), UV-A (320–400 nm), and photosynthetically active radiation (PAR) (400–700 nm) through the water column for eight sites in Doubtful Sound, New Zealand.

DISCUSSION

This research examined the response of a number of New Zealand marine species to spatial variation in UV-R, in an initial attempt to understand how, and the degree to which, local marine species could potentially respond to any future increases in incident UV-R in the marine environment. The research was undertaken in Doubtful Sound, where we observed a gradient of water column UV-R transmission along a transect of the fiord. We took a “snapshot” of the ambient UV-R environment, which showed decreasing UV-B penetration ($K_d = 1.59\text{--}6.42$ m^{-1}) with increasing distance into the fiord, and a concurrent decrease in the percentage of UV-B reaching 1 m depth ranging from 0.16% to 20.35%.

Although we only made a single survey of the light environment, a permanent gradient in UV-B penetration is likely to exist in Doubtful Sound. Previous research has found that the high absorption of UV-wavelengths can be attributed to the large concentrations of galvanic substances found in the low salinity surface layer (Davis-Colley 1992). Furthermore, the thickness of this low salinity water increases with increasing distance into Doubtful Sound and is a consistent feature throughout the year (Gibbs 2001; Wing et al. 2001).

Our UV-R readings represent a “snapshot” of intensities, and we did not measure UV-R over the entire season. Previous research (McKenzie et al. 1996) has examined daily, seasonal, and latitudinal

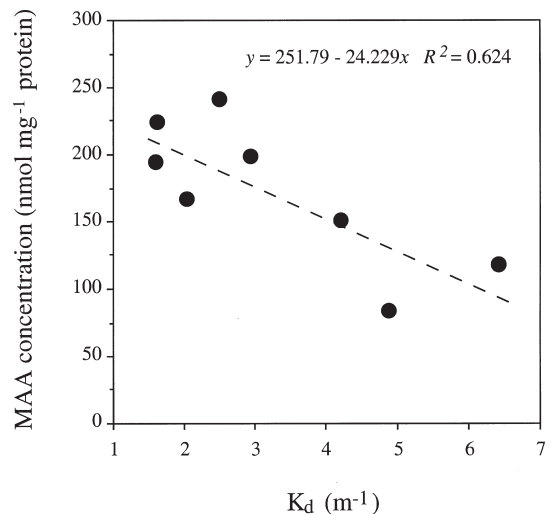


Fig. 6 Plot showing mycosporine-like amino acid (MAA) concentration (nmol mg^{-1} protein) in *Evechinus chloroticus* gonad versus ultraviolet-B radiation (UV-B) attenuation (K_d m^{-1}) at each site in Doubtful Sound, New Zealand. Regression analysis of K_d versus \ln transformed MAA concentrations indicated a significant relationship between the two variables ($F_{(1,6)} = 9.683$, $P = 0.021$).

variation in UV-R at two sites (Lauder, Central Otago (45°35'S, 169°68'E, 370 m altitude) and Leigh, Northland (36.3°S, 174.8°E, 27 m altitude)) in New Zealand. Although Doubtful Sound is not one of the sites examined and has a very different

climate to Lauder, they are at a similar latitude and longitude (45°18'S, 166°58'E). Therefore, this latter site can provide some indication of the degree that UV-R can vary with time in Doubtful Sound.

Day-to-day variation in UV dose varied less than 10% as a function of changes in total ozone column, but by 25–30% as a function of cloud cover. Seasonally, UV dose in the winter was 5–10% that of the summer as a result of changes in day length and zenith angle. The influence of cloud cover is relevant to Doubtful Sound because it is often cloudy and rainy (although rainfall is not seasonal in Doubtful Sound).

We examined the degree to which algae respond to differences in ambient UV-R through the production of sunscreens MAA. Concentrations varied between species and spatially within Doubtful Sound. For *E. radiata* and *A. lyallii* concentrations of MAAs were highest at the outer site (Febrero Point), where the transmission of UV-B will be highest over the long term. For *Rhododymenia* spp. however, which we sampled at all sites, MAA concentrations were not significantly correlated with UV-R attenuation along the fiord gradient.

Previous research has often found a correlation between ambient UV-R and MAA concentration (Banaszak et al. 1998; Franklin et al. 2001; Hoyer et al. 2001; Krabs et al. 2002; Apprill & Lesser 2003; Huovinen et al. 2004). This includes correlating algal depth and ambient UV-R intensities with MAA concentration (Bischof et al. 2000; Hoyer 2001), correlating geographic variation in ambient UV-R and MAA concentrations (Huovinen 2004), or correlating temporal changes in MAA concentration and ambient UV-R (Aguilera et al. 2002; Apprill & Lesser 2003). *In vitro* experiments and manipulation of ambient UV-R have confirmed these observations, noting that the production and accumulation of MAAs can be induced by exposure to UV-R and blue wavelengths (Franklin et al. 2001; Krabs et al. 2001; Apprill & Lesser 2003).

The degree that MAA concentrations can vary as a function of UV-R exposure is species specific. For example, Hoyer et al. (2001) examined MAA concentrations in Antarctic algae and found that although some algae adjusted MAA concentrations relative to ambient intensities, other species either had constantly high MAA concentrations or no capacity to synthesise these sunscreens. For those species that could adjust MAA concentrations, total content varied between 1.5 and 20-fold (Hoyer et al. 2001). Similarly, Apprill & Lesser (2003) found the depth-related difference in MAA concentration

to be approximately twice that for *Laminaria saccharina*.

In this respect, our limited survey of New Zealand algae yielded similar results. First, the highest MAA concentrations were found in *Apophlaea lyallii*, a low intertidal species. Second, we could divide our species into those that appear to adjust MAA concentration depending on ambient UV-R (*A. lyallii*, *E. radiata*), and those with no MAAs (*U. lactuca*) or MAA concentrations not apparently correlated with ambient UV-R (*Rhododymenia*). For those species that appear to respond to UV-R, the differences in MAA concentrations changed 7-fold for *E. radiata* and 3-fold for *A. lyallii*. *Rhododymenia* either does not produce high concentrations of MAAs in general or ambient UV-R levels within Doubtful Sound are not high enough to evoke a sunscreen response. Indeed, based on our UV-R readings on 23 October, the depth to which the 1% incident UV-R ranged (from 0.71 to 2.89 m), is shallower than the depth of *Rhododymenia*.

Average MAA concentrations in *E. chloroticus* ranged spatially from 83.9 to 224.3 nmol mg⁻¹ protein during our October survey, and between 1.85 and 14.12 nmol mg⁻¹ DW at three sites over a 1-year period. The closest comparison is between *Evechinus* and the northern temperate *Strongylocentrotid* species. For these species, average MAA concentrations range from 0.0052 to 0.525 nmol mg⁻¹ DW for samples taken from Washington State (Lamare & Hoffman unpubl. data), and between 1.63 and 8.86 nmol mg⁻¹ DW for Gulf of Maine populations (Carroll & Shick 1996; Adams et al. 2001). Average concentrations in *Evechinus* are comparable.

The concentrations of MAAs in *E. chloroticus* can be influenced by diet, demonstrated by our laboratory manipulations which resulted in significant differences in MAA concentration, ranging from 8.54 to 96.65 nmol mg⁻¹ DW depending on diet. The finding is consistent with similar diet manipulation experiments carried out on the sea urchin *Strongylocentrotus droebachiensis* (Adams & Shick 1996; Carroll & Shick 1996; Adams et al. 2001). Concentrations of MAAs were higher in those individuals fed a diet containing red algae compared with those fed a *Laminaria saccharina* diet.

Our findings would suggest that MAA concentrations in wild stocks of *Evechinus* could be influenced by dietary sources of MAAs. If this were so we would expect to see higher MAA concentrations in *Evechinus* populations inhabiting areas of higher ambient UV-R (and higher environmental MAA concentrations). Indeed, there was a

significant relationship between MAA concentration and the attenuation of UV-B (Fig. 6), with MAA concentration increasing with greater penetration of UV-B. This is consistent with the suggestion that the environment influences MAA concentrations in *Evechinus*.

Ambient UV-R itself does not appear to directly induce higher rates of MAA uptake in other species of sea urchins (Adams et al. 2001), therefore the positive correlation between ambient UV-R level and MAA concentration would likely be of dietary origin. We could not however, detect a significant spatial difference in MAA concentration along the entire length of Doubtful Sound (i.e., *Rhodomenia*) and we could not correlate concentrations of MAAs in *E. chloroticus* with concentration in the four algal species that we examined.

Could this apparent inconsistency reflect the fact that *Evechinus* was not feeding on the algae species we examined? *Evechinus* is known to eat a range of algae including *E. radiata* and *U. lactuca* (Andrew 1988) but it is unknown if they consume *Rhodomenia* or *A. lyallii*. The diet of *E. chloroticus* is varied and although it is primarily a herbivore, it undoubtedly predated encrusting sponges and smaller sessile invertebrates such as the blue mussel, *Mytilus* (Ayling 1981; Witmann & Grange 1998). We did not present concentrations of MAAs for other possible food sources although we have made some preliminary measures of the concentrations of MAAs in the blue mussel, *Mytilus galloprovincialis*, which are found at the outer sites of Doubtful Sound and appear to have high but variable MAA concentrations (53–5036 nmol mg⁻¹ protein, Lamare & Lesser unpubl. data). Therefore, it is possible that *Evechinus* is ingesting high concentrations of MAAs from other constituents of its diet (i.e., blue mussels) that vary spatially within Doubtful Sound.

Seasonal changes in gonad MAA concentrations will also be related to reproductive cycles. *E. chloroticus* is known to spawn in Doubtful Sound between December and April (Lamare 1999; Lamare et al. 2002) depending on location. Measures of gonad index during this research failed to identify a clear spawning period (where index increases and then decreases pre- to post-spawning respectively). For Causet Cove and Deep Cove, there is an increase in the index over the year indicating gametogenesis but not spawning. During this time, MAA concentrations vary but tend to decrease during summer as the gonad index reaches its maximum. There is a similar decrease in MAA concentration at Espinosa Point, but gonad index tends to decrease

over time. It is therefore difficult to assess the relationship between MAA accumulation and investment in eggs during gametogenesis and spawning. The decrease in MAA concentration between December and April would be consistent with an investment of MAAs in the gametes that are released during spawning. A drop in gonad index (indicative of spawning) was not, however, observed. Previous examination of the sea urchin *Strongylocentrotus droebachiensis* has found that MAA concentrations vary seasonally, increasing during gametogenesis and being highest before spawning (Adams et al. 2001). Similarly, for the Antarctic sea urchin, *Sterechinus neumayeri*, MAA concentration was related to gametogenic cycle with MAA content decreasing during the spawning period (Karentz et al. 1997). These findings suggest a large investment of MAAs in gametes, consistent with the high concentrations of MAAs measured in sea urchin eggs (Adams & Shick 1996, 2001). In this respect *Evechinus* is no exception. Our diet manipulation experiments indicated a high rate of MAA investment in *Evechinus* eggs ranging from 8.5 to 96.6 nmol mg⁻¹ DW depending on diet.

The broader aim of this research was to understand the degree to which New Zealand marine organisms could be influenced by, and mitigate the effects of, increased UV-R—in other words, if they can maintain their fitness and long-term survival under conditions of increasing ambient UV-R. It is therefore important to put in context, spatial and temporal differences in MAA concentrations in marine algae and sea urchins in terms of fitness. For the algae, fitness is directly influenced by MAA concentration when exposed to higher UV-R intensity, with plants containing higher MAA concentrations able to maintain photosynthetic capacity and exhibiting less DNA damage and photooxidation (Dunlap & Yamamoto 1995; Shick & Dunlap 2002; Apprill & Lesser 2003).

Evechinus chloroticus fitness will also be influenced by MAA concentration. For example, *Evechinus* has a long-lived (30–60 days) planktotrophic embryonic and larval stage that is relatively small (100–1000 µm) and transparent (Lamare & Barker 1999). These embryos represent a key life-history stage undergoing rapid DNA replication, and they can be sensitive to UV-R (Adams & Shick 2001; Lesser & Barry 2003). Factors controlling their susceptibility to UV-R will influence larval survival and ultimately species fitness. The degree that MAAs are invested in eggs and the concentration of MAAs in sea urchin eggs directly influences the

susceptibility of eggs and larvae to UV-R (Adams & Shick 1996, 2001). Therefore, our observation that *Evechinus* found in areas of higher ambient UV-R have higher gonadal MAA concentrations (and hence higher MAA concentrations in embryos) could be an example of this species maintaining fitness under a higher UV-R regime. The results of our diet manipulation experiments, which showed egg MAA concentrations can vary by an order of magnitude depending on diet, reinforce the potential that dietary difference can make on embryo fitness.

It should be noted that although increases in MAA content as a response to increased ambient UV-R is found in a number of organisms (see reviews by Dunlap & Shick 1998; Shick & Dunlap 2002), other marine species do not demonstrate this phenomena. We have already cited work by Hoyer et al. (2001) who found that some algae neither contain MAAs nor produce MAAs in response to increased UV-R. Other examples exist for marine animals, such as the anemone *Anthopleura*, where MAA content is related to their phylogeny and not environmental factors (Shick et al. 2002).

In addition to light, it is possible that MAA concentrations are influenced by the lower surface salinities in Doubtful Sound. This is because MAAs are water soluble osmolytes (Shick & Dunlap 2002) and it is possible that their concentrations could be altered in algae exposed to hyposaline conditions (i.e., at the inner fiord sites). There has been no specific research on the osmoregulation of MAAs that we can cite, although the effects of low salinity on other osmolytes has been examined in macroalgae species (Karsten et al. 1991; Bisson & Kirst 1995) that would suggest the influence of salinity on MAA concentrations warrant further investigation. *E. chloroticus* is never found in the low salinity layer and migrates up and down the rock wall as the thickness of the low salinity layer changes. Therefore, it is isoosmotic with the sea water and should not experience changes in osmolyte concentration as a result of low salinity exposure.

Huovinen et al. (2004) note that MAA concentrations are generally higher in geographic locations where there are higher ambient UV-R intensities; therefore, it will be interesting to compare New Zealand MAA concentrations given our higher ambient UV-R levels. We only quantified MAA concentrations in four algae species, and are presently undertaking a comprehensive survey of MAA concentration in New Zealand marine algae and invertebrates. The results of this larger survey will provide a greater understanding on the degree

to which MAAs could provide protection to New Zealand marine species if southern New Zealand experiences further increases in incident UV-R.

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