

Temporal concentrations of sunscreen compounds (Mycosporine-like Amino Acids) in phytoplankton and in the New Zealand krill, *Nyctiphanes australis* G.O. Sars

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This study investigated the relationship between seasonal changes in ambient UV-R, and sunscreen concentrations in phytoplankton and krill. Concentrations of mycosporine-like amino acid (MAA) sunscreens were quantified in phytoplankton communities and in the krill Nyctiphanes australis over a 1-year period off the Otago Coast, New Zealand. Ambient UV-B and UV-A ranged from a minimum mean daily dose of 2.19×10^4 kJ day⁻¹ and 0.73×10^6 kJ day⁻¹ in June, to a maximum in January of 20.19×10^4 kJ day⁻¹ and 4.88×10^6 kJ day⁻¹, respectively. Concentrations of MAAs (consisting almost entirely of Mycosporine-glycine) in the phytoplankton community were lowest in August (5.6 nmol μg^{-1} Chl) when UV-R irradiances were minimal and highest in January (41.4 nmol μg^{-1} Chl) when UV-R irradiances were maximal. Nyctiphanes australis was found to contain five identified MAAs (mycosporine-glycine, shinorine, Porphyra-334, palythine and palythinol) and several unknown UV-R absorbing compounds. Concentrations ranged from 4.73 to 15.51 nmol mg^{-1} dw, with little indication of a seasonal cycle that could be correlated with changes in either phytoplankton MAA concentrations or ambient UV-R irradiances. The findings suggest that krill are neither accumulating MAAs in response to changes in MAA concentrations in their phytoplankton food, or that MAA concentrations in krill are increased in response to higher ambient UV-R irradiances. Concentrations of MAAs in krill body parts (carapace, legs, eyes, antennae, muscle) were similar (4.89 – 5.98 nmol mg^{-1} dw), with the exception of the carapace (2.03 nmol mg^{-1} dw).

INTRODUCTION

Mycosporine-like amino acids (MAAs) are an important and ubiquitous group of sunscreen compounds that have been found to reduce the potential detrimental effects of ultraviolet radiation (UV-R) in many marine organisms (see review by Shick and Dunlap, 2002). They report that MAAs are synthesized through the

shikimate pathway, which is restricted to bacteria, algae and fungi. The compounds are found in organisms at higher trophic levels through dietary assimilation (Shick and Dunlap, 2002).

The concentrations of MAAs in organisms are often positively correlated with UV-R exposure and with ambient UV-R levels. For example, Maragos (Maragos, 1972) noted that MAA concentrations (unidentified at the

time and referred to as just S-320) increased at higher ambient UV-R irradiances (i.e. with decreasing depth) in the coral *Porites lobata*. Similarly, a positive correlation between ambient UV-R and MAA concentration has been reported for the sea urchin *Evechinus chloroticus* (Lamare *et al.*, 2004). Direct evidence of UV-induced MAA synthesis has been demonstrated by Karsten *et al.* (1998), who found that MAA synthesis in macroalgae was induced through exposure of the plant to UV-R. Similarly, Shick (Shick, 2004) experimentally showed that in the coral, *Stylophora pistillata*, MAA synthesis in zooxanthallae increased with increasing UV-R exposure. In phytoplankton, UV-R induced production of MAAs is not universal among all species. While MAAs are often induced by exposure to UV-R (Hannach and Sigleo, 1998; Sinha *et al.*, 1998), they can also be produced in response high PAR levels (Carreto *et al.*, 1990, 2002; Helbling *et al.*, 1996; Jeffrey *et al.*, 1999; Callone *et al.*, 2006). In addition, in some species, UV-R exposure does not result in the synthesis of MAAs (Helbling *et al.*, 1996; Hannach and Sigleo, 1998) and may even inhibit their production (Carreto *et al.*, 2002; Carignan *et al.*, 2002).

The dietary origin of MAAs in higher trophic organisms (Shick and Dunlap, 2002) means that MAA concentration and composition can be directly influenced by diet. For example, the concentrations of MAAs have been altered experimentally by manipulation of diets in sea urchins (Adams and Shick, 1996; Carroll and Shick, 1996; Lamare *et al.*, 2004) and in the sea hare, *Aplysia dactylomela* (Carefoot *et al.*, 2000). Less information is available on the trophic accumulation of MAAs through pelagic organisms, particularly the transfer of MAAs from phytoplankton to herbivorous zooplankton. Whitehead *et al.* (Whitehead *et al.*, 2001) examined the transfer of MAA through phytoplankton, herbivorous pteropods (*Limacina helicina*) and predatory pteropods (*Clione antarctica*), and determined a concentration factor of 4.8 and 2.4, respectively, through the first and second trophic transfers. Newman *et al.* (Newman *et al.*, 2000) examined in the laboratory, the uptake of MAAs by Antarctic krill, *Euphausia superba*, from their phytoplankton diet, *Phaeocystis antarctica*. They observed that both the type and concentration of MAA in the krill differed depending on the MAA composition and concentration in the phytoplankton, which in turn were positively correlated with *in vitro* UV-R exposure. These experiments suggest that any detrimental effect of increases in UV-R exposure for zooplankton that might arise, for example from ozone depletion, could be mitigated by higher MAA concentrations in phytoplankton, and ultimately the zooplankton.

Whether or not such a mechanism is occurring *in situ* in species such as krill has not been examined directly, and it is still unknown if MAA concentrations in

zooplankton are correlated with concentrations in their phytoplankton food or with ambient UV-R irradiances. To address this question, the relationship between ambient UV-R irradiances and MAA concentration and composition in phytoplankton and the euphausiid, *Nyctiphanes australis* G.O Sars was established over a 1-year period.

Nyctiphanes australis is restricted to New Zealand and southeastern Australia neritic waters (Sheard, 1953; Bary, 1956). The krill reach an adult length of 15–19 mm, which can be very abundant, and form an important component of the diets of sea birds and commercial fish species (Ritz and Hosie, 1982), thus making them a key link between primary producers and higher trophic levels. It is abundant along the Otago coast, SE New Zealand, and occurs in large swarms at the sea surface, where it experiences high irradiances during the day (M.D. Lamare, personal observation). Although *N. australis* will feed on zooplankton such as copepods (Pilditch and McClatchie, 1994) and detritus, it is primarily herbivorous (Dalley and McClatchie, 1989; Ritz *et al.*, 1990) with phytoplankton grazing rates influenced by sea temperature and phytoplankton composition and density. Clearance rates have been estimate as high as 5000 cells animal⁻¹ h⁻¹ (Haywood and Burns, 2003).

This research describes temporal changes in the composition and concentration of MAAs in *N. australis* and in phytoplankton communities in coastal waters off the Otago Peninsula, New Zealand. Concentrations of MAAs in both communities are compared with seasonal changes in ambient UV-R irradiances in the region.

METHOD

Sampling sites and times

Monthly collections were made from three sampling areas within the Otago Harbour and off the Otago Peninsula, New Zealand. Sampling locations were recorded using a GPS and occurred within an area from 45°47.47'S to 45°49.82'S and from 170°37.46'E to 170°43.59'E. The location of samples varied depending on the distribution of krill patches on the day of sampling. Because of the patchiness of *N. australis*, our initial plans to compare spatial differences in MAA concentrations within the study area were abandoned, and concentrations of MAAs were averaged from the results of krill caught throughout the entire area. Samples of *N. australis* were collected from surface waters (<2 m depth) using a 1.5 m × 2 m plankton net with a 500 µm mesh size towed for 15 min at ≈0.5 m s⁻¹. After capture, krill were placed in plastic bags and kept on ice and, on

landing, immediately were rinsed with MilliQ water and stored at -60°C . Later, samples were lyophilized, ground to a fine powder and stored at -60°C prior to High Performance Liquid Chromatography (HPLC) analysis.

On each sampling day, nine 1-L seawater samples were collected from 2 m depth and stored in the dark (<6 h) prior to filtering. The water was pre-filtered through a $100\ \mu\text{m}$ nylon mesh, and then passed through a Whatman GF/C glass fiber filter. The filters were folded in half, wrapped in aluminum foil and stored at -80°C prior to further biochemical analysis.

Surface UV-R irradiances

Ambient instantaneous UV-B (280–315 nm, expressed as W m^{-2}), UV-A (315–380 nm, W m^{-2}) and PAR (400–700 nm, $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) levels were recorded every 5 min at the University of Otago Campus, Dunedin ($45^{\circ}52'\text{S}$, $170^{\circ}31'\text{E}$). Measurements were made using Skye High-Output Sensors (Skye Instrumentation, UK), models SKU 420 and SKU 430 for UV-A and UV-B, respectively, while PAR was measured using a Li-Cor LI190SB quantum sensor (LiCor Biosciences, USA).

Biochemical analyses

MAA extractions and HPLC analysis

MAAs from krill were extracted by placing 50 mg of each sample in 5 mL 100% HPLC grade MeOH, sonicating for 5 min, and soaking overnight in the dark at 4°C . Prior to HPLC analysis, extracts were centrifuged at $1677\ \text{g}$ for 1 min and then passed through a $0.22\ \mu\text{m}$ filter. The efficiency of the extraction process was tested with serial extraction of five replicate krill samples, and was found to be $81.2\% \pm 0.4$ for mycosporine-glycine, $91.3 \pm 0.4\%$ for shinorine, $86.6 \pm 0.6\%$ for porphyra-334, $86.3 \pm 0.8\%$ for palythine and $85.1 \pm 2.3\%$ for palythanol. While $<100\%$ extraction efficiencies indicate that our measurements of MAAs are likely to be under-estimates, we did not adjust our measured MAA concentrations in case there were temporal differences in extraction efficiencies among samples.

MAAs were extracted from the filtered phytoplankton samples by immersion in 4 mL of 100% HPLC grade MeOH. Samples were sonicated for 5 min then left in the dark for 24 h at 4°C . Samples were then centrifuged at $1677\ \text{g}$ for 5 min, and the supernatant passed through a $0.22\ \mu\text{m}$ filter prior to HPLC analysis.

HPLC analysis was made on a Shimadzu SPD-6AV instrument fitted with a Phenomenex C8 Octyl guard

column ($4 \times 3\ \text{mm}$) and a Phenosphere C8 analytical column ($250 \times 4.6\ \text{mm}$). Separation of the extracts was undertaken under isocratic conditions using an aqueous mobile phase of 75% MeOH and 0.1% glacial acetic acid, at a flow rate of $0.3\ \text{mL min}^{-1}$ (pressure ≈ 90 psi). Between 5 and 25 μL (typically 10 μL) was injected for each sample, and the absorbance of the extracts monitored at 334 nm. MAAs were identified by co-chromatography with a mixed secondary standard of mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythanol and palythene, as well as primary standards of shinorine, porphyra-334 and palythine. Primary and secondary standards were obtained from *Palythoa* sp. (Zoanthidea), *Porphyra* sp. (Rhodophyta), *Anthopleura elegantissima* (Actiniaria), *Prochloron* sp. (Prochlorobacteriae) and *Pocillopora damicornis* (Scleractini). There were several additional peaks in chromatographs that could not be identified. Quantification of individual peaks was made using response factors calculated from MAA standards (as described by Apprill and Lesser, 2003) using the Shimadzu VPCClass v5.032 software. MAA concentrations in krill were expressed as $\text{nmol mg}^{-1} \text{ dw}$, while MAA concentrations in phytoplankton were expressed as $\text{nmol MAA per } \mu\text{g total chlorophyll}$ ($\text{nmol } \mu\text{g}^{-1} \text{ Chl}$).

Spectrophotometry

To explore the presence UV-R absorbing compounds, MeOH extracts were analysed spectrophotometrically with a Beckman DU70 spectrophotometer (Beckman Instruments, USA) using a 1 cm pathlength cell. The absorbance was recorded between 280 and 700 nm at a 1 nm interval.

Chlorophyll analysis

Chlorophyll pigments were extracted from filtered phytoplankton samples using 10 mL of 90% acetone (Jeffrey and Humphrey, 1975) and placed in the dark for 24 h at 4°C . Total chlorophyll concentration was then determined spectrophotometrically using methods previously described (Jeffrey and Humphrey, 1975).

MAA distribution within different krill body parts

The concentrations of MAAs in the eyes, feeding limbs (plus gills and chromophores), carapace, antennae and muscle tissue were quantified for krill caught on 20 February 2006. After removal, each body part was washed in MilliQ water. Samples of each body part type from 100 krill were pooled, lyophilized and stored at -60°C prior to HPLC analysis.

RESULTS

Ambient surface irradiance (March 2005 to February 2006)

Surface UV-B (290–320 nm) irradiances ranged from a maximum in December 2005 of 1.44 W m^{-2} to a minimum 0.2 W m^{-2} in June 2005 (Table I). Total monthly UV-B dose and mean daily dose were greatest in January 2006 ($6.26 \times 10^6 \text{ kJ month}^{-1}$ and $20.19 \times 10^4 \text{ kJ day}^{-1}$, respectively), and lowest in June 2005 ($0.66 \times 10^6 \text{ kJ month}^{-1}$ and $2.19 \times 10^4 \text{ kJ day}^{-1}$, respectively). Surface UV-A (320–380 nm) showed a similar trend (Table I), with maximum irradiance recorded in December (32.5 W m^{-2}) and minimum irradiance in June (6.4 W m^{-2}). Total monthly UV-A dose and mean daily dose ranged from $15.1 \times 10^7 \text{ kJ month}^{-1}$ and $4.88 \times 10^6 \text{ kJ day}^{-1}$, respectively, in January 2006 down to $2.19 \times 10^7 \text{ kJ}$ and $0.73 \times 10^6 \text{ kJ day}^{-1}$, respectively. Maximum surface PAR (400 to 700 nm) varied 3.09-fold over the year of sampling (Table I) with minimum irradiances in June ($700 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) to a maximum in December ($2167 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$).

HPLC chromatography

Spectrophotometric analysis indicated the presence of UV-R absorbing compounds in *N. australis* and in phytoplankton filtrates (Fig. 1). Using HPLC, we were able to quantify a total of five MAAs (Mycosporine-glycine, Shinorine, Porphyrin-334, Palythine, and Palythanol) using secondary MAA reference compounds (Fig. 2). There were also three significant unknown absorption peaks (Fig. 2).

MAA concentrations

Phytoplankton

Only mycosporine-glycine was detected in phytoplankton filtrates, with the exception of August 2005 filtrates, when two other UV-R absorbing substances were detected. These did not correspond with our five known MAAs and remained unidentified. MAA concentrations in phytoplankton (i.e. $0.2 \mu\text{m}$ filtrates from 1L of seawater) ranged from $41.4 \text{ nmol } \mu\text{g}^{-1} \text{ Chl}$ in January 2006 to a measured low of $5.6 \text{ nmol } \mu\text{g}^{-1} \text{ Chl}$ in August 2005 (Fig. 3). One-way ANOVA indicated that there was a significant difference ($F_{(10, 77)} = 14.024, P < 0.001$) in MAA concentrations among months. Tukey HSD *post hoc* comparisons between months indicate the differences were associated with the significantly ($P < 0.05$) lower concentrations in August and September, and with the significantly ($P < 0.05$) higher concentrations recorded in January.

Monthly changes in MAA concentrations were not tightly correlated with seasonal changes in UV-B, UV-A and PAR irradiances. There was, however, a general trend of low MAA concentrations during the late-winter months when irradiances are low, and highest MAA concentrations in January coinciding with the maximum daily irradiances. Changes in mean daily UV-B doses during the period of phytoplankton sampling (Fig. 3) demonstrate this trend, with highest MAA concentrations and UV-B daily doses occurring in January ($41.4 \text{ nmol } \mu\text{g}^{-1} \text{ Chl}$ and $20.19 \times 10^4 \text{ kJ day}^{-1}$, respectively). A similar pattern could be demonstrated for UV-A and PAR.

Nyctiphanes australis

The five MAAs identified in *N. australis* and relative molar proportions (%) were mycosporine-glycine (31.1%), shinorine (36.8%), porphyrin-334 (20.3%), palythine (5.4%) and palythanol (0.8%). Mycosporine-glycine, shinorine, porphyrin-334 were found in all

Table I: Maximum irradiance, monthly dose and mean daily dose of UV-B, UV-A and maximum irradiance of PAR for upper Otago Harbour between 1 March 2005 and 28 February 2006

	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
UV-B (290–320 nm)												
Maximum irradiance (W m^{-2})	0.98	0.62	0.33	0.20	0.26	0.45	0.61	1.00	1.17	1.44	1.37	1.27
Monthly dose ($\text{kJ} \times 10^6$)	2.99	1.90	1.02	0.66	0.77	1.33	2.06	3.41	4.49	5.58	6.26	4.04
Mean daily dose ($\text{kJ} \times 10^4$)	9.66	6.35	3.28	2.19	2.49	4.28	6.88	11.00	14.98	18.00	20.19	14.42
UV-A (320–380 nm)												
Maximum irradiance (W m^{-2})	21.6	14.6	9.0	6.4	8.4	12.9	17.4	25.5	28.4	32.5	30.9	29.8
Monthly dose ($\text{kJ} \times 10^7$)	7.12	5.05	3.08	2.19	2.64	4.23	6.22	9.60	11.60	13.50	15.10	9.82
Mean daily dose ($\text{kJ} \times 10^6$)	2.29	1.68	0.99	0.73	0.85	1.36	2.07	3.09	3.87	4.35	4.88	3.50
PAR (400–700 nm)												
Maximum irradiance ($\mu\text{mol quanta m}^{-2} \text{ s}^{-1} \times 10^3$)	1.62	1.33	0.91	0.7	0.83	1.18	1.41	1.83	1.95	2.16	2.10	2.06

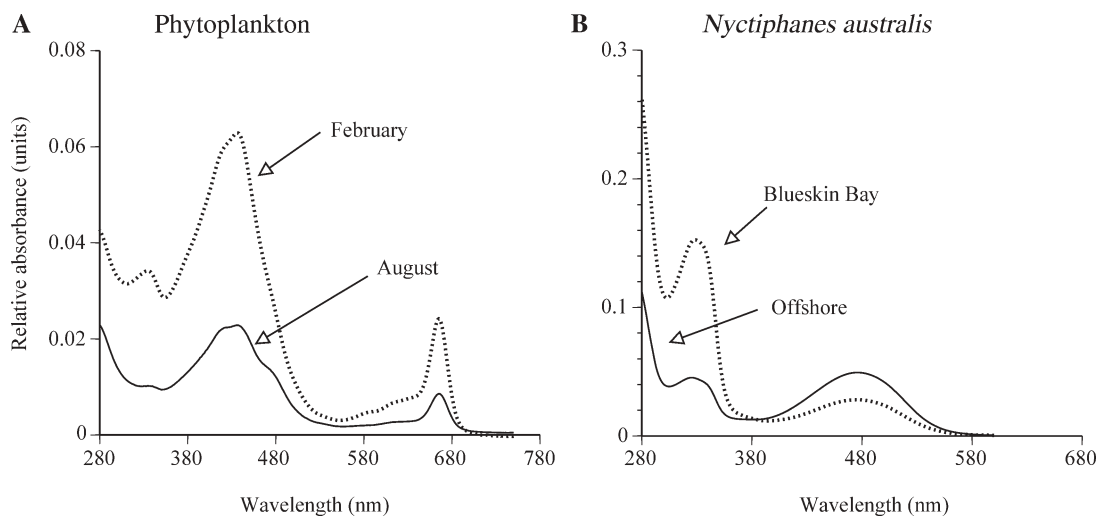


Fig. 1. Spectrographs of MeOH extracts of: **(A)** phytoplankton filtrates from Otago Harbour on 19 August 2005 and 20 February 2006, and; **(B)** *Nyctiphanes australis* collected from two sites in the study area (Blueskin Bay and Offshore) on 21 December 2005. The absorption peak at 330 nm in extracts is attributed to MAAs.

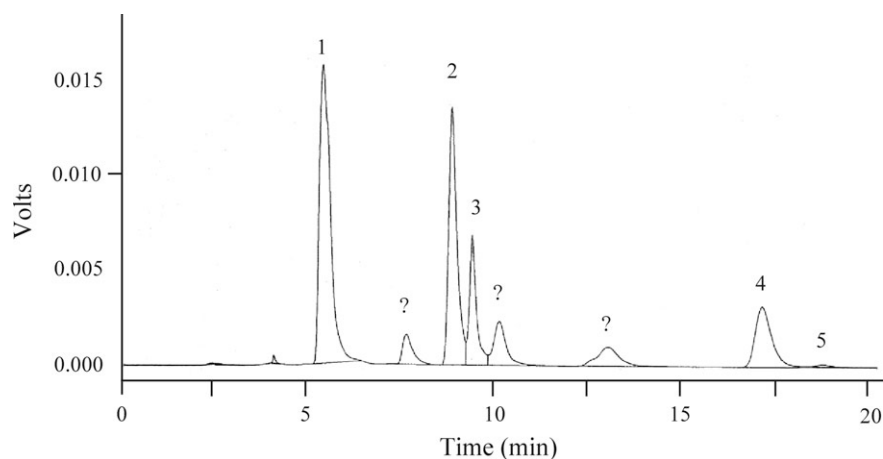


Fig. 2. HPLC chromatographs of a MeOH extract of *Nyctiphanes australis* collected 20 February 2006. MAAs are; 1, Mycosporine-glycine; 2, Shinorine; 3, Porphyra-334; 4, Palythine; 5, Palythiol. Three significant unidentified peaks eluted at 7.37, 9.84 and 12.74 min.

samples, palythine was detected at nine sampling times, and palythiol was detected at eight sampling times (Fig. 4). The relative proportions of the different MAAs varied little over the course of the study, with monthly proportions ranging from 23.2 to 37.4% mycosporine-glycine, 34.7 to 45.9% shinorine, 13.8 to 34.1% Porphyra-334, 0 to 11.9% palythine, and 0 to 1.1% palythiol (Fig. 4).

Average MAA concentration in *N. australis* varied 3-fold over the course of the year (Fig. 5), ranging from a maximum of $15.51 \text{ nmol mg}^{-1} \text{ dw}$ in April 2005 to a minimum in January of $4.73 \text{ nmol mg}^{-1} \text{ dw}$. Apart from the peak in MAA concentration in April,

concentrations tended to fluctuate between 4.7 and $8.5 \text{ nmol mg}^{-1} \text{ dw}$. One-way ANOVA indicated that there was a significant difference ($F_{(9,122)} = 10.609$, $P < 0.001$) in the MAA concentration among months. Tukey HSD *post hoc* comparisons between months indicate the differences were associated with the significantly ($P < 0.05$) higher concentration in April compared with any other month.

There was no correspondence between krill MAA concentrations and monthly UV-R irradiances. There was also no direct correspondence between Krill MAAs and MAA concentrations in phytoplankton (Fig. 6). In both instances, highest MAA concentrations in krill (i.e.

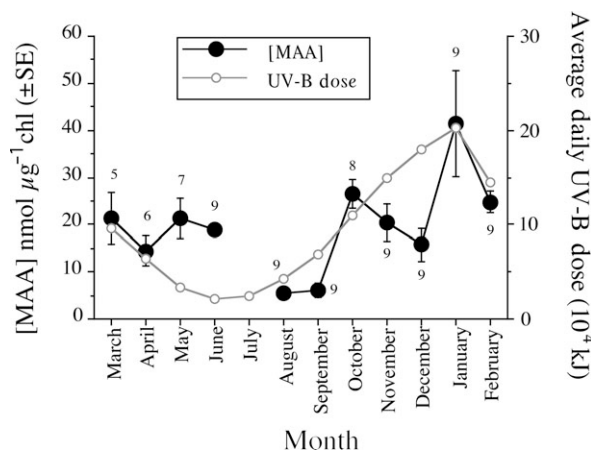


Fig. 3. Average daily UV-B dose (kJ) per month and average MAA concentration (nmol µg⁻¹ Chl) in phytoplankton (1 L seawater filtrates) collected in Otago Harbor and off the Otago Coast between March 2005 and February 2006. Number adjacent to each point is the sample size.

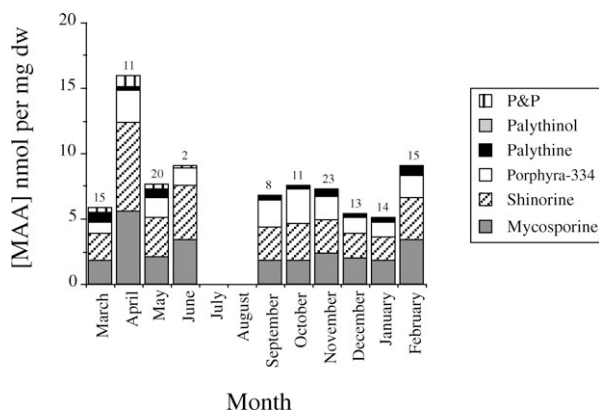


Fig. 4. Average concentration of mycosporine-glycine, shinorine, Porphyra-334, palythine and palythanol (nmol mg⁻¹ dw) in *Nyctiphanes australis* collected between March 2005 and February 2006. The category P&P represents data from chromatographs were palythine and palythanol formed a merged peak that could not be separated by HPLC analysis. Number above each bar is the sample size.

April) occurred approximately three months later than highest irradiances and highest phytoplankton MAA concentrations.

Nyctiphanes australis body parts

The lowest total MAA concentration was determined in the carapace (2.03 nmol mg⁻¹ dw, Table II), with the remaining dissected body parts having concentrations ranging from 4.89 to 5.98 nmol mg⁻¹ dw. There was very little variation in the percentage composition of MAA types among body parts, with the range of percentages of mycosporine-glycine ranging from 37.8 to 43.3%, shinorine from 25.6 to 33.5% and Porphyra-334 from 19.1 to 22.1%.

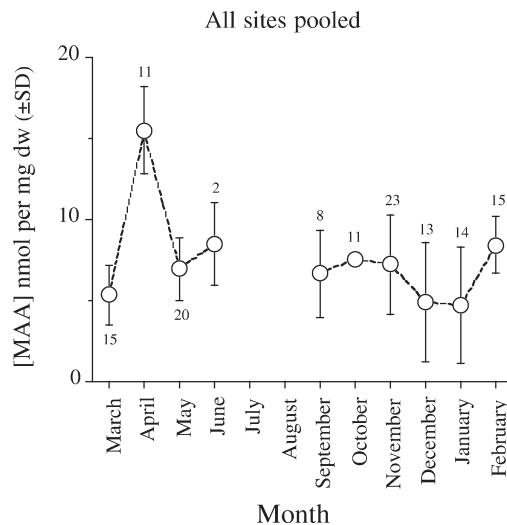


Fig. 5. Average total MAA concentration (nmol mg⁻¹ dw) in *Nyctiphanes australis* collected between March 2005 and February 2006. Number adjacent to each point is the sample size.

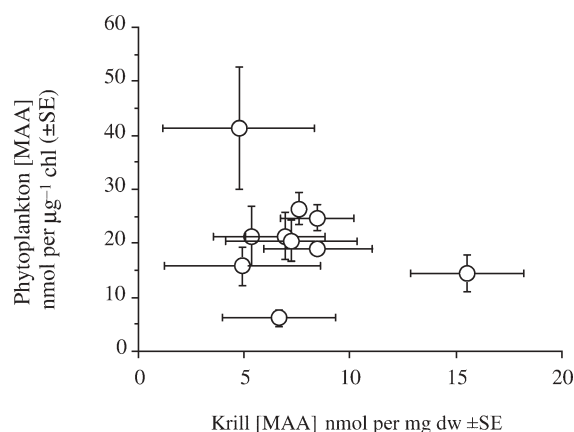


Fig. 6. Average total MAA concentration (nmol mg⁻¹ dw) in *Nyctiphanes australis* versus average MAA concentration (nmol µg⁻¹ Chl) in phytoplankton (1 L seawater filtrates) collected between March 2005 and February 2006.

DISCUSSION

MAAs are widespread in phytoplankton (Sinha *et al.*, 1998; Gröniger *et al.*, 2000), although cellular concentrations and the capacity to synthesis MAAs vary from species to species. In the present study, MAA concentrations in phytoplankton filtrates ranged from 5.6 to 41.4 nmol µg⁻¹ Chl. Few estimates of *in situ* MAA concentrations in phytoplankton have been reported. Whitehead *et al.* (Whitehead *et al.*, 2001) reported concentrations in phytoplankton assemblages in McMurdo Sound of 8.11 pg Shinorine µg⁻¹ protein and 20.2 pg Porphyra-334 µg⁻¹ protein. We could find no estimates

Table II: Comparative concentration of mycosporine-glycine, shinorine, Porphyrin-334, palythine and palythiol ($\text{nmol mg}^{-1} \text{ dw}$) in the carapace, legs, eyes, antennae and muscle in *Nyctiphanes australis*

	Myco-glycine	Shinorine	Porphyra-334	Palythine	Palythiol	Total
Carapace	0.88 (43.3%)	0.52 (25.6%)	0.45 (22.1%)	0.16 (7.8%)	0.02 (0.9%)	2.03
Legs	2.38 (39.7%)	1.98 (33.1%)	1.22 (20.4%)	0.36 (6.0%)	0.05 (0.8%)	5.98
Eyes	1.99 (37.8%)	1.67 (31.7%)	1.08 (20.5%)	0.43 (8.2%)	0.08 (1.5%)	5.26
Antenna	2.32 (41.2%)	1.81 (32.1%)	1.08 (19.1%)	0.37 (6.5%)	0.06 (1.1%)	5.63
Muscle	1.88 (38.4%)	1.64 (33.5%)	1.04 (21.2%)	0.29 (5.9%)	0.03 (0.6%)	4.89
Whole animal (SE)	3.65 (0.17)	3.47 (0.13)	1.88 (0.14)	0.66 (0.03)	0.09 (0.01)	9.75 (0.44)

Averages are for body parts pooled from 10 individuals collected from Blue Skin Bay on 21 February 2005. Percentages of total for each component are given in parentheses. To get a measure of variance, the average (\pm SE) MAA concentration in whole krill was examined in 10 additional individuals collected on the same day.

expressed in nanomolar concentrations to compare with the present results.

Our study did show that MAA concentrations in phytoplankton filtrates varied over time, and were lowest in the mid-winter months when UV-R was lowest, and highest in mid-summer when highest UV-R occurred. Sinha *et al.* (Sinha *et al.*, 1998) reviewed the role of MAAs in phytoplankton, and concluded that MAA concentrations often increase with increasing *in vitro* UV-R exposure. Researchers (Villafañe *et al.*, 1995; Helbling *et al.*, 1996; Zudaire and Roy, 2001; Hannach and Sigleo, 2004) have shown similar *in vitro* responses to UV-R exposure, although the response is species-specific. There are fewer examples of increases in MAA concentration in response to UV-R *in situ* in marine phytoplankton assemblages. Morrison and Nelson (Morrison and Nelson, 2004) examined changes in relative MAA concentrations in phytoplankton along the Bermuda Atlantic Time Series site (as measured by UV absorption in the range 313–335 nm), and noted that highest absorptions occurred in summer months, and were lowest or absent in winter months. Llewellyn and Harbour (Llewellyn and Harbour, 2003) measured MAA concentrations in English Channel phytoplankton assemblages, and noted a rapid increase in concentrations in spring and summer to a maximum concentration of $8.5 \mu\text{g L}^{-1}$, with the MAA:chlorophyll *a* ratio increasing linearly with *in situ* irradiances. We did not find the same linear relationship, but the observed lower winter and higher summer MAA concentrations are consistent with higher UV-R irradiances inducing MAA synthesis in phytoplankton off the Otago coast. In this respect, the average daily dose of UV-B increased 4.4-fold from winter to summer, while the minimum and maximum MAA concentrations varied 8-fold from August to January.

Apart from ambient surface irradiances, seasonal changes in MAA concentration in phytoplankton assemblages will be influenced by phytoplankton

composition, vertical mixing and the optical properties of the water column. Off the Otago Coast, the upper 200 m of the water column stratifies in the summer (Jillett, 1969; Haywood, 2004), while light attenuation increases 2-fold during spring (Pfannkuche, 2002). Both these features will influence the dose of UV-R to which the phytoplankton are exposed, and hence the extent of any UVR-induced MAA production. In addition, the phytoplankton composition off the Otago coast changes seasonally (Haywood, 2004), being dominated by diatoms (*Chaetoceros socialis* and *Asterionella* sp.) in spring, flagellates (*Phaeocystis* sp.) in the summer, dinoflagellates (*Ceratium* sp., *Heterocapsa* sp. and *Oxytoxum* sp.) and diatoms (*Corethron* sp., *Lichmophora* sp. and *Nitzschia* sp.) in the autumn, and pinnate diatoms (*Pseudo-nitzschia* sp. *Rhizosolenia* sp. and *Pleurosigma* sp.) in the winter. Given that MAA production is species-specific, these compositional changes may contribute to the observed pattern of MAA concentrations.

There is limited information on the concentrations of MAAs in krill. Newman *et al.* (Newman *et al.*, 2000) found a similar suite of MAAs in the Antarctic krill *Euphausia superba* as we observed in *N. australis*, with concentrations lower (mean total MAA concentration of $3.86 \text{ nmol mg}^{-1} \text{ dw}$) in the Antarctic species. These estimates may be lower than *in situ* concentrations as the krill they measured had been in captivity for 52 days. Karentz *et al.* (Karentz *et al.*, 1991) measured MAA concentrations in freshly caught *E. superba* as $4.67 \text{ nmol mg}^{-1} \text{ dw}$, which is $\approx 37\%$ lower than the average MAA concentration of $7.32 \text{ nmol mg}^{-1} \text{ dw}$ observed in *N. australis* over the entire year.

Newman *et al.* (Newman *et al.*, 2000) showed, through experimental manipulation, that krill accumulate MAAs from their phytoplankton diets, and that their MAA concentrations can depend on concentrations of MAAs in phytoplankton. These results would imply that concentrations of MAAs in naturally occurring populations of krill would depend on, and vary with,

concentrations of MAAs in phytoplankton assemblages that it is feeding on. The results of the present research did not find a temporal relationship between MAA concentrations in phytoplankton and *N. australis*. While MAA concentrations in phytoplankton were generally lower in the winter and higher in the summer, concentrations in krill remained relatively consistent during the year except for a peak in early-Autumn (April).

These observations may reflect one or a combination of factors. First, krill may accumulate MAAs at a rate independent of the concentrations in the phytoplankton it consumes. This may be because rates of accumulation in the relatively long-lived krill (probably 12 to 18 mo.) are relatively slow compared with the rate at which MAA concentrations vary in phytoplankton assemblages. It is worth noting that the high April concentrations in *N. australis* followed the summer period, which may represent a lag time in MAA accumulation. Without knowing the rate of MAA accumulation in *N. australis*, it is difficult to know the duration of a lag between changes in MAAs concentrations in phytoplankton and *N. australis*, although it likely exists. For example, Newman *et al.* (Newman *et al.*, 2000) showed that changes in MAA concentrations in the laboratory were small after 36 days, and in some cases only evident after 63 days. Alternatively, MAAs in *N. australis* may represent the near-saturation concentrations found in krill over the year (although this would not explain the peak in concentrations in April). Another possible explanation of the present observations is that *N. australis* accumulate MAAs from sources other than phytoplankton. This is plausible given the omnivorous nature of the *N. australis* diet that includes copepods (Pilditch and McClatchie, 1994), which themselves will likely contain MAAs. Indeed, the greater range of MAAs found in krill compared with those found within the phytoplankton assemblages would be consistent with an accumulation of MAAs from a range of food sources.

Nyctiphanes australis does not appear not to mitigate higher UV-R irradiances in the summer through co-accumulation of higher MAA concentrations (consuming and accumulating greater amounts of MAAs synthesized by summer phytoplankton assemblages) as has previously been suggested for *E. superba* by Newman *et al.* (2000). This is an important observation, as co-accumulation has been proposed as a potential mechanism for mitigating effects of higher UV-R irradiances in species like *E. superba* during processes such as ozone depletion (Newman *et al.*, 2000).

The temporal pattern of MAA concentration will have implications for the photobiology of *N. australis*. The lack of an increase in MAAs during summer months suggests that this krill species does not have a

greater sun-screening potential during months when UV-R irradiances are highest. If measured MAA concentrations are insufficient to completely block harmful UV-R, then it is possible that krill are under greater UV-induced stress during the summer months. Indeed, the intensities of UV-R recorded off Otago in the summer (up to 1.44 W m^{-2} and 32.5 W m^{-2} for UV-B and UV-A, respectively) are similar or higher than intensities that have been reported to cause *in situ* mortality and DNA damage in other marine zooplankton (e.g. Karentz *et al.*, 1991; Malloy *et al.*, 1997; Browman and Vetter, 2002; Lamare *et al.*, 2007), including Antarctic krill, *Euphausia superba*. For this latter species, Newman *et al.* (Newman *et al.*, 1999) found that *in vitro* survival time was significantly reduced to approximately 30 to 50% of dark controls when exposed to UV-B irradiances ranging from 0.378 to 0.918 W m^{-2} and UV-A ranging 5.23 to 5.73 W m^{-2} of UV-A.

Newman *et al.* (Newman *et al.*, 2003) later showed experimentally that *Euphausia superba* could reduce exposure to UV-B by increasing its depth when illuminated with high PAR and UV-A irradiances. In contrast, *Nyctiphanes australis* appears not to reduce UV-R exposure by altering their vertical distribution, either daily or seasonally. *Nyctiphanes australis* are often observed at the sea surface during daylight hours (M.D. Lamare personal observation; Ritz and Hosie, 1982; O'Brien 1988), with surface swarming occurring throughout the year (Bradford 1972; Ritz and Hosie, 1982; O'Brien, 1988). It should be noted that the swarming in itself, and resulting densities greater than $450\,000$ individuals m^{-3} (O'Brien, 1988), will significantly reduce UV-R exposure at the sea surface through substantial "self-shading" of individuals by con-specifics.

When discussing the role of MAAs for UV-R protection in krill, it is important to know how MAAs are distributed throughout the body of the animal. In *N. australis*, concentrations throughout the body were similar (ranging from 4.89 to $5.98 \text{ nmol mg}^{-1} \text{ dw}$) with the exception of the carapace that had a much lower concentration ($2.03 \text{ nmol mg}^{-1} \text{ dw}$). The widespread distribution throughout *N. australis* is not surprising given the relatively transparent krill; however, the lower concentrations in the carapace is unexpected given it is the surface that would receive the greatest incident UV-R. Indeed, other photoprotective compounds such as carotenoids have been found to be concentrated in the carapace of krill (Grynbaum *et al.*, 2005). The carapace is moulted during growth of *N. australis* [every 4 to 10 days at temperatures of 15°C and 10°C , respectively (Hosie and Ritz, 1983)], and the lower carapace MAA concentrations could be due to the removal of the compounds during moulting, or

the time required for MAAs to be incorporated into new carapace tissue.

In conclusion, while *N. australis* inhabits surface waters where it is likely to experience high incident UV-R, and indeed utilizes MAAs as photoprotectants, it does not appear to adjust MAA concentrations either passively (through co-accumulation in the phytoplankton diet) or actively in response to higher summer MAA concentrations. MAA concentrations were lowest in the carapace and may be a result of regular moulting of these body parts. Given the importance of the carapace for photoprotection, it would be useful to examine MAA fluxes within the structure during the moulting cycle.

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