Annals of Tropical Research 24(2):1-22
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Survey of ultraviolet radiation-absorbing mycosporine-like amino acids in adult females of planktonic copepods

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ABSTRACT

Lacuna D. G. 2002. Survey of ultraviolet radiation-absorbing mycosporine-like amino acids in adult females of planktonic copepods. *Ann. Trop. Res.* 24(2):1-22.

The presence of UV-absorbing mycosporine-like amino acids (MAAs) from nine planktonic calanoid copepod species were investigated. MAA concentrations from methanol extracts of adult females were analyzed by High Performance Liquid Chromatography (HPLC). A total of 4 MAAs were identified, namely mycosporine-glycine, palythine, porphyra-334 and shinorine. Although all the experimental copepods had MAAs, the occurrence of these compounds varied among species. For *Acartia omorii, Calanus sinicus* and *Pontella spinicauda*, 4 MAAs were identified; *Pontellopsis tenuicauda* and *P. yamadae*, had 3 MAAs while *Acartia sinjiensis, Centropages abdominalis, Paracalanus* sp. and *Sinocalanus tenellus* had only one MAA. Concentrations of total MAAs were also assessed. Total MAA concentrations ranged from 3.53 µg mgC^{-1} (*Pontella spinicauda*) to 0.003 µg mgC^{-1} (*Sinocalanus tenellus*). Since UV-absorbing MAAs act as a biochemical defense or sunscreen against the harmful wavelengths of UVB, increase content of these compounds, specifically MAAs with UVB-absorbing properties, in all neustonic copepods (*Pontella spinicauda, Pontellopsis tenuicauda* and *P. yamadae*) may suggest a physiological adaptation to high UV exposure which is the natural condition in neustonic environment.

Keywords: UV-absorbing compounds, copepods, mycosporine-like amino acids, neuston, plankton

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INTRODUCTION

Solar energy coming from the sun that reaches the edge of the earth's atmosphere is approximately 1,394 Wm-2 and has a spectrum characterized as follows: (1) UV radiation which is typically divided into 3 regions namely UVC (200-280 nm), UVB (280-320 nm) and UVA (320-400 nm); (2) photosynthetically active radiation or PAR (400-700 nm); and infrared radiation or IR (>700 nm). The relative solar spectral distribution outside the atmosphere comprises 51% in the IR region, 41% in the PAR and 8% in the UV region where 6.5% is UVA and 1.5% is UVB. Passing through the atmosphere, the radiation is subjected to scattering and absorption which reduces its intensity by ~35% before it reaches the earth's surface. As a result, the spectral distribution at the earth's surface differs from that experienced at the edge of the atmosphere. The amount of scattering and absorption is due to the different layers of the atmosphere surrounding the earth (deMora *et al.* 2000). Although various layers of the atmosphere are responsible for the attenuation of solar radiation, it is in the stratospheric layer where 90% of the ozone is created, that greatly attenuates the most biologically harmful wavelengths of UVB radiation. Reduction in the concentration of stratospheric ozone due to anthropogenic inputs of chlorofluorocarbons resulted in an increase influx of biologically damaging UVB radiation reaching the earth's surface and to ecologically significant depths in the ocean (Karentz and Lutze 1990, Smith *et al.* 1992). First discovered over the Antarctic (Farman *et al.* 1985), ozone depletion has also been documented in the Arctic and mid-latitudes (Blumthaler and Ambach 1990, Stolarski *et al.* 1992). Of immediate importance to this global problem are the threats to biological ecosystems since these shorter wavelengths damage the biological processes, of which the principal target is the DNA molecule, although protein and pigment damage may also occur (Cladwell 1981).

In the marine ecosystem, the impact of enhanced UVB penetration will likely be focused on the disruption of the food web dynamics. Phytoplankton which form the foundation of the aquatic food chain are affected by enhanced UVB as clearly demonstrated in the reduced photosynthetic rate (Boucher and Prezelin 1996, Lesser 1996), nitrogen uptake (Lohman *et al.* 1998) and carbon fixation (Sundback *et al.* 1997) and induced DNA damage

(Buma *et al.* 1996). In the second trophic level, constituted by primary herbivores (zooplankton), increased UVB radiation induced reproduction impairment (Karanas *et al.* 1979, 1981, Lacuna and Uye 2000, 2001), reduced zooplankton survival and development rate (Karanas *et al.* 1979, 1981, Damkaer and Dey 1983, Dey *et al.* 1988, Keller *et al.* 1997, Saito *et al.* 1998, Kouwenberg *et al.* 1999, Nozais *et al.* 1999, Lacuna and Uye 2000, 2001), induced sex shift (Chalker-Scott 1995) and zooplankton deformation (Naganuma *et al.* 1997, Kouwenberg *et al.* 1999, Lacuna and Uye 2000, 2001).

To counteract harmful effect of UVB radiation, aquatic organisms have evolved a biochemical defense, in the presence of photoprotective compounds such as mycosporine-like amino acids or MAAs, that aid survival (Karentz *et al.* 1994, Siebeck *et al.* 1994). The UV-absorbing compounds, or MAAs, were first discovered in fungi and were associated with increased sporulation, hence their name mycosporine (Leach 1965, Favre-Bonvin *et al.* 1976). These substances have strong absorption maxima from 310 to 360 nm, which corresponds to the spectrum of environmentally and biologically relevant UVB and UVA. It is assumed that MAA may function as UV filter or sunscreens that blocks the penetration of harmful wavelengths of UV upon reaching the DNA of the cell. Although direct evidence showing MAAs' primary function as UV protection is scarce, their assumed sunscreen role is implied by their strong absorption at wavelengths spanning UV radiation. MAAs are solely synthesized by the algae, bacteria and fungi via the shikimate pathway (Favre-Bonvin *et al.* 1987, Shick *et al.* 1999) and it is assumed that these compounds are bioaccumulated in the marine animals from their food source or diet (Karentz *et al.* 1994).

The most dominant herbivorous zooplankton in the marine food web dynamics are copepods because they play a vital link between phytoplankton and planktivorous fish (Uye *et al.* 1986). For example, in the Inland Sea of Japan, the important copepod species are *Acartia omorii*, *Calanus sinicus*, *Centropages abdominalis* and *Paracalanus* sp. that are particularly abundant in spring and early summer (Uye and Shimazu 1997). In the brackish-water lagoon, Lake Nakaumi, the dominant planktonic copepods are *Acartia sinjiensis* and *Sinocalanus tenellus* which are abundant in summer and winter-spring, respectively. Any perturbations in these copepod communities due to

UVB induced damage, would possibly result to changes in fisheries productivity not only in these two ecosystems but also in other Japanese marine and brackish-waters.

To date, only one marine planktonic copepod, *Calanus propinquus*, was studied for the presence of MAAs (Karentz *et al.* 1991). The aim therefore of this paper is to identify and assessed the concentration of UV-absorbing compounds in the adult females of 9 planktonic copepods.

MATERIALS AND METHODS

Sampling period and collection of samples

Sampling of marine water column dwelling copepods, viz. *Acartia omorii, Calanus sinicus*, *Centropages abdominalis* and *Paracalanus* sp. were carried out from the pontoons located at Ondo, Kure, during the periods from May 1996 to November 1999. Brackish-water copepods, viz. *Acartia sinjiensis* were collected in May 1999 while *Sinocalanus tenellus* were sampled irregularly from October 1997 to May 1999 in Lake Nakaumi. Marine neustonic copepods, viz. *Pontella spinicauda*, *Pontellopsis tenuicauda* and *P. yamadae*, were sampled in September 1997, July 1998 and May 1999 in the Inland Sea of Japan during cruises of T & R/V Toyoshio Maru.

Collections were made by oblique hauls of a conical plankton net (diameter:0.45 m, mesh opening:300μm) fitted with a 1 l volume cod-end. The contents of the cod-end were transferred into 7 l insulated stainless steel containers containing surface water where the animals were sampled and brought back to the laboratory in Higashi-Hiroshima. The time for transportation was ca.3 h from Lake Nakaumi and 1 h from Ondo and Kure harbors. *Pontella spinicauda*, *Pontellopsis tenuicauda* and *P. yamadae* were collected in the Inland Sea of Japan by towing a neuston net for 10 min

with a speed of 1m sec^{-1}.

Analysis of Mycosporine-like amino acids (MAAs)

Immediately after zooplankton collections, about 500 adult females of each copepod species were sorted in a glass dish, from which they were individually picked by a pair of forceps, dipped in filtered sea water and placed on glass fiber filters (GF/F). The filters with copepods were transferred into plastic tubes (volume=8 ml) and were kept at -30°C before MAA analysis.

During the MAA analyses, adult females were extracted using methanol and the UV absorption spectra was recorded with a Shimadzu spectrophotometer. MAAs were separated and quantified by high-liquid performance chromatography (HPLC). Absorbances of MAAs were recorded at 280, 313 and 340 nm to indicate the wavelength range of maximum absorption. MAAs were identified by co-chromatography and comparison of wavelength ratios with standards. Total content of specific MAAs in each sample was calculated from HPLC peak areas with calibration factors determined by analysis of standards and corrected by the extraction coefficient.

The final concentrations are presented as micrograms of a specific MAA per milligram carbon of each copepod species. This is obtained by dividing the total content of a specific MAA with the carbon weight of each copepod species obtained by converting the prosome length using the length-weight regression equations established for some copepod species by Uye (1982).

RESULTS

Spectrophotometric analyses of methanol extracts of adult females of each copepod species are presented in Fig.1. For neustonic copepods, *Pontellopsis tenuicauda* and *P. yamadae* showed maximum absorbance peak at 310 nm while *Pontella spinicauda* at 310 and 334 nm. For water column dwelling copepods, *Acartia sinjiensis*,*Calanus sinicus*, *Paracalanus* sp. and *Sinocalanus tenellus* showed a maximum absorbance peak at 334 nm while *Centropages abdominalis* and *Acartia omorii* at 310 and 320 nm, respectively.

HPLC separations of methanolic extracts of adult females of each copepod species are shown in Fig. 2. The chromatograms indicate the presence of 4 mycosporine-like amino acids identified as mycosporine-glycine, palythine, porphyra-334 and shinorine, the structures of which are given in Fig. 3. The neustonic copepod, *Pontella spinicauda*, and the water column dwelling copepods, *Acartia omorii* and *Calanus sinicus*, had all the 4 MAAs (as mentioned above). Other neustonic species such as *Pontellopsis tenuicauda* and *P. yamadae* contained 3 MAAs, such as mycosporine-glycine, palythine and porphyra-334. On the other hand, only one MAA was identified for the rest of the water column dwelling copepods. *Acartia sinjiensis*, *Paracalanus* sp. and *Sinocalanus tenellus* contained shinorine while *Centropages abdominalis* had mycosporine-glycine.

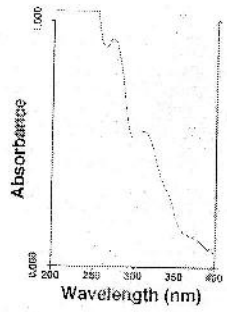
List of the experimental copepods with notes on the type of MAA(s) present, maximum absorbance, total number of MAAs, individual content and total MAA concentration is presented in Table 1. The total concentration of MAAs is generally higher in adult females of neustonic copepods (ranged: 3.53 to 0.54 $\mu g\ mgC^{-1}$) and some water column dwelling species such as *Acartia omorii* and *Calanus sinicus* (3.19 and 2.65 $\mu g\ mgC^{-1}$,respectively) compared to the rest of the water column dwelling copepods (ranged: 0.55 to 0.003 $\mu g\ mgC^{-1}$). *Pontellopsis yamadae* and *P. tenuicauda* were largely dominated by high concentrations of mycosporine-glycine (65%) (Fig. 4); *Pontella spinicauda* contained high amount of porphyra-334 (53.3%) and mycosporine-glycine (36.5%); *Acartia omorii* and *Calanus sinicus* had high

Table 1. Wavelength of maximum (λ_{max} nm) in UV range for methanol extract concentration of individual MAAs (μg mg C^{-1}), total number of MAAs (#), total MAA concentration (Σ, μg mg C^{-1}) for each copepod species

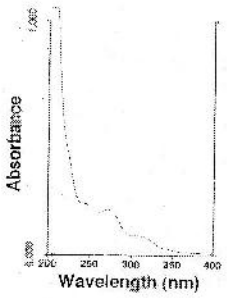
Copepod Species	λ_{max}	Palythine	Shinorine	Porphyra-334	Mycosporine-glycine	#	(Σ)
Neustonic Copepods							
1. *Pontellopsis yamadae*	310	0.16	0	0.23	0.72	3	1.11
2. *P. tenuicauda*	310	0.08	0	0.11	0.35	3	0.54
3. *Pontella spinicauda*	334 & 310	0.25	0.11	1.88	1.29	4	3.53
Water-column dwelling Copepods							
1. *Acartia sinjiensis*	334	0	0.05	0	0	1	0.05
2. *Acartia omorii*	320	1.5	0.30	0.49	0.90	4	3.19
3.*Calanus sinicus*	334	0.15	0.29	1.46	0.75	4	2.65
4. *Centropages abdominalis*	310	0	0	0	0.55	1	0.55
5.*Sinocalanus tenellus*	334	0	0.003	0	0	1	0.003
6. *Paracalanus sp.*	334	0	0.42	0	0	1	0.42

concentrations of palythine (47%) and porphyra-334 (55.1%), respectively.

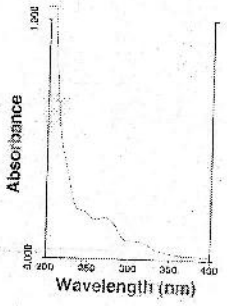
The results demonstrated that neustonic copepods contained large concentrations of mycosporine-glycine which has a maximum absorbance at the UVB region while some water column dwelling species, *Acartia omorii* and *Calanus sinicus*, contained high concentrations of palythine and porphyra-334, respectively, the maximum absorbance peak of which is within the UVA wavelengths. The rest of the water column dwelling copepods contained one MAA of low concentrations.



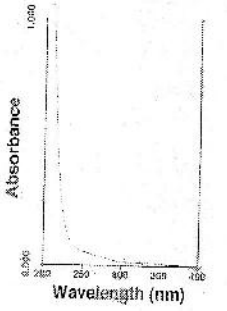
Pontella spinicauda



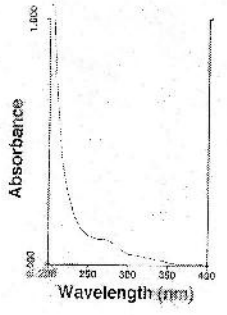
Pontellopsis tenuicauda



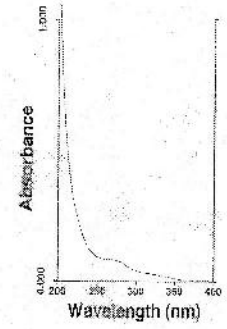
Pontellopsis yamadae



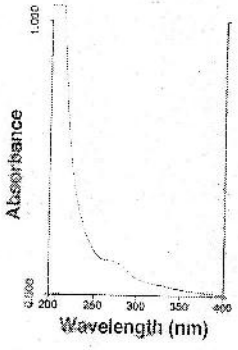
Acartia sinjiensis



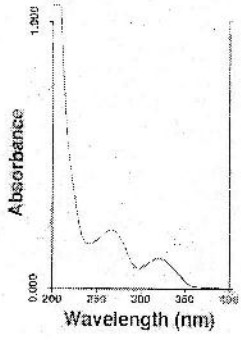
Centropages abdominalis



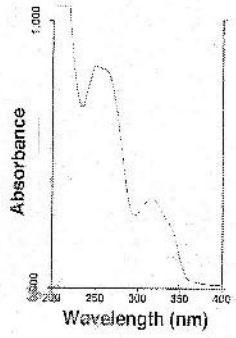
Paracalanus sp.



Sinocalanus tenellus

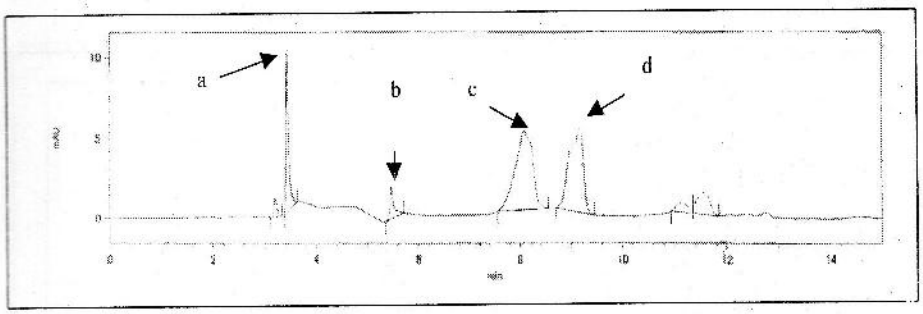


Acartia omorii



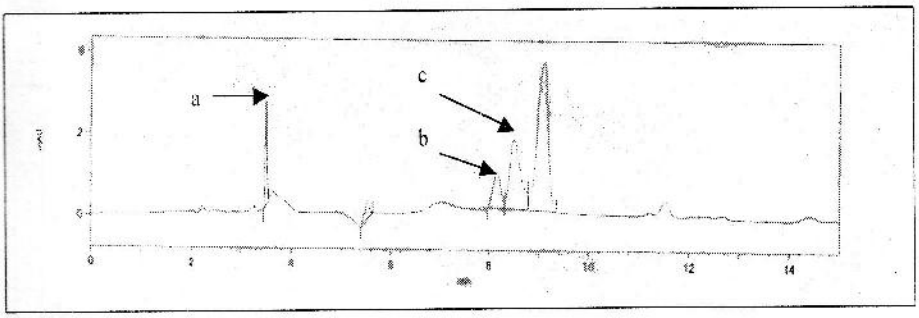
Calanus sinicus

Fig. 1. Spectrophotometric scan of the methanol extract of adult females of planktonic copepods.



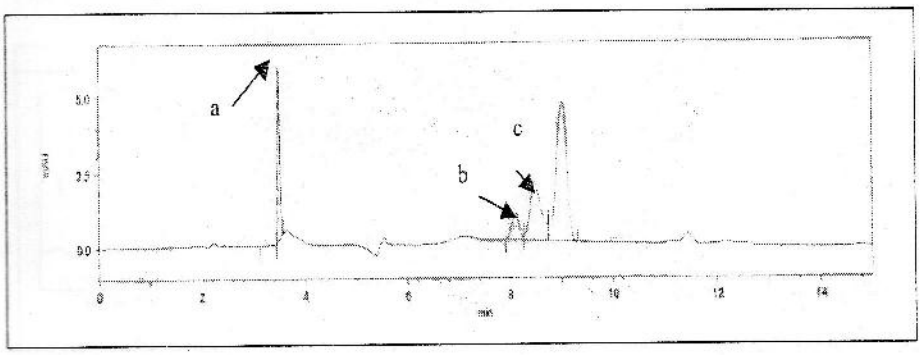
Pontella spinicauda

Legend : a:palythine; b=shinorine; c=porphyra-334; d=mycosporine-glycine



Pontellopsis yamadae

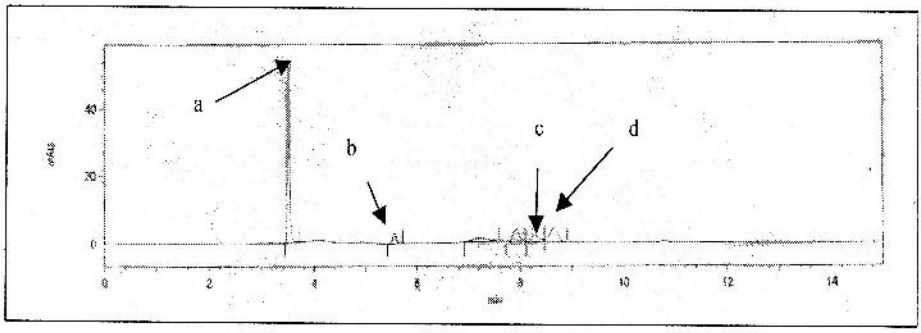
Legend: a=palythine; b=porphyra-334; c=mycosporine-glycine



Pontellopsis tenuicauda

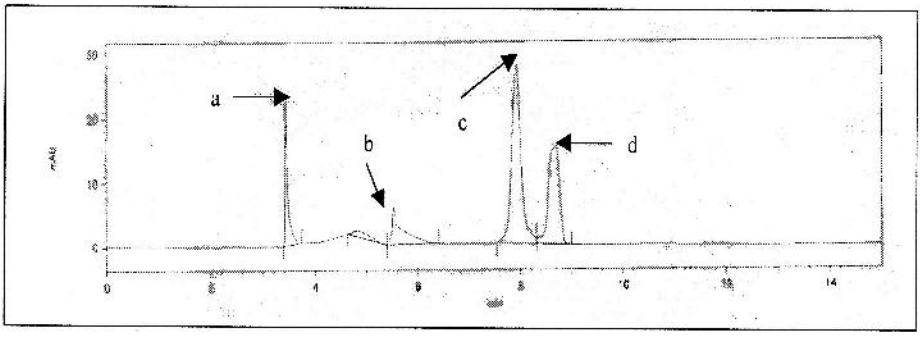
Legend:a=palythine; b=porphyra-334; c=mycosporine-glycine

Fig. 2. High performance liquid chromatographic separation of MAAs from methanol extracts of adult females of planktonic copepods.



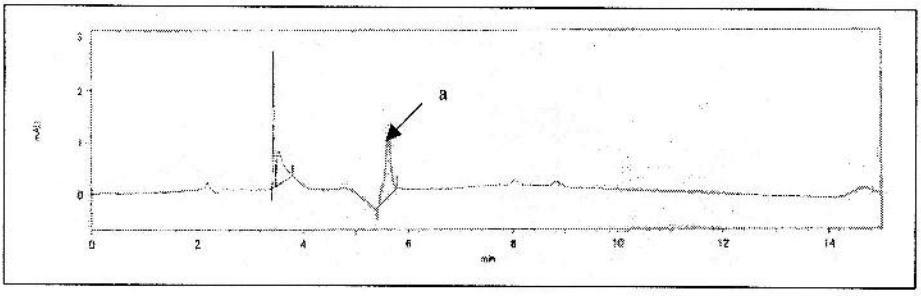
Acartia omorii

Legend: a=palythine; b=shinorine; c=porphyra-334; d=mycosporine-glycine



Calanus sinicus

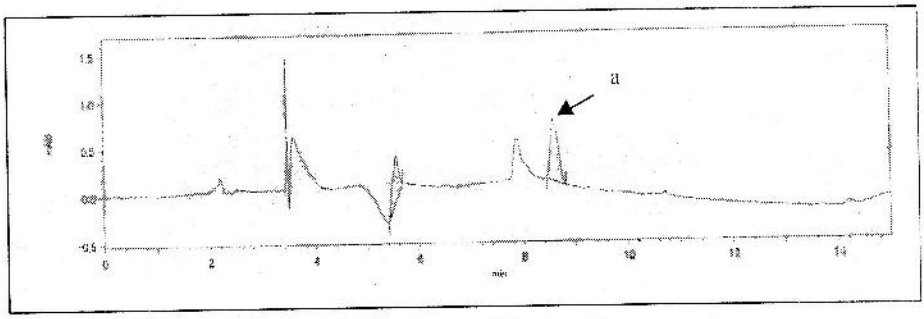
Legend:a=palythine; b=shinorine; c=porphyra-334; d=mycosporine-glycine



Acartia sinjiensis

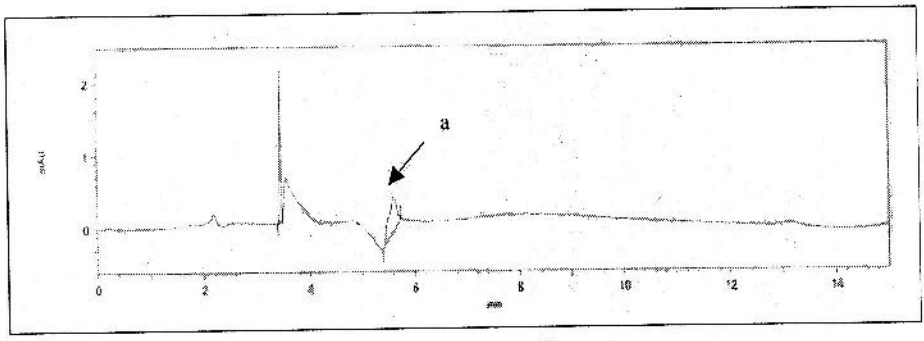
Legend: a= shinorine

Fig. 2. Continued...



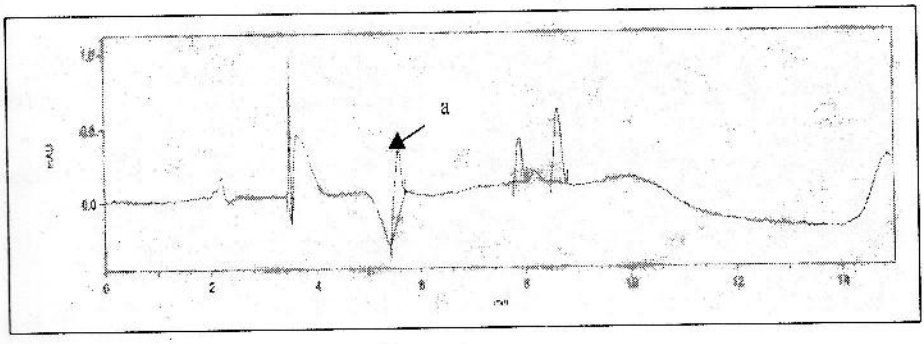
Centropages abdominalis

Legend:a=mycosporine-glycine



Sinocalanus tenellus

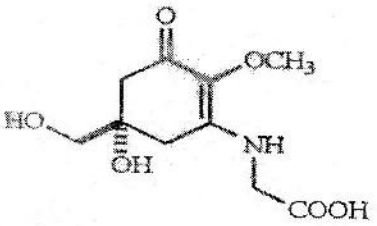
Legend: a=shinorine



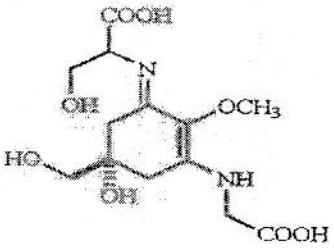
Paracalanus sp.

Legend: a=shinorine

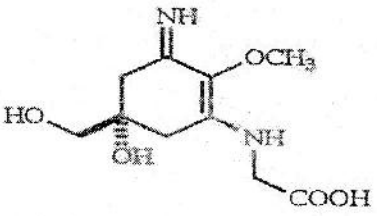
Fig. 2. Continued...



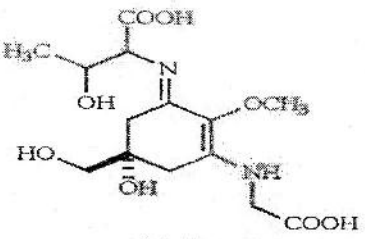
Mycosporine-glycine
(λ_{max} 310 nm)



Shinorine
(λ_{max} 334 nm)

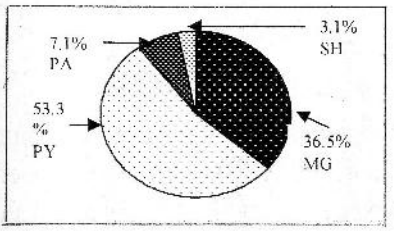


Palythine
(λ_{max} 320 nm)

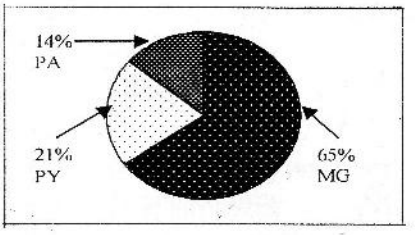


Porphyra-334
(λ_{max} 334 nm)

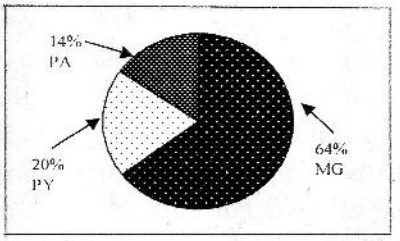
Fig. 3. Chemical structures of mycosporine-like amino acids from the methanol extract of adult females of planktonic copepods.



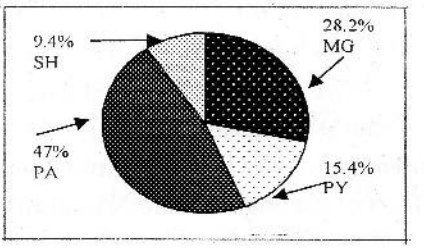
Pontella spinicauda



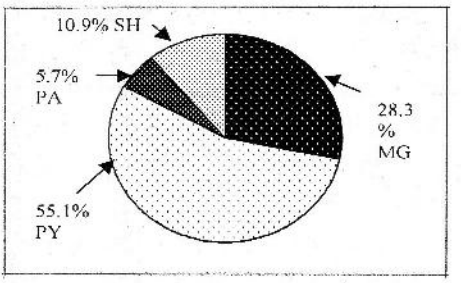
Pontellopsis yamadae



Pontellopsis tenuicauda



Acartia omorii



Calanus sinicus

Legend: PA=Palythine; PY=Porphyra-334; MG=Mycosporine-glycine; SH=Shinorine

Fig. 4. Relative concentration (%) of individual MAAs in the adult females of neustonic copepods and some water column dwelling copepods.

DISCUSSION

MAAs have previously been isolated from a variety of fungi and marine organisms (Arpin and Bouillant 1981, Nakamura 1982) but have not been reported in terrestrial plants. In marine organisms, identical MAAs have been found in diverse species from temperate and tropical latitudes (Yoshida and Sivalingham 1970, Sivalingham *et al.* 1976, Ito and Hirata 1977, Takano et al. 1978 a,b, 1979, Chiocarra *et al.* 1980, Tusjino *et al.* 1980, Nakamura *et al.* 1981,1982, Dunlap and Chalker 1986, Dunlap *et al.* 1989) and from the Antarctic (Karentz *et al.* 1991). The results of the present survey indicate that MAAs are also common in some planktonic copepods from the Inland Sea of Japan and Lake Nakaumi. The widespread occurrence of MAAs at all latitudes suggest a conservative trait in the evolution of organisms (Karentz *et al.*, 1991). This may be practically true since during the origins of life on earth, where ozone layer was void in Earth's original atmosphere, UVB intensities then were much higher compared to the present situation (Fischer 1965, Cloud 1968, Margulis 1981). The biological hazard created by the absence of ozone is considered to be primarily responsible for the confinement of early life to the oceans. The oxygenation of the atmosphere, as a result of the evolution of photosynthesis, resulted to the presence of UV-filtering ozone at the earth's atmosphere. This process has taken over 1 billion years to attain the present atmosphere conditions. The first life forms to succeed in shallow-water areas were subjected to high levels of UVB and had to develop effective methods for protection and repair. Organisms have probably always had an array of defenses to minimize UV exposure and a variety of repair mechanisms to deal with UV-induced damage. As a result, many tolerance mechanisms for UV are common across many forms of life (Mitchell and Karentz 1993). Therefore, protection from UV exposure would have been an important phenotypic feature in the early evolution of eukaryotic organisms and in the subsequent processes of speciation and natural selection. Over the course of evolution, this genetic information for biochemical defense against UV exposure has been retained in fungi and marine organisms. Other groups that must cope with more constant and direct UV irradiation, such as terrestrial plants, have developed more elaborate protective mechanisms, including morphological

adaptations (i.e. leaf shape and orientation), pigments (i.e. anthocyanins, carotenoids), and other UV-protecting compounds (i.e. furanocoumarins, flavanols) (Caldwell 1981, Zangerl and Berenbaum 1987, Tevini and Teramura 1989).

To date, fourteen different MAAs have been chemically identified, described and characterized in a wide variety of marine animals including amphipod, bryzoan, chaetognatha, copepod, ctenophore, fish, isopod, planarian, polychaete, ribbon worm, sea anemone, snail (Karentz *et al.* 1991), brine shrimp (Grant *et al.* 1985), clam (Ishikura et al. 1997, Karentz *et al.* 1991), corals (Gleason 1993), euphausiids (Nakamura *et al.* 1982, Newman *et al.* 2000), sea cucumber (Shick et al. 1992, Badaranyake and Roucher 1999, Karentz *et al.* 1991), sea hare (Carefoot et al. 1998), sea urchin (Adams and Shick 1996, Carroll and Shick 1996), starfish (Nakamura *et al.* 1982, Karentz *et al.* 1991) and one copepod, *Calanus propinquus* (Karentz *et al.* 1991). This study reports the presence of MAAs in marine and brackish-water planktonic copepods from the Inland Sea of Japan and Lake Nakaumi.

Among the 9 planktonic copepods analyzed in this study, the neustonic species and some water column-dwelling copepods (*Acartia omorii* and *Calanus sinicus*) contained 3 or 4 MAAs while the rest of the water column-dwelling species had only one MAA. The cumulative effect of having more than one MAA with different maximum absorption between 310 and 334 nm is to widen the copepods' UV-screening capabilities so that the MAAs' protective role will increase across a broader range of wavelengths. Since MAAs absorb UV between 310 to 360 nm, it is assumed that they provide protection from UV exposure that is comparable to the melanin pigmentation response of human skin (Karentz 1994). All species of the neustonic copepods examined had mycosporine-glycine, porphyra-334 and shinorine. The presence of the compound palythine in addition to the 3 mentioned MAAs was also separated and identified in the neustonic copepod *Pontella spinicauda* and some water column-dwelling copepods such as *Acartia omorii* and *Calanus sinicus*.

Biologically harmful radiation of UVB can reach between 11-17 m depth in the ocean (Montecino and Pizarro 1995) and even a short residence time in the surface waters could result in detrimental exposure to UVB. The neustonic species of copepods occur in the hyponeuston, the layer between the surface

and about 5 cm depth (Mauchline *et al.* 1998). This neustonic environment received the highest and strongest doses of UVB radiation. In order to adapt to such harsh condition, the neustonic copepods must evolve a mechanism that ensures their protection and survival against the damaging effect of UVB. Since the neustonic copepods examined in the present study do not exhibit migration to deeper waters, it is assumed that the presence of high concentrations of mycosporine-glycine,which has a maximum absorbance in the UVB region, may act as sunscreen or protection against UVB, hence allowing them to maintain its population in such harsh environment. On the other hand, the water column dwelling species examined in this study were dominated by MAAs which absorb the UVA wavelengths but not the damaging radiation of UVB. This may be the reason why this group occur as pseudoneuston, organisms whose maximum concentration occur in the hyponeuston but are much deeper than those inhabited by the neustonic copepods. A portion of these pseudoneustonic populations enters the hyponeuston at night during diel vertical migration (Mauchline *et al.* 1998). Although this behavior is common for the water column dwelling species examined in the study, there is no direct evidence that they can actually detect and avoid UVB. Instead, this behavior is induced by escape from visual predators (Bollens and Frost 1990; Huang *et al.* 1993).

Limited information is available on the biosynthesis and functions of MAAs. It is obvious that these compounds are synthesized by algae, but it is not known if UV protection is their primary function or is instead a secondary role. There is no evidence yet that MAAs are synthesized *de novo* by animals but it was proven that they are bioaccumulated in the animal tissues through ingestion of plants containing these MAAs, as reported in the sea urchin, *Strongylocentrotus droebachiensis* (Carroll and Shick 1996); or through translocation from symbionts (Karentz *et al.* 1991, Shick *et al.* 1992); or ingestion of preys that may have fed on MAA-rich plants.

Regardless of whether the UV protective properties of MAAs are a primary or secondary function or whether MAAs are synthesized or bioaccumulated, the existence of these compounds within a cell may shield internal components and organelles from the full impact of UV radiation. The presence of these natural UV filter systems, specifically UVB filters, in high

concentrations in the adult females of neustonic copepods may imply that these organisms have some degree of biochemical defense or protection from physiologically harmful wavelengths of UVB, hence allowing them to survive and proliferate in neustonic environment where UV intensities are strong.

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