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Large variability in the concentration of mycosporine-like amino acids among zooplankton from lakes located across an altitude gradient

Abstract—The qualitative and quantitative composition of mycosporine-like amino acids (MAAs), a family of intracellular UV-absorbing compounds, were investigated in zooplankton from 15 lakes located in the Central Alps between 913 and 2,485 m above sea level. The lakes differed in their UV water transparency (1% attenuation depth, $Z_{1\%}$, at 320 nm: 1.1–25.6 m) and maximum depth (Z_{max}: 3–133 m), thus offering the possibility to test the influence of different UV exposure conditions of zooplankton on the concentration of MAAs. Seven distinct MAAs were detected, but shinorine (maximum absorption: 334 nm) was the predominant compound. In the copepods *Cyclops abyssorum, C. abyssorum tatricus,* and *Acanthodiaptomus denticornis,* the total MAA concentration ranged from 0.01 to 3.1% of the dry weight. In the rotifers *Keratella cochlearis* and *Polyarthra dolichoptera,* MAAs were also found; however, these compounds were undetectable in *Asplanchna priodonta* as well as in the cladocerans *Daphnia hyalina, D. longispina, Bosmina longispina,* and *Chydorus sphaericus.* The total concentration of MAAs in populations of *Cyclops* spp. and phytoplankton collected simultaneously was not associated ($r^2 = 0.09$, $P > 0.05$), suggesting a different dynamic in the accumulation of these compounds. The variability in the concentration of MAAs, however, was related with the diffuse attenuation coefficient at 320 nm ($r^2 = 0.74$, $P < 0.001$) and the fraction of the water column to which 1% of the surface irradiance at 320 nm ($Z_{1\%}:Z_{max}$) penetrated ($r^2 = 0.86$, $P < 0.001$). These relationships suggest that the prevailing UV exposure condition in the lakes is a major determinant of the concentration of MAAs found in zooplankton. Our data support the hypothesis that MAAs, together with other photoprotective compounds, play a major role in minimizing the damaging effects of solar UV radiation in zooplankton from transparent lakes.

The increase of the solar ultraviolet radiation (UVR, 290–400 nm) flux with elevation and the high water transparency observed in most mountain lakes, particularly those located above the tree line (Morris et al. 1995; Sommaruga and Psenner 1997; Laurion et al. 2000), suggest that zooplankton, as well as other organisms, are potentially stressed by high fluxes of UVR. However in these ecosystems, zooplankton have evolved different strategies to either avoid (e.g., diel vertical migration) or protect themselves from UVR (e.g., through the synthesis or accumulation of pigments such as melanin and carotenoids that absorb directly or indirectly the energy of solar radiation).

Several studies with freshwater copepods have demonstrated the photoprotective role of carotenoids (Hairston 1976; Byron 1982). The concentration of carotenoids in calanoid copepods increases with lake altitude as well as with the shallowness of the system (Byron 1982). About 10-fold higher carotenoid concentrations are usually observed in freshwater calanoid copepods than in cladocerans (Hessen 1992). Melanin, which absorbs UVR directly, is mainly observed in arctic and alpine *Daphnia,* as well as in other cladocerans (Hessen and Sørensen 1990). Generally, pigmented species are less sensitive to solar radiation than unpigmented ones (Hairston 1976; Byron 1982; Ringelberg et al. 1984).

Mycosporine-like amino acids (MAAs) are a family of water-soluble, low–molecular weight compounds with high molar extinction coefficients (ϵ: 28,100–50,000 M cm^{-1}) and absorption maxima between 309 and 360 nm. Chemically, MAAs are composed of a cyclohexenone or cyclohexenimine ring substituted with different amino compounds. To date, approximately 19 different MAAs have been described in different aquatic and terrestrial organisms, including bacteria, cyanobacteria, lichens, phytoplankton, macroalgae, invertebrates, and fish (*see* Karentz et al. 1991; Dunlap and Shick 1998).

Recently, MAAs have been identified for the first time in the freshwater copepod *Cyclops abyssorum tatricus* from an alpine lake (Sommaruga and Garcia-Pichel 1999). MAAs have also been detected in the marine copepod *Calanus propinquus* (Karentz et al. 1991) and may be common in other freshwater zooplankton species (e.g., in *Boeckella gracilipes* because UV absorption peaks [~334 nm] have been observed in extracts of this species (Tartarotti et al. 1999).

Knowledge about the occurrence of MAAs in different zooplankton taxa, as well as information on the factors affecting the concentration of these compounds, is limited. Consequently, in this study, our objectives were (1) to determine how widely distributed MAAs are among different freshwater zooplankton species and (2) to relate MAA concentrations with parameters reflecting the prevailing UV exposure conditions of zooplankton, such as the UV water transparency and the fraction of the water column exposed to 1% of the surface UV irradiance.

Fifteen lakes located between 913 and 2,485 m above sea level in the Central Alps (Austria and Italy) were sampled in October 1998 during the overturn period (Table 1). The maximum depth (Z_{max}) of the lakes ranged from 3 to 133 m, and the diffuse attenuation coefficient (K_d) of UVR at 320 nm ranged from 0.18 to 4.34 m^{-1} (Table 1; Laurion et al. 2000), corresponding to attenuation depths, that is, 1% of the surface irradiance ($Z_{1\%}$) of 25.6–1.1 m, respectively. The wavelength of 320 nm (one of the four nominal wavelengths in the UV range measured by the PUV 500A profiling radiometer from Biospherical Instruments) was chosen to provide information on the attenuation of UVR because it lies at the border between UV-B (290–320 nm) and UV-A (320–400 nm) radiation.

Zooplankton samples were collected from a rubber boat by vertical net (55-μm mesh size) tows made at the center of the lake around noon. In the laboratory, the organisms were kept at 4°C in the dark until further processing within 48 h, but in most cases within 24 h. Crustacean plankton

Table 1. Latitude, longitude, altitude, maximum depth (Z_{max}), diffuse attenuation coefficient at 320 nm (K_{d320}), and fraction of the water column to which 1% of the surface UV radiation at 320 nm penetrated ($Z_{1\%}:Z_{max}$) in each sampled lake. The 1% attenuation depth ($Z_{1\%}$) was calculated by dividing 4.605 (the natural log of 100) by K_d. Except for Karersee, data on Z_{max} and K_{d320} are from Laurion et al. (2000).

Lake	Latitude (N)	Longitude (E)	Altitude (m a.s.l.)	Z_{max} (m)	K_{d320} (m^{-1})	$Z_{1\%}:Z_{max}$
Piburgersee (PIB)	47°11′	10°53′	913	24.6	3.32	0.06
Achensee (ACH)	47°27′	11°42′	929	133	2.24	0.02
Pragser Wildsee (PRA)	46°41′	12°05′	1,489	36	2.01	0.06
Karersee (KAR)	46°25′	11°35′	1,519	17	1.44	0.19
Durnholzer See (DUR)	46°44′	11°25′	1,560	13	1.36	0.26
Obernbergersee (OBB)	46°59′	11°24′	1,590	15	0.84	0.37
Antholzer See (ANT)	46°53′	12°10′	1,640	38	0.97	0.12
Sebensee (SEE)	47°21′	10°56′	1,650	14	0.60	0.55
Obersee (OBR)	46°53′	12°12′	2,016	26.7	1.09	0.16
Lichtsee (LIC)	47°01′	11°24′	2,104	6.6	4.34	0.16
Klammsee (KLA)	46°58′	12°07′	2,258	3	1.50	1.02
Mittlerer Plenderlesee (MPL)	47°12′	11°03′	2,317	5.7	0.50	1.62
Oberer Plenderlesee (OPL)	47°12′	11°02′	2,344	7.5	0.18	3.41
Gossenköllesee (GKS)	47°13′	11°00′	2,417	9.9	0.24	1.94
Rotfelssee (ROT)	47°14′	11°00′	2,485	5.5	0.24	3.49

and the rotifer *Asplanchna priodonta* were concentrated on a net sieve of 100-μm mesh size and washed several times with tap water to remove phytoplankton. Under the stereomicroscope, CO_2-narcotized copepods were separated into different life stages (nauplii only found in Rotfelssee), while cladocerans were separated into juvenile and adult stages. Individuals were carefully transferred with a pipette and placed on a wet glass GF/F fiber filter (Whatman) kept on an ice pack. The number of individuals per filter ranged between ~10 and 100 (copepods) and up to 800 (cladocerans). In some cases, because of the very low number of individuals present in the sample, the different developmental stages of the copepods were pooled on one filter. Rotifers (*Keratella cochlearis, Polyarthra dolichoptera*) were concentrated onto a GF/F filter. To estimate their abundance under the inverted microscope, an aliquot was taken before filtration. All filters were frozen at −80°C until extraction within 1 month.

UV-absorbing compounds were extracted in 20% aqueous methanol (v:v; MeOH) for 24 h at 4°C, followed by a 2-h extraction in a water bath at 45°C (Garcia-Pichel and Castenholz 1993; Sommaruga and Garcia-Pichel 1999). The efficiency of the extraction procedure was >95%. Filters were sonicated on an ice bath, and the extracts were cleared using a 0.1-μm pore size Anodisc filter (Whatman). To determine the presence of UV-absorbing compounds, the extracts were scanned in a spectrophotometer (double-beam Hitachi U-2000) between 250 and 750 nm, using a 5-cm pathlength quartz cuvette. The extracts were subsequently evaporated to dryness under vacuum in 2-ml Eppendorf microcentrifugation tubes using a SpeedVac concentrator (Savant) at 45°C. The samples were stored at −80°C for further characterization using high-performance liquid chromatography (HPLC).

The dried extracts were resuspended in 0.5–2 ml of 55% MeOH (v:v), and 10-μl aliquots were injected in a Brownlee 5-μm pore size RP-8 column (4.6 mm interior diameter by 25 cm) protected with an RP-8 guard column for isocratic reversed-phase HPLC analysis. Samples were run with a mobile phase of 0.1% acetic acid in 55% aqueous MeOH (v:v) at a flow rate of 0.5 ml min^{-1}. The MAAs in the eluate were detected by online UV spectroscopy. All samples were later crosschecked with another HPLC equipped with a diode array detector (Dionex) to confirm the different MAAs identified. Moreover, most samples were run with a mobile phase of 0.1% acetic acid in 25% aqueous MeOH (v:v). The MAAs were identified by comparison with published retention times and by cochromatography with purified standards obtained from F. Garcia-Pichel or with standards prepared from marine invertebrate extracts (*Aplysia dactylomela* eggs obtained from D. Karentz; *Anthopleura* sp. and *Palythoa* sp. obtained from M. Shick). The total content of specific MAAs in each sample was calculated from HPLC peak areas, using published molar extinction coefficients (Ito and Hirata 1977; Takano et al. 1978, 1979; Tsujino et al. 1979, 1980 and references therein). The molar extinction coefficient for asterina-330 was assumed to be the same as that of palythinol (Dunlap et al. 1989). Concentrations of MAAs were normalized to the dry weight of zooplankton, which was estimated by the relationship between body length and dry weight calculated for these taxa (Bottrell et al. 1976).

Spectrophotometric analyses of extracts from all copepod species examined showed prominent peaks or absorption shoulders in the UV range. In the cyclopoid copepods, *Cyclops abyssorum* and *C. abyssorum tatricus*, the absorption maximum was found at ~330 nm (Fig. 1a–c), whereas in the calanoid copepod *Acanthodiaptomus denticornis* (only present in Piburgersee), the maximum was detected at 357 nm (Fig. 1d). The methanolic extracts of *C. abyssorum tatricus* from Gossenköllesee showed two peaks: one with very high absorption at 328 nm and another at 373 nm (Fig.

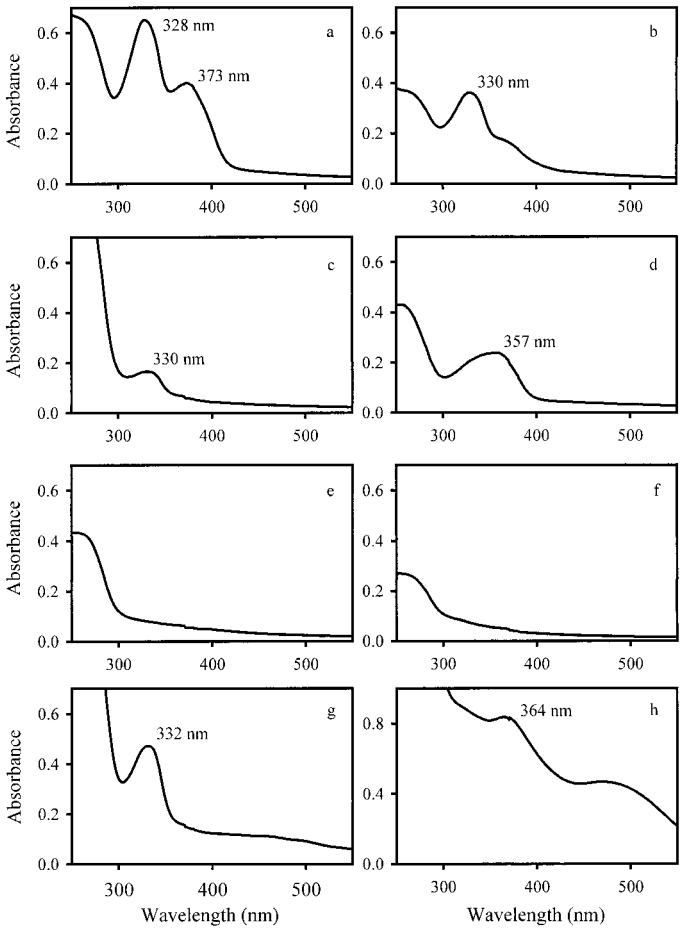

Fig. 1. Absorbance of aqueous methanolic (20%) extracts made of (a) *Cyclops abyssorum tatricus,* GKS; (b) *C. abyssorum tatricus,* MPL; (c) *C. abyssorum,* ACH; (d) *Acanthodiaptomus denticornis,* PIB; (e) *Daphnia longispina,* PIB; (f) *Bosmina longispina,* ACH; (g) *Polyarthra dolichoptera,* SEE; (h) *Keratella cochlearis,* LIC. Abbreviations of the lakes are given in Table 1.

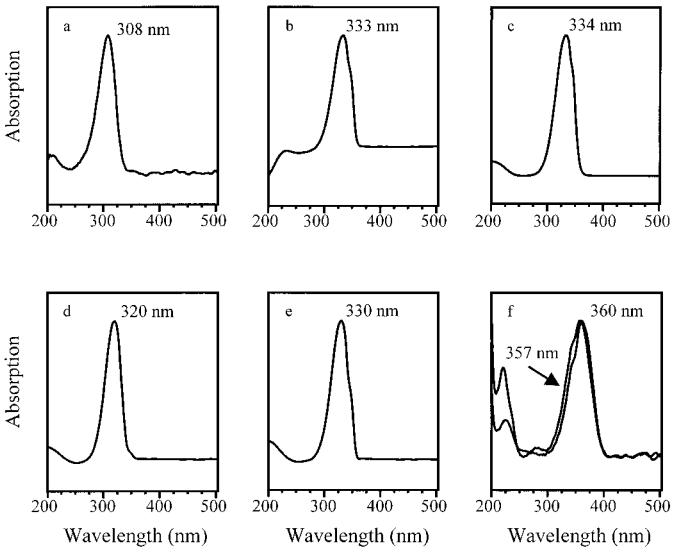

Fig. 2. Absorption spectra of the seven different MAAs detected in zooplankton ordered by their average retention time (Rt) in min: (a) mycosporine-glycine, Rt = 4.4; (b) shinorine, Rt = 6.6; (c) porphyra-334, Rt = 7.5; (d) palythine, Rt = 13.1; (e) asterina-330, Rt = 13.9; (f) MAA 357, Rt = 16.3, and palythene, Rt = 17.2. Values indicate the wavelength at maximum absorption. Samples were run with a mobile phase of 0.1% acetic acid in 55% aqueous methanol (v/v) at a flow rate of 0.5 ml min^{-1}.

1a). For the different cladoceran taxa (*Daphnia hyalina, D. longispina, Bosmina longispina,* and *Chydorus sphaericus*), there was no distinct absorption in the UV range (Fig. 1e,f). Methanolic extracts from the rotifers showed absorption maxima at 332 nm (*P. dolichoptera*) and 364 nm (*K. cochlearis*) (Fig. 1g,h). In *A. priodonta,* no absorption peak in the UV range was observed (data not shown).

Six different MAAs were identified in the zooplankton of the 15 lakes investigated: mycosporine-glycine, shinorine, porphyra-334, asterina-330, palythine, and palythene. The absorption spectra of the different MAAs identified are shown in Fig. 2. One unknown UV-absorbing compound was frequently found in copepods and rotifers with a maximum absorption at 357 nm, probably usujirene, the *cis*-isomer of palythene (Tsujino et al. 1979), and designated as MAA 357 hereafter.

All copepod species and life stages examined contained at least two distinct MAAs (Fig. 3). Only *C. abyssorum tatricus* from Gossenköllesee and Mittlerer Plenderlesee and *A. denticornis* contained detectable amounts of all seven MAAs. Shinorine was present in all copepod species and developmental stages, whereas palythine and asterina-330 were found in 86 and 79%, respectively, of the copepods. Sixty-four percent of all copepods contained MAA 357 and palythene; 43% had mycosporine-glycine. Porphyra-334 was detected in 29% of the copepods examined. Shinorine showed the highest concentrations in *C. abyssorum* and *C. abyssorum tatricus* (up to 95% of the total MAA concentration; Fig. 3g), whereas MAA 357 and palythene were the dominant MAAs in *A. denticornis* (up to 34% of the total MAA concentration; Fig. 3h). The highest concentration of a specific MAA (0.022 μg shinorine μg^{-1} dry wt) was found in nauplii of *C. abyssorum tatricus* from Rotfelssee (mean total MAA concentration: 0.025 $\pm$ 0.008 μg μg^{-1} dry wt). Total MAA concentrations in copepods ranged from 0.01 to 3.1% of the dry weight.

The rotifers *P. dolichoptera* and *K. cochlearis* had five and six MAAs, respectively (Fig. 3o,p). Total MAA concentrations represented 0.3 and 0.01% of the dry weight for *P. dolichoptera* and *K. cochlearis,* respectively. Shinorine was the most abundant MAA in *P. dolichoptera* (89% of total MAA concentration) and *K. cochlearis* (38%) (Fig. 3o,p). In agreement with the results from the spectrophotometric analyses, no MAAs were found by HPLC analyses in the rotifer *A. priodonta* or in any cladoceran species examined.

The variability in the concentration of MAAs was only assessed for the species present in most of the studied lakes (i.e., *C. abyssorum,* including its alpine form *C. abyssorum tatricus*). The concentration of MAAs in those populations increased exponentially with lake altitude ($r^2 = 0.86$, $P < 0.001$; Fig. 4a), as well as with the UV water transparency (e.g., K_d at 320 nm [$r^2 = 0.74$, $P < 0.001$; Fig. 4b]). Although lake altitude and K_d were not significantly correlated

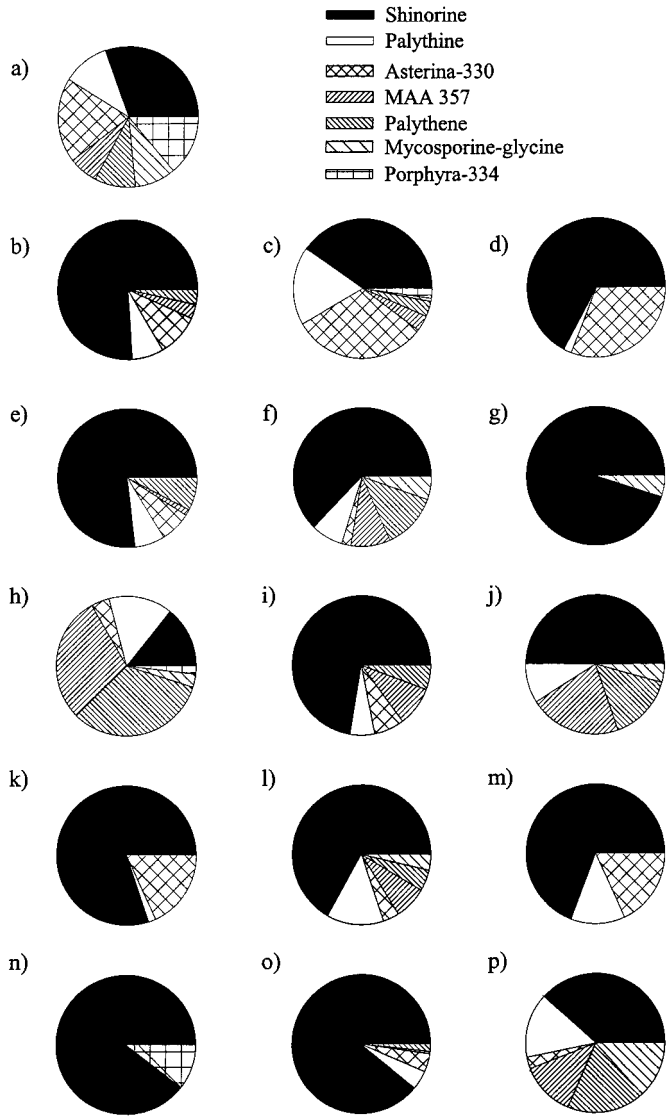

Fig. 3. Relative contribution (%) of the different compounds to the total MAA concentration in (a) *C. abyssorum tatricus*, MPL; (b) *C. abyssorum tatricus*, OPL; (c) *C. abyssorum tatricus*, GKS; (d) *C. abyssorum tatricus*, ROT; (e) *C. abyssorum tatricus*, OBB; (f) *C. abyssorum tatricus*, LIC; (g) *C. abyssorum*, ACH; (h) *A. denticornis*, PIB; (i) *C. abyssorum*, ANT; (j) *C. abyssorum tatricus*, OBE; (k) *C. abyssorum tatricus*, KLA; (l) *C. abyssorum tatricus*, PRA; (m) *C. abyssorum tatricus*, KAR; (n) *C. abyssorum tatricus*, DUR; (o) *P. dolichoptera*, SEE; (p) *K. cochlearis*, LIC. Abbreviations of the lakes are given in Table 1.

($r = -0.480$, $P > 0.05$) in our data set, these parameters are known to be associated (Laurion et al. 2000), so that the correlation found between altitude and concentration of MAAs may be indirect. The fraction of the water column to which 1% of the surface UVR at 320 nm penetrated ($Z_{1\%}$: Z_{max}, Table 1) explained even a higher percentage of the variability in MAA concentration than the K_d ($r^2 = 0.86$, $P < 0.001$; Fig. 4c). The 1% value used here does not refer to any critical level at which damage to zooplankton occurs but was selected to be consistent with other studies on plankton.

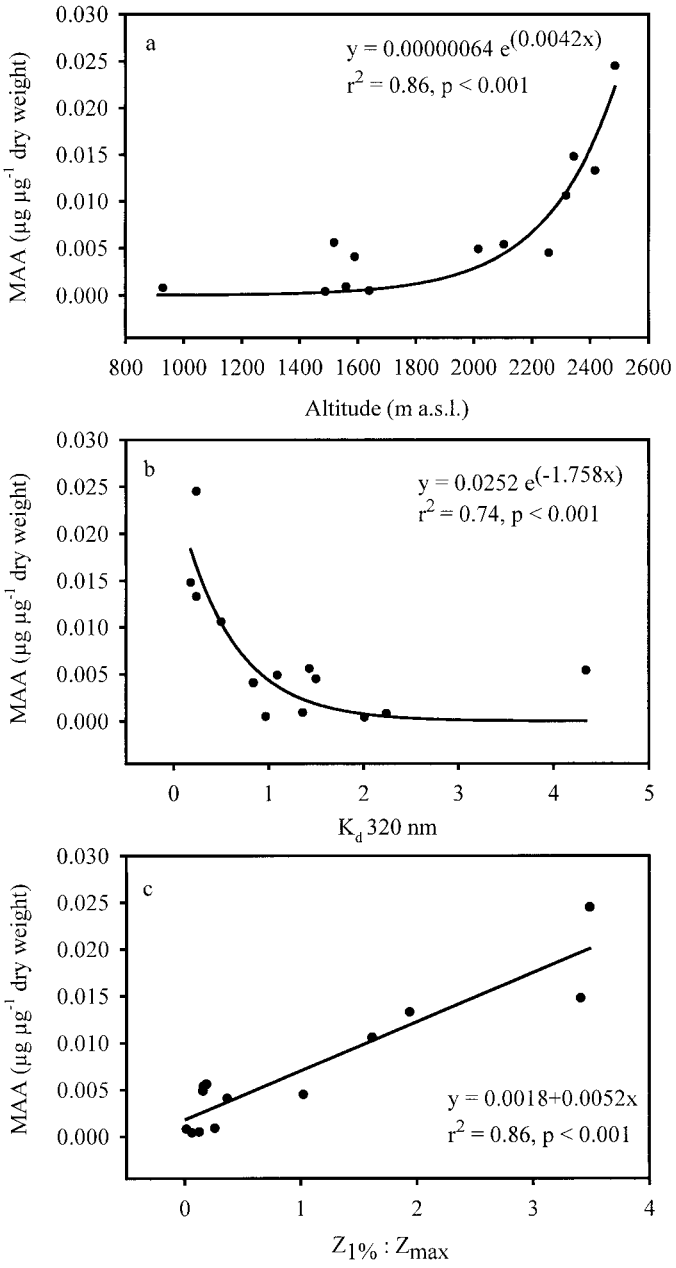

Fig. 4. Relationship between the total concentration of MAAs in populations of the copepod *Cyclops abyssorum* and *C. abyssorum tatricus* (mean for all life stages) and (a) lake altitude, (b) the diffuse attenuation coefficient (K_d) at 320 nm and (c) the fraction of the water column to which 1% of the surface UV radiation at 320 nm penetrated ($Z_{1\%}$: Z_{max}). See Table 1 for explanations on the latter parameter.

Our results show that MAAs are widespread among different freshwater cyclopoid and calanoid copepod species. However, a large variability in the concentration of MAAs (~14-fold) was observed among populations of *C. abyssorum* and *C. abyssorum tatricus* (Fig. 4). The lakes selected in our study represent a wide range of UV exposure conditions for planktonic organisms, as assessed from K_d and the ratio $Z_{1\%}$: Z_{max}. Notably, the last five lakes in the series shown in Table 1 differed largely from the others in these param-

eters. Furthermore, the lakes are located at different altitudes, a factor known to affect the magnitude of the instantaneous flux of solar UVR reaching the surface. Interestingly, we did find a strong correlation of the concentration of MAAs in *Cyclops* populations with lake altitude, as well as with K_d and $Z_{1\%} : Z_{max}$ (Fig. 4). The late copepodid stages and adults of *Cyclops* spp. are known to vertically migrate and stay deep in the water column during daytime (Tartarotti et al. 1999 and references therein); however, in transparent and shallow lakes, they will still be exposed to considerable UV radiation. Thus, the ratio $Z_{1\%} : Z_{max}$ may be considered as a proxy to assess the physical UV refuge available to zooplankton in the lakes. The ecological relationships observed suggest that the prevailing UV exposure condition in the lakes is a major determinant of the amount of MAAs accumulated by zooplankton. This is in agreement with studies reporting that differences in the MAA concentrations in marine invertebrates are associated with UV exposure conditions of the habitat (e.g., shallow vs. deep or the latitudinal gradient of UV irradiance) (Karentz et al. 1991; Banaszak et al. 1998).

Interpretation of the above-mentioned relationships, however, needs to be done in the context of the origin of MAAs for these organisms. Because metazoans apparently lack the putative shikimate acid pathway needed to synthesize MAAs (Bentley 1990), the UV-absorbing compounds found in zooplankton have most likely an algal dietary origin, as reported for other invertebrates (Carrol and Shick 1996). Consequently, it is important to consider that the variation in the concentration of MAAs among copepod populations may be associated with that of phytoplankton. Testing this association in field studies, however, can be difficult if the timescale for changes in the concentration of MAAs in phytoplankton is different from that in zooplankton. Furthermore, in species that change food habits during its ontogeny, like *Cyclops abyssorum*, MAAs may have an intermediary origin. Newman et al. (2000) have shown that starved *Euphasia superba* can maintain constant concentrations of MAAs for up to 35 d. In contrast, phytoplankton can more rapidly adjust MAA concentrations (i.e., hours to days) (Villafañe et al. 1995; Sommaruga unpubl. data). These antecedents suggest that the concentrations of MAAs in *Cyclops* populations must not necessarily relate to those found in phytoplankton at the time of sampling. In fact, when we compared the concentration of MAAs between copepods and phytoplankton collected simultaneously from the same studied lakes (Laurion et al. 2000), no significant correlation was found ($r = 0.09$, $P > 0.05$; Fig. 5).

Nevertheless, one might hypothesize that in the absence of nutrient limitation or other inhibitory factors, concentrations of MAAs in phytoplankton would be higher at certain periods (e.g., at high UV irradiance) in lakes where the prevailing UV exposure conditions suggest UV stress for these organisms. Indeed, the concentration of MAAs in phytoplankton (normalized to biomass) is higher in lakes with low K_d values, although in many cases a large variability for the same attenuation coefficient value exists (Laurion et al. in prep.). The concept of coacclimation proposed by Newman et al. (2000) provides a functional mechanism by which MAA concentrations in consumers can be increased by an indirect response of algae to enhanced UV exposure. These authors proposed that grazers like *E. superba* can coacclimate to enhanced UV exposure by ingesting larger amounts of MAAs produced by phytoplankton in response to periods of high UVR or by UV-mediated changes in species composition within the algal community favoring the presence of UV-tolerant species with a higher capacity to synthesize MAAs. We have no information about which species of phytoplankton are able to synthesize MAAs in the study lakes. However, experiments in one of the studied lakes (Gossenköllesee) have shown that the phytoplankton community structure during summer is dominated in the epilimnion by UV-tolerant species (Halac et al. 1997).

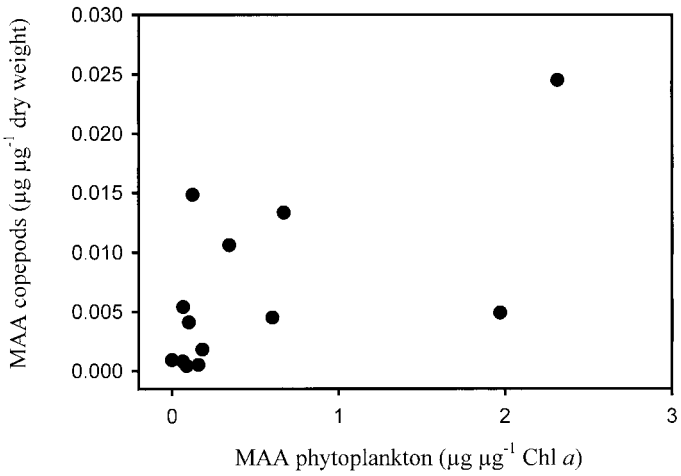

Fig. 5. Comparison between the total concentration of MAAs in copepods (μg μg^{-1} dry wt; mean for all life stages) and phytoplankton (μg μg^{-1} chlorophyll *a*) sampled during October 1998. Data on concentrations of MAAs in phytoplankton are from Laurion et al. (2000). The point in the upper right corner was not considered for the statistical analysis.

The highest concentration of MAAs in zooplankton was found in nauplii of *C. abyssorum tatricus*. In contrast to the late stages of this species, nauplii often thrive close to the water surface during daytime. Thus, having high concentrations of MAAs may be crucial for minimizing UV damage in the early developmental stages. Because the first two naupliar stages do not feed, the first batch of MAAs probably derives from the mother. Afterwards, nauplii most likely begin to feed on microplankton and start to bioaccumulate MAAs. The same process is known for the transfer of carotenoids in the freshwater calanoid copepod *Diaptomus nevadensis* (Hairston 1978), whose nauplii contain between 3 and 10 times higher carotenoid concentrations than the adults.

Although MAAs were present in the rotifers *P. dolichoptera* and *K. cochlearis*, these compounds were undetectable in the transparent predatory rotifer *A. priodonta*, as well as in the cladocerans *D. hyalina, D. longispina, B. longispina*, and *C. sphaericus*. Absence of MAAs is known for certain taxonomic groups of marine organisms (Karentz et al. 1991). However, in the case of *A. priodonta*, we cannot discard the possibility that the number of individuals extracted ($n = 60$) was too low for the detection of MAAs. Interestingly, this

species seems to be highly sensitive to solar UVR (Williamson et al. 2001). Regarding the absence of MAAs in cladocerans, we have no valid explanation because these species are known to feed mainly on phytoplankton, and up to 800 individuals were extracted in one sample. On the other hand, these cladoceran populations came from lakes with high K_d and $Z_{max} > 15$ m (ACH, PIB, LIC, KLA; *see* Table 1); thus, during daytime, they may occupy water layers receiving low UVR. Nevertheless, in the same lakes, MAAs were found in copepods, albeit at low concentrations. One can only speculate about a lack of, or inefficient, transport systems for MAAs in the gut of these cladoceran species. If the absence of MAAs proves to be general for cladocerans, this may be an explanation, in combination with the generally low concentration of carotenoids (Hessen 1992), for their high UV sensitivity (Zagarese et al. 1994; Williamson et al. 2001).

The occurrence of several MAAs with different absorption maxima in one species represents a clear advantage in terms of sunscreen capability. In most organisms, one compound accounts for most of the UV absorption, whereas the contribution by other MAAs is relatively minor (Dunlap and Chalker 1986; Garcia-Pichel and Castenholz 1993). Our data show that the rotifer and copepod species examined have a broad UV filtering capacity, with two to seven MAAs per species. However, as found in other organisms, one MAA was responsible for most of the absorption (*Cyclops* spp.: shinorine; *A. denticornis*: palythene). The difference in the dominant MAAs found between cyclopoid and calanoid copepods may reflect the distinct composition of their diets because these groups are considered carnivorous/omnivorous and herbivorous/omnivorous, respectively.

The only study on MAAs of freshwater zooplankton is that of Sommaruga and Garcia-Pichel (1999), who identified shinorine, asterina-330, mycosporine-glycine, and palythine in *C. abyssorum tatricus* from Gossenköllesee (i.e., the same species and lake examined in the present study). Our present data confirm the presence of the same MAAs in this species, but also show the existence of other compounds like palythene, porphyra-334, and MAA 357. In addition, the MAA concentration in the copepodid stages C III/IV collected in October was lower than that reported for summer by Sommaruga and Garcia-Pichel (1999). For example, the concentrations of shinorine, palythine, and mycosporine-glycine were ~2.7, 2.6, and 1.6 times lower in October, while that of asterina-330 was ~1.7 times higher. Altogether, these data suggest the existence of qualitative and quantitative temporal changes of MAAs in zooplankton requiring further study.

As discussed above, the concentration of MAAs in zooplankton and phytoplankton was not correlated. Discrepancies in the qualitative composition of MAAs between these two planktonic groups were also observed (data not shown), which can also be attributed to the different dynamic in the accumulation of MAAs in zooplankton. However, two additional nonexclusive explanations that need to be tested are (1) other sources of MAAs than phytoplankton and (2) potential chemically and bacterially mediated transformations of MAAs in the copepod's gut, as suggested for marine invertebrates (Dunlap and Shick 1998).

The high concentrations of MAAs observed in many populations of copepods and rotifers suggest that these sunscreens constitute an important adaptation to minimizing UV damage in those aquatic ecosystems where exposure to high solar UVR is significant. The high efficiency of absorption of UVR by MAAs, combined with the wide range of wavelengths screened in the UV-B and UV-A bands, may explain the high UV tolerance found in copepod species from transparent alpine lakes (Tartarotti et al. 1999). The relative contribution of MAAs, as well as other photoprotective compounds and strategies (e.g., photoreactivation) to minimize damage, still need to be tested.

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References

BANASZAK, A. T., M. P. LESSER, I. B. KUFFNER, AND M. ONDRUSEK. 1998. Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAs) in marine organisms. Bull. Mar. Sci. **63:** 617–628.

BENTLEY, R. 1990. The shikimate pathway: A metabolic tree with many branches. Crit. Rev. Biochem. **25:** 307–384.

BOTTRELL, H. H., AND OTHERS. 1976. Review of some problems in zooplankton production studies. Nor. J. Zool. **24:** 419–456.

BYRON, E. R. 1982. The adaptive significance of calanoid copepod pigmentation: A comparative and experimental analysis. Ecology **63:** 1871–1886.

CARROL, A. K., AND J. M. SHICK. 1996. Dietary accumulation of UV-absorbing mycosporine-like amino acids (MAAs) by the green sea urchin *Strongylocentrotus droebachiensis.* Mar. Biol. **124:** 561–569.

DUNLAP, W. C., AND B. E. CHALKER. 1986. Identification and quantitation of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. Coral Reefs **5:** 155–159.

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———, D. MCB. WILLIAMS, B. E. CHALKER, AND A. T. BANASZAK. 1989. Biochemical photoadaptations in vision: UV-absorbing pigments in fish eye tissues. Comp. Biochem. Physiol. **93B:** 601–607.

———, AND J. M. SHICK. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: A biochemical and environmental perspective. J. Phycol. **34:** 418–430.

GARCIA-PICHEL, F., AND R. W. CASTENHOLZ. 1993. Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl. Environ. Microbiol. **59:** 163–169.

HAIRSTON, N. G. 1976. Photoprotection by carotenoid pigments in the copepod *Diaptomus nevadensis.* Proc. Natl. Acad. Sci. USA **73:** 971–974.

———. 1978. Carotenoid photoprotection in *Diaptomus kenai.* Verh. Int. Ver. Limnol. **20:** 2541–2545.

HALAC, S., M. FELIP, L. CAMARERO, S. SOMMARUGA-WÖGRATH, R. PSENNER, J. CATALÁN, AND R. SOMMARUGA. 1997. An in situ enclosure experiment to test the solar UV-B impact on microplankton in a high-altitude mountain lake. I. Lack of effect on phytoplankton species composition and growth. J. Plankton Res. **19:** 1671–1686.

HESSEN, D. O. 1992. DNA-damage and pigmentation in alpine and arctic zooplankton as bioindicators of UV-B radiation. Verh. Int. Ver. Limnol. **25:** 482–486.

———, AND K. SØRENSEN. 1990. Photoprotective pigmentation in alpine zooplankton populations. Aqua Fenn. **20:** 165–170.

ITO, S., AND Y. HIRATA. 1977. Isolation and structure of a mycosporine from the zoanthid *Palythoa tuberculosa.* Tetrahedron Lett. **28:** 2429–2430.

KARENTZ, D., F. S. MCEUEN, M. C. LAND, AND W. C. DUNLAP. 1991. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: Potential protection from ultraviolet exposure. Mar. Biol. **108:** 157–166.

LAURION, I., M. VENTURA, J. CATALAN, R. PSENNER, AND R. SOMMARUGA. 2000. Attenuation of ultraviolet radiation in mountain lakes: Factors controlling the among- and within-lake variability. Limnol. Oceanogr. **45:** 1274–1288.

MORRIS, D. P., AND OTHERS. 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. Limnol. Oceanogr. **40:** 1381–1391.

NEWMAN, S. J., W. C. DUNLAP, S. NICOL, AND D. RITZ. 2000. Antarctic krill (*Euphasia superba*) acquire a UV-absorbing mycosporine-like amino acid from dietary algae. J. Exp. Mar. Biol. Ecol. **255:** 93–110.

RINGELBERG, J., A. L. KEYSER, AND B. J. G. FLIK. 1984. The mortality effect of ultraviolet radiation in a translucent and in a red morph of *Acanthodiaptomus denticornis* (Crustacea, Copepoda) and its possible ecological relevance. Hydrobiologia **112:** 217–222.

SOMMARUGA, R., AND F. GARCIA-PICHEL. 1999. UV-absorbing mycosporine-like compounds in planktonic and benthic organisms from a high-mountain lake. Arch. Hydrobiol. **144:** 255–269.

———, AND R. PSENNER. 1997. Ultraviolet radiation in a high mountain lake of the Austrian Alps: Air and underwater measurements. Photochem. Photobiol. **65:** 957–963.

TAKANO, S., D. NAKANASHI, D. UEMURA, AND Y. HIRATA. 1979. Isolation and structure of a 334 nm UV-absorbing substance, Porphyra-334 from the red alga *Porphyra tenera* Kjellman. Chem. Lett. (Chem. Soc. Japan, Tokyo) **1979:** 419–420.

———, D. UEMURA, AND Y. HIRATA. 1978. Isolation and structure of two new amino acids, palythinol and palythene, from the zoanthid *Palythoa tuberculosa.* Tetrahedron Lett. **49:** 4909–4912.

TARTAROTTI, B., S. CABRERA, R. PSENNER, AND R. SOMMARUGA. 1999. Survivorship of *Cyclops abyssorum tatricus* (Cyclopoida, Copepoda) and *Boeckella gracilipes* (Calanoida, Copepoda) under ambient levels of solar UV radiation in two high mountain lakes. J. Plankton Res. **21:** 549–560.

TSUJINO, I., K. YABE, AND M. SAKURAI. 1979. Presence of the near 358 nm UV-absorbing substances in red algae. Bull. Fac. Fish, Hokkaido Univ. **30:** 100–108.

———, ———, AND I. SEKIKAWA. 1980. Isolation and structure of a new amino acid, shinorine, from the red alga *Chondrus yendoi,* Yamada et Mikami. Bot. Mar. **23:** 65–68.

VILLAFAÑE, V. E., E. W. HELBLING, O. HOLM-HANSEN, AND B. E. CHALKER. 1995. Acclimatization of Antarctic natural phytoplankton assemblages when exposed to solar ultraviolet radiation. J. Plankton Res. **17:** 2295–2306.

WILLIAMSON, C. E., O. G. OLSON, S. E. LOTT, N. D. WALKER, D. R. ENGSTROM, AND B. HARGREAVES. 2001. Ultraviolet radiation and zooplankton community structure following deglaciation in Glacier Bay, Alaska. Ecology **82:** 1748–1760.

ZAGARESE, H. E., C. E. WILLIAMSON, M. MISLIVETS, AND P. ORR. 1994. The vulnerability of *Daphnia* to UV-B radiation in the northeastern United States. Arch. Hydrobiol. Beih. Ergeb. Limnol. **43:** 207–216.

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Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*

Abstract—The hypothesis that the low food quality of nontoxic, ingestible cyanobacteria for *Daphnia* is due to the absence of long-chained polyunsaturated fatty acids (PUFAs) was tested. *Synechococcus elongatus,* which is well assimilated by *Daphnia* and is deficient in PUFAs, was chosen as a model food organism. A newly devised method was used to load single fatty acids and mixtures of fatty acids on beads of bovine serum albumin, which then were added as supplements to cyanobacterial suspensions. Growth rates of juvenile *Daphnia galeata* on *Synechococcus elongatus* were low and were not enhanced by the addition of C_{18}-PUFAs and C_{20}-PUFAs singly or together on beads. Supplementation of lipids from the green alga *Scenedesmus obliquus* significantly enhanced growth of *D. galeata,* indicating that the low quality of cyanobacterial carbon is due to a deficiency in a lipid other than PUFAs. The low food quality of *Synechococcus elongatus* was