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Complementary UV protective compounds in zooplankton

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Abstract

Zooplankton accumulate several groups of photoprotective compounds to shield against damaging ultraviolet radiation (UV). One of these groups, the carotenoids, makes the animals more conspicuous to visually hunting predators, whereas others, such as the mycosporine-like amino acids (MAAs) may not. The blend of photoprotective compounds is therefore important for the UV defense but also for the ability to escape predation through crypsis. Here we assess laboratory and field data from different latitudes to examine how UV, predation threat, and pigment availability (in food) affects the mixture of UV-protective compounds in copepods. Overall, the blend of MAAs and carotenoids was partly explained by the availability of MAAs in the food, the UV-threat, and the presence of predators. Copepods upregulated their MAA content when UV threat was increasing (i.e., if MAAs were abundant in food), and in field data this accumulation only occurred at high levels of predation threat. If MAAs were scarce, copepods instead compensated with higher carotenoid accumulation. However, when there was a high predation threat this carotenoid compensatory effect was disadvantageous, and low concentrations of both MAAs and carotenoids at high UV-threat resulted in lower reproduction. In all, these results showed that carotenoids and MAAs are complementary substances, i.e., one is high when the other is low, and copepods are, hence, able to adjust their blend of different UV-protective compounds to optimize their defenses to the threats of UV and predation. These defense systems may buffer against direct food-web interactions and help the zooplankton to survive in environments with high UV threat.

Pigmentation is a widespread phenomenon in nature with several important functions. One of the most obvious examples is chlorophyll, which harvests the solar energy during photosynthesis. Other functions of pigments include vivid coloration in plants to attract pollinators or as deposits used as sexual ornamentation. For example, birds and fish deposit brown melanins and red carotenoids in their bodies to indicate high fitness (Niecke et al. 2003; Pike et al. 2007). Deficiency of pigments can, however, be detrimental, illustrated by the common salmon disease M-74 that may arise due to a deficiency of thiamine and caroteniods (Pettersson and Lignell 1999).

Aquatic zooplankton also display a wide range of pigmentation. For example, in arctic and high-altitude areas the common cladoceran *Daphnia* sometimes has a melanized carapace suggested to work as an ultraviolet radiation (UV) sunscreen (Hebert and Emery 1990; Hessen 1994; Hansson et al. 2007). Calanoid copepods, on the other hand, typically have a red carotenoid pigmentation (Hairston 1979*a*). These deeply colored populations are usually found at high-latitude or high-altitude areas, but pigmentation is also observed in temperate regions (Byron 1982; Hansson 2000; Hansson 2004). In a series of studies Hairston (1979*a*) concluded that carotenoids mainly function as photoprotectants in high-light environments, since carotenoids are antioxidants neutralizing photoproduced radicals (Goodwin 1986). Zooplankton with lower levels of protective pigmentation indeed suffer from greater mortality when exposed to UV radiation (Ringelberg et al. 1984; Hessen 1996). It was also early established that large size and noncryptic coloration is disadvantageous to zooplankton that are exposed to visually selective fish predators (Hrbácek 1961; Brooks and Dodson 1965; Hairston 1979*a*). Recently the predation and photoprotection results have been integrated, demonstrating that copepods are plastic in their pigmentation, making a trade-off between high and low pigmentation in relation to the prevalent fish : UV threat ratio (Hansson 2004; Hylander et al. 2009).

Other substances with UV protection abilities, the mycosporine-like amino acids (MAAs), have also been observed in copepods (Sommaruga and Garcia-Pichel 1999; Hansson et al. 2007). These substances have an absorption maximum between 310 nm and 360 nm (Sinha et al. 2007) and are thus invisible in visible light. Accumulation of MAAs can therefore be hypothesized to function as an alternative strategy, compared to the carotenoids, giving UV protection but not making the animals more conspicuous to visually hunting predators. The seasonal variation in the blend of MAAs and carotenoids adds to this hypothesis with high caroteniod concentrations in spring corresponding to a relatively high UV threat and a low predation threat (Hansson 2004). But during summer, high MAA concentrations and low carotenoid concentrations have been observed that coincide with the highest fish predation threat during a season (Hansson 2004; Moeller et al. 2005; Persaud et al. 2007). MAAs have been detected in several zooplankton species including rotifers and copepods (Tartarotti et al. 2001), and it is well established that they function as sunscreens

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protecting against damage from harmful levels of UV radiation (Shick and Dunlap 2002). Like the carotenoids, the MAAs have to be accumulated from the algal food since these substances are not produced de novo by animals (Goodwin 1986; Bandaranayake 1998).

In natural systems there is a strong positive correlation between altitude and zooplankton MAAs as well as carotenoid concentrations (Byron 1982; Tartariotti et al. 2001). The increase in these compounds with altitude is interpreted as protection against increasing UV exposure. This is confirmed by a positive correlation between MAAs in zooplankton and the ratio between the 1% attenuation depth of UV and the maximum lake depth (Tartarotti et al. 2001; Tartarotti et al. 2004). The levels of carotenoids are usually highest in lakes without fish compared with lakes with fish predators (Hansson 2000). The levels of carotenoids, furthermore, fluctuate over the year with peaks in spring and autumn, and these fluctuations are correlated with the ratio of prevalent UV to predation threat (Hansson 2004). Thus, the blend of carotenoids and MAAs in copepods is important for both the UV defense and the ability to escape predation through crypsis. The levels of both carotenoids and MAAs have in laboratory experiments been shown to be inducible and to increase with increasing UV exposure (Moeller et al. 2005; Hansson et al. 2007). Upon exposure to fish cues, the amounts of carotenoids, however, decrease (Hansson 2004; Hylander et al. 2009). In natural systems the blend of carotenoids and MAAs is not well described, and only few studies have measured carotenoids and MAAs simultaneously and then only in a few lakes at a time (Moeller et al. 2005; Hansson et al. 2007; Persaud et al. 2007).

Here we focus on factors that regulate the blend of carotenoids and MAAs in calanoid copepods. Because the ability to show a plastic response to environmental change is likely to infer costs on fitness variables (DeWitt et al. 1998), we hypothesize that there would be an inverse relationship between the substances because it would be too costly to accumulate both substances simultaneously. Hence, by comparing data from a large latitudinal gradient with samples from subarctic to temperate and dry-temperate systems, we tested whether carotenoids and MAAs were complementary substances, i.e., if one is high when the other is low. The field observations are confirmed by a meta-analysis, and costs of different MAA and carotenoid blends are also evaluated in a mechanistic laboratory experiment.

Methods

A total of 37 lakes from three different regions were sampled. Among these, 13 were from the subarctic Abisko region in northern Sweden (68°N, 18°E); 12 were from a temperate area in southern Sweden (57°N, 15°E); and 12 were from a warm and dry area in New Mexico, USA (33°N, 104°W, defined as dry mid-latitude desert and steppe climate), hereafter denoted subarctic, temperate, and dry-temperate, respectively. The chemical and physical characteristics of the lakes are shown in Table 1. The subarctic lakes are generally ice free during 3 months to 4 months per year (Karlsson and Byström 2005), and dissolved organic carbon (DOC) content ranged from 2.6 mg L^{-1} to 11.7 mg L^{-1}. Lakes in this area have scarce populations of arctic char (*Salvelinus alpinus*) and ninespine stickleback (*Pungitius pungitius*). The temperate area is dominated by coniferous forests, and the studied lakes had a DOC content ranging from 3.7 mg L^{-1} to 26.8 mg L^{-1}. The lakes contain fish populations dominated by roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*). The dry-temperate New Mexico area is characterized by arid climate, clear skies, and intense solar radiation. Salinity levels range from 4 ppt to 29 ppt, and DOC levels in the studied dry-temperate lakes ranged from 1.2 mg L^{-1} to 17.0 mg L^{-1}. The fish community is dominated by Pecos pupfish (*Cyprinodon pecosensis*), Pecos gambusia (*Gambusia nobilis*), and plains killifish (*Fundulus zebrinus*).

Sampling was performed during summer 2007 at all sites: in Abisko from 26 June to 06 July, in Småland from 02 July to 24 July, and in New Mexico from 28 July to 06 August. In each lake calanoid copepods were collected for pigment analysis (carotenoids and MAAs), with net hauls (mesh size <300 μm) from the shore (subarctic samples) or from a boat (temperate and dry-temperate samples). Copepods were transported to the laboratory in filtered water (grade GF/C glass microfiber filter, Whatman) for gut evacuation in a dark and cool bag. At the lake, surface water was pre-filtered through a 50-μm filter (to remove zooplankton), and then 0.1–1.5 liters were filtered through a filter (grade GF/F glass microfiber filter, Whatman) for MAA and chlorophyll analysis in seston. From the same water a subsample for nutrients was taken. Finally, a sample for DOC was taken by filtering water through a 0.2-μm filter in the subarctic and dry-temperate samplings (25-mm membrane filter, Whatman) and through GF/F filters in the temperate sampling (Whatman). Samples for DOC, nutrients, and chlorophyll were stored at -20°C and were analyzed according to standard methods (total organic carbon, Schimadzu 6500; mass spectrometer, ICP MS ELAN-6000; visible spectrophotometer, Beckman DU 800).

In the laboratory live copepods were stranded on a filter (mesh size: 5–10 μm), and under a stereo microscope two samples (for carotenoids and MAAs respectively) of 25–100 individuals each were collected from all lakes. The copepods were all calanoid copepods (*Eudiaptomus* sp. and *Leptodiaptomus* sp.) with similar morphological features, i.e., evenly distributed pigment deposits and about 1 mm long (only adults and later-stage copepodites, egg-bearing females always excluded). Copepod and seston samples were then stored at -80°C until they were analyzed within 7 months.

Carotenoid and MAA quantification followed standard methods (Tartarotti and Sommaruga 2002) and are described in detail in Hansson et al. (2007) and Hylander et al. (2009). In short, carotenoid samples were extracted in ethanol (95%) and quantified with a spectrophotometer (Beckman DU 800) at 474 nm, the absorption peak for common carotenoids in copepods, i.e., astaxanthin and its esters (Hairston 1979*a*; Hansson 2000, 2004). MAAs were extracted in 25% methyl alcohol (MeOH) in water and were

Table 1. Physical and biological parameters from the sampled lakes. All parameters apart from altitude are tested together with copepod MAA and carotenoid content in MANOVA analysis (altitude omitted due to zero variance in sub-tropical samples). *p* values for multiple comparisons (Tukey's test) in the MANOVA are given for the different combinations of regions. TN = total nitrogen; TP = total phosporus; DOC = dissolved organic carbon; Subarc. = subarctic; dry-t. = dry-temperate; temp. = temperate; n.a. = not available.

Lake	Altitude (m asl)	Z_{max} (m)	TN (μg L^{-1})	TP (μg L^{-1})	Chlorophyll (μg L^{-1})	DOC (mg L^{-1})*	Attenuation depth, 320 nm, 1%	Seston MAAs (ng L^{-1})
Subarctic								
Almberga	382	6.0	220	2.3	0.6	6.4	0.41	7
Frans 1	490	2.5	560	4.9	1.7	10.3	0.21	53
Guossaja	570	6.5	664	8.4	0.5	7.8	0.41	6
Långsjön	375	5.9	212	2.3	0.3	2.6	4.64	48
Njusakjau	400	6.9	261	2.6	1.1	5.1	0.48	50
Rouzut	710	8.5	222	2.7	0.5	4.4	0.82	43
Sjö 434	434	9.0	455	2.1	0.5	3.9	1.08	0
Sjö v Långsj	370	3.3	226	2.6	1.5	5.3	0.96	133
Sjö v Solb N	415	2.1	940	10.3	2.1	11.4	0.24	72
Sjö v Solb S	415	4.5	313	4.5	0.7	7.5	0.41	133
Tjabrak	508	14.0	300	2.3	0.4	4.9	0.66	6
Vid Vassijau	600	3.0	146	3.3	0.5	3.1	2.43	323
Voure	712	8.5	177	1.9	0.4	4.2	0.68	46
Sign. temp. (*p* value)	n.a.	=0.003	=0.02	=0.06	<0.001	=0.014	<0.001	=0.16
Sign. dry-t. (*p* value)	n.a.	=0.94	=0.001	<0.001	=0.16	=0.47	=0.99	<0.001
Temperate								
Borrasjön	170	7.0	1219	11.0	4.2	25.1	0.02	20
Boskvarasjön	251	19.7	655	8.3	10.8	17.0	0.03	0
Burken	228	7.0	434	10.1	5.9	11.0	0.07	21
Fiolen	226	10.0	517	7.9	7.3	6.4	0.14	0
Hjärtsjön	274	7.0	369	2.8	2.1	4.2	0.18	17
Idesjön	189	18.0	628	7.0	5.1	13.5	0.05	13
Juven	227	11.0	830	14.8	10.8	20.4	0.03	0
Kinnen	127	16.0	940	9.9	6.0	22.8	0.03	0
Klintsjön	232	18.0	264	3.6	4.1	3.7	0.33	39
Linnerydsjön	136	8.0	1306	21.4	28.5	26.8	0.02	888
Skärlen	212	28.0	298	4.2	2.6	3.9	0.05	43
Älgarrydsjön	210	9.0	522	9.2	5.1	11.8	0.05	0
Sign. sub-arc. (*p* value)	n.a.	=0.003	=0.02	=0.06	<0.001	=0.014	<0.001	=0.16
Sign. dry-t. (*p* value)	n.a.	=0.002	=0.36	=0.05	<0.001	=0.22	<0.001	<0.001
Dry-temperate								
1	1070	5.5	959	10.0	2.7	9.2	0.97	9098
2	1070	5.6	667	n.a.	2.7	7.0	1.14	8426
7	1070	9.3	547	10.0	0.9	4.8	1.73	235
9	1070	6.2	838	20.0	1.6	8.4	0.75	2948
10	1070	2.9	1103	50.0	3.7	11.1	0.56	24056
11	1070	6.6	1581	40.0	1.1	11.2	0.39	12712
19	1070	3.3	2218	110.0	12.8	17.0	0.36	1589
20	1070	4.2	750	20.0	4.3	6.0	0.85	1838
32	1070	1.8	1122	20.0	1.1	11.7	0.30	17977
37	1070	14.5	497	40.0	0.5	3.0	0.90	1829
38	1070	2.1	826	40.0	2.1	1.2	8.00	1505
59	1070	4.3	222	20.0	0.5	2.5	0.97	3585
Sign. sub-arc. (*p* value)	n.a.	=0.94	=0.001	<0.001	=0.16	=0.47	=0.99	<0.001
Sign. temp. (*p* value)	n.a.	=0.002	=0.36	=0.05	<0.001	=0.22	<0.001	<0.001

* DOC dry-temperate from 2005.

analyzed according to conventional methods in high-performance liquid chromatography (HPLC; Tartarotti and Sommaruga 2002; Tartarotti et al. 2004; Hansson et al. 2007). The concentration of photoprotective compounds was normalized to dry weight calculated from published relationships between length and dry weight for calanoid copepods (Bottrell et al. 1976; Persson and Ekbohm 1980). To assure that algal pigments would not influence the results, the copepods were kept in tap water for at least 1 h before sampling. No peaks were observed at the absorption maximum of chlorophyll (665 nm), indicating that gut evacuation had been effective and that carotenoids and MAAs from algae in the gut were not included in the analysis.

To get an estimate of the UV threat in each lake, defined here as the daily UV radiation at a depth of 0.1 m ($I_{0.1}$), we first calculated the intensities of UVA reaching the lakes during 24 h using the tropospheric ultraviolet and visible radiation (TUV) model (http://cprm.acd.ucar.edu/Models/TUV/Interactive_TUV/) after corrections for the atmospheric ozone thickness using the total ozone-mapping spectrometer (TOMS) (http://toms.gsfc.nasa.gov/teacher/ozone_overhead_v8.html). Daily radiation was preferred since subarctic areas have midnight sun during summer. To be able to account for previous UV exposure, a mean radiation value was calculated by taking the mean UV-A radiation of the sampling date and of 4 d prior to the sampling date. For each simulation, sampling date, altitude, latitude and longitude, and overhead ozone column were varied to obtain a compound variable for the UV threat. Surface albedo was set to 0.1 in all calculations. Since UV radiation is attenuated to different extents in natural waters, the daily UV-A threat averaged over 5 d at a depth of 0.1 m was finally calculated from $I_{0.1} = I_0 e^{(-K_{320} \times 0.1)}$ (Hansson 2004), where I_0 was the averaged daily UV-A radiation (above the surface) estimated by the TUV model. The diffuse attenuation coefficient at 320 nm (K_{320}) and the 1% attenuation depth were calculated using the relationship between the absorption coefficient (A_{320}) and the diffuse attenuation coefficient (Kirk 1994; Morris et al. 1995). To obtain A_{320}, water samples were analyzed for absorbance at 320 nm (Beckman DU 800 spectrophotometer).

Laboratory study—A laboratory experiment was performed in Lund, southern Sweden (55.7°N, 13.5°E) to examine factors determining the blend of photoprotective compounds in copepods. Tap water and 200 calanoid copepods (*Eudiaptomus gracilis*) were added to cylindrical plastic UV-opaque containers (height: 0.19 m; diameter: 0.21 m; volume: 5 liters). Copepods were collected from a nearby lake (Dalby Quarry) by net hauls (mesh size: 300 μm). The quarry is about 10 m deep and has an A_{320} of 2.0. The containers were illuminated by eight fluorescent lamps (36 W, UV-A-340, Q-Panel) mounted 0.1 m above the containers. Lamps were on at a 15 : 9 light : dark cycle, producing a UV-A intensity of 9.3 W m^{-2}. These lamps are commonly used to simulate solar radiation in the UV wavelength range (Hansson 2004; MacFayden et al. 2004). In treatments not exposed to UV, radiation was restricted by use of Plexiglas (Röhm GS 233), effectively cutting off radiation below 370 nm, i.e., the UV-A and UV-B range. In treatments that were exposed to UV, radiation was admitted by UV-transparent Plexiglas (Röhm GS 2458). There were no differences, however, in transmittance of photosynthetically active radiation (PAR) between Plexiglas types (Hansson et al. 2007).

Experimental treatments were applied from 06 March 2008 until 18 March 2008. Six different treatments, each with four replicates, were applied randomly to the containers. Treatments were the following: (1) PAR and MAA-rich food (r); (2) the complete solar spectrum and MAA-rich food (UVr); (3) the complete solar spectrum and MAA-poor food (UVp); (4) PAR, MAA-rich food, and fish threat (Fr); (5) the complete solar spectrum with MAA-rich food plus fish threat (UVFr); and (6) the complete solar spectrum with MAA-poor food plus fish threat (UVFp).

Fish threat was mimicked by adding 25 mL of frozen and filtered (grade GF/C filter, Whatman) water coming from a 10-liter aquarium where 10 roaches (*Rutilus rutilus*, 0+ or 1+; length <50 mm) had been feeding on zooplankton for 2 d. Fish cues were added every second day to fish treatments, and the similar amount of nontreated water (from aquaria without fish) was added to all other treatments. The copepods were fed by adding cultured phytoplankton, *Scenedesmus* sp. and *Peridinium inconspicuum*, to an initial total concentration of around 25 μg C L^{-1} in all treatments. Total carbon content increased over time and never went below this limit. There were no differences in carbon content among treatments throughout the experiment [repeated-measures annalysis of variance (ANOVA), $F_{5,18} = 1.8$, $p = 0.17$]. Both algal species were cultured in Modified Woods Hole medium (MWC; Guillard and Lorenzen 1972) at 20°C and PAR. Treatments that had MAA-rich food, apart from *Scenedesmus*, initially also received around 80,000 cells L^{-1} of the MAA-producing *P. inconspicuum* (Hansson et al. 2007). Treatments that were MAA poor received around 760 cells L^{-1} of *P. inconspicuum*. To make total carbon content similar in all treatments, MAA-poor treatments received slightly more *Scenedesmus* compared with MAA-rich treatments. The number of *P. inconspicuum* decreased throughout the experiment, and final concentrations were around 53,000 cells L^{-1} in MAA-rich treatments and around 500 cells L^{-1} in MAA-poor treatments. Algal cells were counted in an inverted light microscope (Olympus CKX 41), and there were differences in the number of *Peridinium* cells among treatments (repeated measures analysis of variance; RM ANOVA, $F_{5,18} = 17.9$, $p < 0.001$), with significant differences between MAA-rich and MAA-poor treatments ($p < 0.05$), but there were no significant differences within MAA-rich or MAA-poor treatments ($p > 0.05$).

We collected copepods for pigmentation analysis initially and after 12 d of treatment. For each pigment analysis, 40 copepods per replicate (*E. gracilis*; adults and later stage copepodites) of approximately the same size were collected (one sample for carotenoids and one for MAAs; egg-bearing females were always excluded). Animals were kept in tap water for at least 1 h for gut evacuation and then measured at 40× magnification (Olympus SZ 40) before freezing at −80°C. Carotenoids were analyzed as above, and the MAAs were quantified spectrophotometrically (Beckman DU 800) at 333 nm, which is the absorption peak for the most common MAA in the studied copepods, i.e., shinorine (Hansson et al. 2007). MAAs were then expressed as optical density per copepod at 333 nm (OD_{333} cop^{-1}) where $OD_{333} = -\log_{10}(1 - A_{333})$. A_{333} is the absorbance per liter at 333 nm. Copepod reproduction was estimated by counting the number of produced nauplii and the number of produced egg sacks (i.e., number of egg-carrying females).

Statistical tests, meta-analysis, and calculations—Statistical differences in MAA and carotenoid content in

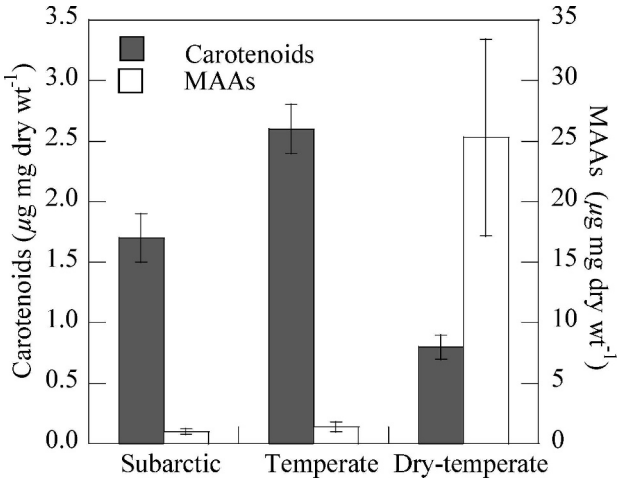

Fig. 1. Concentrations (±1 SD) of carotenoids (μg mg dry wt^{-1}) and MAAs (μg mg dry wt^{-1}) in subarctic, temperate, and dry-temperate copepods (n = 13, 12, 12, respectively).

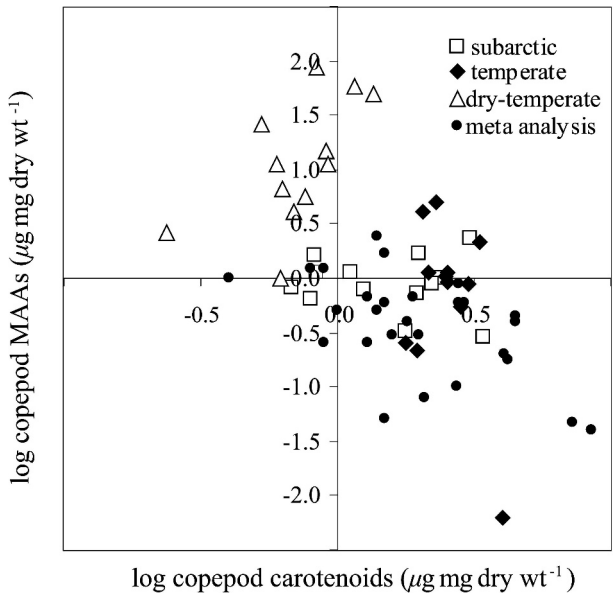

Fig. 2. Relationship between MAA (μg mg dry wt^{-1}) and carotenoid (μg mg dry wt^{-1}) concentrations in subarctic, temperate, and dry-temperate copepods and from the literature (Moeller et al. 2005; Hansson et al. 2007; Persaud et al. 2007). There is a negative relationship between MAAs and carotenoids in samples from this study and also between MAAs and carotenoid readings from the literature ($p < 0.05$).

copepods, as well as physical parameters, were tested in a multivariate analysis of variance (MANOVA). Relationships were analyzed with linear regressions and correlations. Statistical differences in carotenoids, MAAs, and reproduction (number of nauplii produced and egg-sack prevalence) in the laboratory experiment were tested with a one-way ANOVA followed by Tukey's test or, when variances were not homogeneous, with a Kruskal-Wallis test followed by nonparametric Mann-Whitney *U*-tests for multiple comparisons. When necessary, data were transformed to meet assumptions for the tests. In one case (lake 38 in the dry-temperate samples) MAA analysis failed, and therefore we only have a carotenoid reading from this lake. All analyses were performed in SPSS 15.0 for Windows (SPSS). Data for the meta-analysis were collected from figures and tables in Moeller et al. (2005), Hansson et al. (2007), and Persaud et al. (2007). (Data from Lake Earnest were omitted due to possible contamination of phytoplankton carotenoids and from Rock pool 4 due to uncertainties of life stage analyzed.) In some cases samples from the same lake were taken approximately every month throughout a year. Because these samplings were separated by a considerable amount of time, they are treated as independent samplings. As a proxy of fish predation pressure, total fish biomass per hectare was estimated from published relations between total phosphorus and standing crop (Hanson and Legget 1982), a relationship that has been confirmed and used in several studies (Griffiths 2006; Hansson et al. 2007).

Results

Field survey—Along the latitudinal gradient, MAA levels in copepods ranged from not detectable to 58 μg mg dry wt^{-1}. The levels were low in subarctic and temperate animals (1.0 μg mg dry wt^{-1} and 1.4 μg mg dry wt^{-1}) compared with dry-temperate samples (25.3 μg mg dry wt^{-1}), and the variance increased with decreasing latitude (Fig. 1). Carotenoid concentrations on the other hand, were higher in subarctic and temperate regions, with mean values of 1.7 μg mg dry wt^{-1} and 2.6 μg mg dry wt^{-1} respectively, compared with the dry-temperate copepods that had a mean of 0.8 μg mg dry wt^{-1} (Fig. 1). Copepod carotenoid levels ranged from 0.2 μg mg dry wt^{-1} to 4.0 μg mg dry wt^{-1} throughout the latitudinal gradient. Overall, there were differences in both MAAs and carotenoids among geographical areas (MANOVA, $F_{18,50} = 18.5$, $p < 0.001$), with more MAAs in the dry-temperate copepods compared to temperate and subarctic copepods ($p < 0.001$, Tukey's test). There were, however, no significant differences in MAA levels between temperate and subarctic copepods (Tukey's test, $p = 0.86$). Carotenoid levels were different among all regions [Tukey's test, $p = 0.015$ (subarctic and temperate), $p = 0.001$ (subarctic and dry-temperate), $p < 0.001$ (temperate and dry-temperate)]. The biological and physical parameters assessed were, furthermore, different among regions (MANOVA, $F_{18,50} = 18.5$, $p < 0.05$; *see* Table 1 for multiple comparisons). When analyzing for covariance between MAAs and carotenoids and the other parameters, the only significant parameter that co-varied with MAA content was DOC, with higher MAA levels at low DOC [multivariate analysis of covariance (MANCOVA), $F_{2,25} = 3.7$, $p = 0.038$]. Carotenoids did not co-vary with any of the parameters.

The relationship between MAA and carotenoid content in copepods was negative, i.e., when MAA concentrations were high, the carotenoid concentrations were generally low (Pearson Correlation, $r = -0.510$, $p = 0.002$, $n = 36$; Fig. 2). Plotting data on MAAs and carotenoids in calanoid copepods from the literature (Moeller et al. 2005; Hansson et al. 2007; Persaud et al. 2007) revealed a

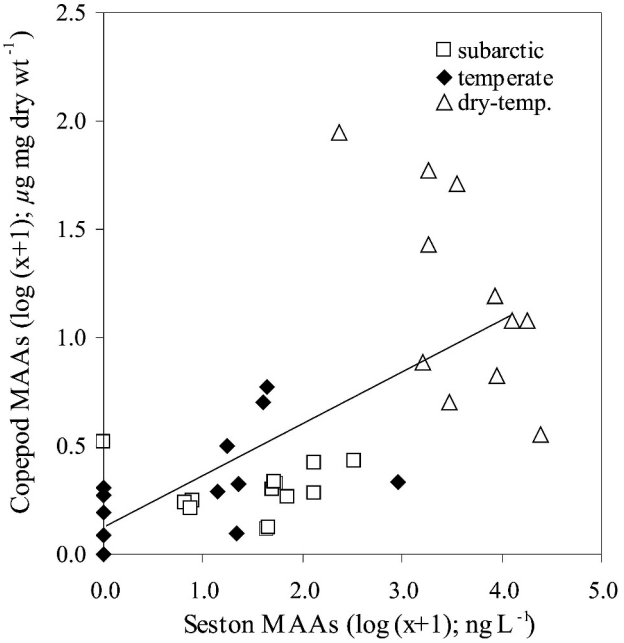

Fig. 3. Relationship between MAA concentration in copepods (μg mg dry wt^{-1}) and in seston (μg L^{-1}). There is a positive relationship between seston MAA concentrations and copepod MAA concentrations ($p = 0.005$).

negative relationship with a similar slope as the data from our study (Fig. 2; Pearson Correlation, $r = -0.552$, $p = 0.003$, $n = 27$). Seston MAA concentrations along the latitudinal gradient ranged from not detectable to 24 μg L^{-1}, and there was a positive relationship between MAA content in copepods and seston (Fig. 3; $r^2 = 0.214$, $F_{1,34} = 9.2$, $p = 0.005$). The inverse relationship between MAAs and carotenoids in copepods was also tightly connected with the observation that carotenoid concentrations never were high when levels of MAAs in seston were high.

When plotting MAAs and carotenoids over different UV threats (i.e., $I_{0.1}$), copepods from different regions accumulated these substances in different ways. There was an overall positive relationship between MAAs and UV threat (Fig. 4; $r^2 = 0.208$, $F_{1,34} = 8.9$, $p = 0.005$). Broken down into the different areas, there were positive relationships in temperate and dry-temperate areas between UV threat and MAAs ($r^2 = 0.582$, $F_{1,9} = 13.9$, $p = 0.004$ and $r^2 = 0.430$, $F_{1,9} = 6.8$, $p = 0.028$, respectively), but there was no significant relationship in subarctic samples ($r^2 = 0.065$, $F_{1,11} = 0.8$, $p = 0.40$). For carotenoids there was overall no significant relationship between pigment and UV threat ($r^2 = 0.070$, $F_{1,35} = 2.6$, $p = 0.12$). When broken down into the different areas, however, the opposite pattern arises for the MAAs, with positive relationships between carotenoids and UV threat in subarctic regions and non-significant relationships in temperate and dry-temperate samples ($r^2 = 0.346$, $F_{1,11} = 5.8$, $p = 0.034$; $r^2 = 0.062$, $F_{1,10} = 0.6$, $p = 0.43$, and $r^2 = 0.012$, $F_{1,10} = 0.1$, $p = 0.74$, respectively).

Mean lengths of copepods from subarctic, temperate, and dry-temperate regions were 0.9 ± 0.1 mm, 1.1 ± 0.1 mm, and 1.0 ± 0.1 mm, respectively (mean ± 1 SD). There were differences in length among regions, with temperate copepods being slightly larger than copepods from the other regions (one-way ANOVA, $F_{2,34} = 5.2$, $p = 0.01$, Tukey's test). There were, however, no significant differences in size between subarctic and dry-temperate animals ($p = 0.86$, Tukey's test). In general, there were also no significant relationships between dry-weight–specific content of carotenoids or MAAs and length ($r^2 < 0.001$, $F_{1,35} = 0.001$, $p = 0.98$; $r^2 = 0.069$, $F_{1,34} = 0.16$, $p = 0.69$ for carotenoids and MAAs over length, respectively).

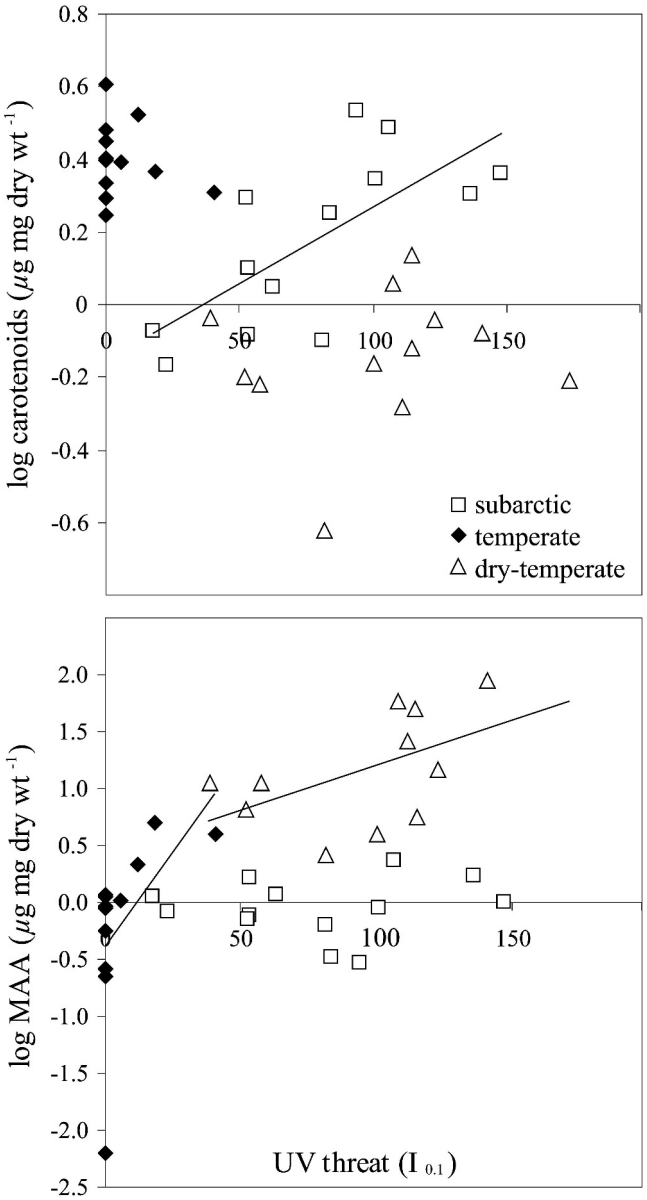

Fig. 4. Copepod carotenoid and MAA concentrations (μg mg dry wt^{-1}) at different UV threats ($I_{0.1}$; upper and lower panel, respectively). Only significant ($p < 0.05$) regression lines are displayed.

Fish biomass for each lake estimated from published relationships between total phosphorus and standing crop (Hanson and Legget 1982) ranged from 900 kg km^{-2} to

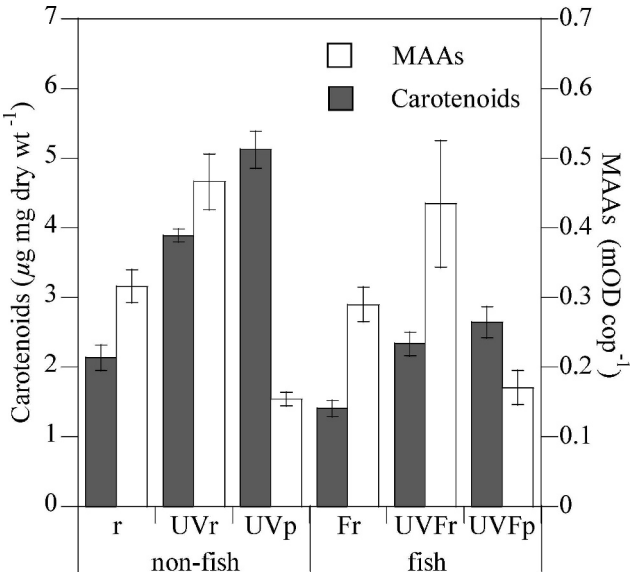

Fig. 5. Carotenoid and MAA concentrations in copepods in the laboratory experiment (±1 SE) Treatments areas follows: (1) PAR and MAA-rich food (r); (2) the complete solar spectrum and MAA-rich food (UVr); (3) the complete solar spectrum and MAA-poor food (UVp); (4) PAR, MAA-rich food, and fish threat (Fr); (5) the complete solar spectrum with MAA-rich food plus fish threat (UVFr); (6) the complete solar spectrum with MAA-poor food plus fish threat (UVFp). MAAs are measured in optical density per copepod (OD cop^{-1}).

16,600 kg km^{-2}. Biomasses were lowest in subarctic lakes (1500 ± 700 kg km^{-2}, mean ± SD) and highest in the dry-temperate lakes (6900 ± 3900 kg km^{-2}, mean ± SD), and temperate lakes had intermediate biomasses (2800 ± 1100 kg km^{-2}, mean ± SD), with differences among all regions (one-way ANOVA, $F_{2,33} = 35.2$, $p < 0.001$; Tukey's test).

Laboratory study—Mean initial concentration of carotenoids was around 2.8 μg mg dry wt^{-1} and there were no significant differences among treatments ($F_{5,18} = 1.2$, $p = 0.34$). On the final sampling date there were, however, differences in carotenoid concentrations among treatments (Fig. 5; $F_{5,18} = 53.8$, $p < 0.001$); Tukey's test revealed differences among several treatments (Table 2). In short, the carotenoids increased with UV radiation and decreased when fish cues were present. Furthermore, the carotenoid levels generally increased when the copepods were in an environment where MAA was scarce without fish predation (UVp) compared with an environment where MAA was more abundant in the food (UVr). In fish treatments, levels of carotenoids were generally lower than in non-fish treatments, but the general trends were similar to the non-fish treatments.

MAA optical density (OD) was higher in UV treatments compared with non-UV treatments if the copepods were fed with MAA-rich food (Fig. 5). Fish treatment did not affect MAA levels. At the final sampling date there were differences in MAA content among treatments (Kruskal-Wallis, $p = 0.003$, df = 5). Multiple comparisons showed differences among treatments with different radiation exposure and if fed with MAA-poor or MAA-rich food ($p < 0.05$; Table 2). There were, however, no differences in copepod MAA content among fish treatments (Mann-Whitney *U*-test, $p > 0.05$; Table 2).

The number of surviving copepods did not differ among treatments ($F_{5,18} = 1.3$, $p = 0.31$). Reproduction, however, was affected, with lowest nauplii production in the UVFp treatment ($F_{5,18} = 11.8$, $p < 0.001$; Fig. 6) and with differences between UVFp and all other treatments (Tukey's test, $p < 0.05$). There was also a difference between UVp and Fr, with slightly higher production of nauplii in Fr. Egg-sack production was lowest in the UVp and in UVFp treatments with differences among treatments ($F_{5,18} = 5.0$, $p = 0.007$). Tukey's test showed significant

Table 2. Statistical differences among treatments in the laboratory experiment (Tukey's test for carotenoids and Mann-Whitney *U*-test for MAAs; significant results in bold). Treatments: (1) PAR and MAA-rich food (r); (2) the complete solar spectrum and MAA-rich food (UVr); (3) the complete solar spectrum and MAA-poor food (UVp); (4) PAR, MAA-rich food, and fish threat (Fr); (5) the complete solar spectrum with MAA-rich food plus fish threat (UVFr); and (6) the complete solar spectrum with MAA-poor food plus fish threat (UVFp).

	(1) r	(2) UVr	(3) UVp	(4) Fr	(5) UVFr	(6) UVFp
Carotenoids						
(1) r	1.00	**<0.001**	**<0.001**	0.10	0.97	0.39
(2) UVr	**<0.001**	1.00	**=0.002**	**<0.001**	**<0.001**	**<0.001**
(3) UVp	**<0.001**	**=0.002**	1.00	**<0.001**	**<0.001**	**<0.001**
(4) Fr	0.10	**<0.001**	**<0.001**	1.00	**=0.02**	**=0.002**
(5) UVFr	0.97	**<0.001**	**<0.001**	**=0.02**	1.00	0.83
(6) UVFp	0.39	**<0.001**	**<0.001**	**=0.002**	0.83	1.00
MAAs						
(1) r	1.00	**=0.03**	**=0.03**	0.69	0.69	**=0.03**
(2) UVr	**=0.03**	1.00	**=0.03**	**=0.03**	1.00	**=0.03**
(3) UVp	**=0.03**	**=0.03**	1.00	**=0.03**	**=0.03**	0.34
(4) Fr	0.69	**=0.03**	**=0.03**	1.00	0.34	**=0.03**
(5) UVFr	0.69	1.00	**=0.03**	0.34	1.00	**=0.03**
(6) UVFp	**=0.03**	**=0.03**	0.34	**=0.03**	**=0.03**	1.00

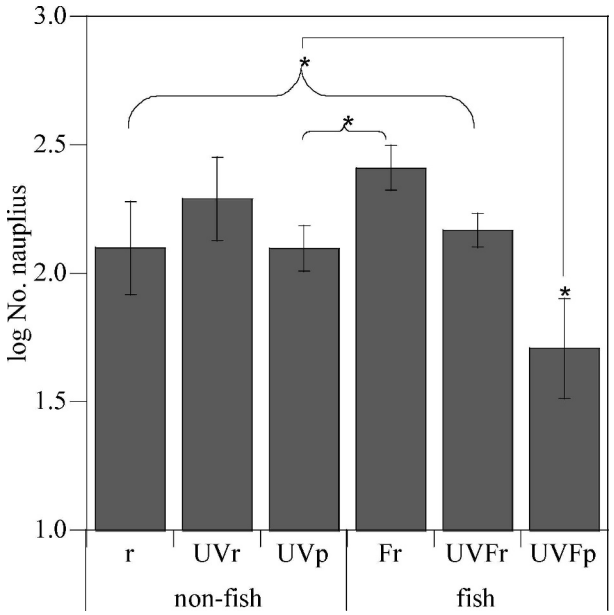

Fig. 6. Number of nauplii produced in the different treatments in the laboratory experiment. Error bars denote ±1 SE. Treatments are the same as in Fig. 5.

differences between r and UVp, r and UVFp, and Fr and UVp.

Discussion

Here we suggest that MAAs and carotenoids are complementary substances, a relationship partly driven by the availability of MAAs in the food source and partly by the levels of UV and predation threats. The effect of UV on the concentrations of MAAs in copepods along the latitudinal gradient is illustrated by an increase in MAAs with lower DOC concentrations. Low DOC waters are typically environments with high UV threat because DOC attenuates UV radiation efficiently (Morris et al. 1995). This is also in accordance with previous results showing that the concentrations of MAAs in copepods increase as the UV exposure increases (Tartarotti et al. 2001; Persaud et al. 2007).

On a UV-threat scale there was a difference among latitudes where copepods in temperate and dry-temperate areas accumulated more MAAs, whereas copepods from the subarctic area instead accumulated more carotenoids when UV threat increased (Fig. 4). This suggests that copepods are subjected to different selective forces when meeting an increasing UV threat. These differences in the pigmentation cocktail could be a matter of differences among species that we sampled or of a phenotypic plastic system where individuals can adjust their level of pigmentation in accordance with the prevalent threats (Hansson 2004; Tollrian and Heibl 2004). Indeed, both genera examined in this study have been shown to be plastic in their UV protective pigmentation (Moeller et al. 2005; Hansson et al. 2007; Hylander et al. 2009). In our laboratory experiments we observed that copepods exposed to UV radiation increased their contents of MAAs and carotenoids, also suggesting a phenotypic response. With MAA-poor food the copepods were, however, unable to induce a higher MAA concentration, but instead compensated with elevated carotenoid accumulation (Fig. 5). This is an effect also observed in our field data, where carotenoid levels were high only when the availability of MAAs in seston was low. The compensatory effect, with increasing carotenoid accumulation when MAA availability is low, was also noted by Moeller et al. (2005) and is possibly one of the mechanisms explaining the inverse relationship between MAAs and carotenoids observed in this study.

Fish predation was highest in the dry-temperate systems and lowest in the subarctic systems, according to TP-fish biomass analysis. Comparisons to direct fish density estimates are not available for all regions, but lakes from the same area as the dry-temperate lakes have a fish density of around 5100 (±4000) kg km^{-2} (W. J. Boeing unpubl., $n = 14$), which is lower than the estimate based on total phosphorus but still more than three times higher than the fish biomass in the subarctic area. Also, predation rate and total possible foraging time can be hypothesized to be higher in the dry-temperate systems because predation rate increases with temperature and because fish may also reproduce multiple times throughout a season in warm climates (Lessmark 1983; Leeuwen et al. 2007). Furthermore, the fish communities of the subarctic lakes are predominately feeding on benthic resources (Karlsson and Byström 2005), adding to an even lower pelagic predation pressure in this area. In all, these data indicate that the predation pressure was highest in the dry-temperate climate and lowest in the subarctic area.

At low fish-predation threat (subarctic), copepods responded by increasing carotenoid concentrations at increasing UV threat, whereas at high predation threat (temperate and dry-temperate), they did not. Instead, when predation threat was high, copepods accumulated MAAs as photoprotective compounds. Our experiment also shows that carotenoid accumulation is only possible at low predation pressure and is interestingly enhanced by low MAA levels (Fig. 5).

When exposed to combined UV and fish threats, we expected that the copepods would decrease their carotenoid concentrations and instead increase their MAA levels because these substances are hypothesized to give UV protection without making the animal conspicuous to visually hunting predators. However, predation threat with consequent decreases in carotenoids did not affect the MAA concentrations. It may be that the response time to increased MAAs at low carotenoid levels is longer than the experimental time or that MAAs were not supplied in sufficient amounts in the algal food. Our field data, however, showed that MAAs increased at high UV levels and high predation threats, which supports the hypothesis of upregulation of MAAs when carotenoids are low. When experimentally exposed to a combination of UV, predation, and low MAA supply, carotenoid compensation was not possible due to the predation threat, and the levels of carotenoids and MAAs dropped despite the fact that the copepods were exposed to UV simultaneously (Fig. 5).

Consequently, the copepods suffered from lower reproduction. However, reproduction in terms of nauplii production did not differ between UV treatments with MAA-rich or MAA-poor food, indicating that food quality and quantity (for reproduction) were similar in MAA-poor and MAA-rich treatments. Apart from lower reproduction, UV radiation exposure has previously also been shown to cause increased copepod mortality (Rautio and Korhola 2002; Hylander et al. 2009).

Why copepods are not accumulating both substances (MAAs and carotenoids) when fish predation is low is unknown, but it is possibly associated with a certain cost to use both defenses simultaneously. For example, accumulation of MAAs may be more costly than accumulation of carotenoids, and thus carotenoids may be preferred in the absence of fish. Although MAA levels occasionally tend to decrease when the animal is released from UV radiation (Hansson et al. 2007), costs for MAA accumulation and retention are rarely assessed and may be very small. Moreover, MAAs are only present in some algal groups, and the levels vary considerably among lakes (Jeffrey et al. 1999; Laurion et al. 2002), suggesting that dietary scarcity of MAAs also may constrain this UV defense (Hylander et al. 2009; this study). Carotenoids, in contrast to MAAs, are typically abundant in nature, and several studies from natural systems have confirmed that carotenoid concentrations in copepods do not seem to be controlled by the availability in seston (Hairston 1979*a*; Byron 1982).

When organisms have several possible inducible defenses, an inverse, trait-compensatory relationship is generally assumed (Dewitt et al. 1999). For example, snails inducing a strong morphological predator defense generally display a low behavioral defense (Rundle and Brönmark 2001). Likewise, zooplankton released from UV exposure reduce their pigmentation, and when again exposed to UV they instead increase their vertical migration (Hansson et al. 2007). When the UV threat is very high, the cost of UV damages may be so high that the trait-compensation concept may not be valid. Copepods in this situation are hypothesized to accumulate both MAAs and carotenoids. This scenario may have been observed anecdotally by Tartarotti et al. (2004), reporting that the highest MAA concentrations were found in the most darkly red-pigmented copepod *B. gibbosa*, a species known to inhabit high UV environments in clear waters at high altitudes.

The concentrations of MAAs in the copepods from our field data also correlated with the availability in the food source even though the variance was high (Fig. 3). Such a relationship was expected because photoprotective compounds commonly are not synthesized by animals and must therefore be derived from food (Goodwin 1986; Shick and Dunlap 2002; Moeller et al. 2005). Similarly, correlations between MAA content in seston and copepods may not always be significant (Tartarotti et al. 2001) because there can be a lag phase of about 3–4 weeks in MAAs in copepods vs. that of their food source (Tartarotti and Sommaruga 2006). This lag phase may also explain some of the variance seen in our seston–copepod relationship.

Low temperatures have also been suggested to induce higher accumulation of photoprotective compounds, because protection may be less effective at colder temperatures (Hairston 1979*b*; García et al. 2008). In our laboratory study accumulation rates were studied at constant temperature, but this relationship was not evident in our field data. For example, MAAs were in the highest concentrations in the warmest climates and lowest in the coldest climate, indicating that other selective forces apart from temperature are more important. Although it seems evident that carotenoids have photoprotective abilities, other benefits like nutritive value have also been suggested (Ringelberg and Hallegraeff 1976). In a series of studies Hairston (1979*a*), however, concluded that the photoprotection hypothesis is the most probable benefit of the carotenoid deposits. Apart from photoprotective compounds like MAAs and carotenoids, zooplankton may also use other defense mechanisms like vertical migration or photo-enzymatic repair to avoid UV damage (MacFadyen 2004; Hansson and Hylander 2009; Hylander et al. 2009). Recent studies, however, indicate that copepods mainly invest in photoprotective pigmentation and less in vertical migration when exposed to UV radiation (Leech and Williamson 2001; Hylander et al. 2009). Repair processes in copepods are also reported by several authors, but their importance in copepods is not well understood (Zagarese et al. 1997; Rocco et al. 2002). DOC in dry climates, like the dry-temperate region, may be photodegraded, leading to high UV transparency. Since our material did not have any outliers with high DOC and low diffuse attenuation coefficient (K_d) values, this issue seems to be of minor importance in this system.

We conclude that copepods from subarctic, temperate, and dry-temperate lakes displayed a negative correlation between MAAs and carotenoids. This relationship was partly driven by the availability of MAAs in the copepod food source, by the UV threat, and by the presence of visually hunting predators. Copepods upregulated their MAA content when UV threat was increasing under the condition that MAAs were abundant in the food. If not, they instead compensated with higher carotenoid accumulation. However, when fish cues were present, this compensatory effect was not possible due to lower carotenoid accumulation; left with both low MAA and carotenoid concentrations in a high UV environment, copepods suffered from lower reproduction rates. Hence, at high levels of predation pressure, copepods preferentially accumulated MAAs when UV threat was increasing. In contrast, accumulation of carotenoids as a response to increasing UV threat was only observed at low levels of predation threat, and this accumulation was enhanced when MAAs were scarce in the phytoplankton. Copepods were, hence, able to adjust their blend of different UV protective compounds to optimize their defenses to the prevalent threats of UV and predation. These defense systems may buffer against direct food-web interactions and help the zooplankton to survive in environments with high UV threat.

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