



UV protective compounds transferred from a marine dinoflagellate to its copepod predator

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ABSTRACT

Planktonic organisms living in surface waters can be exposed to harmful levels of ultraviolet radiation (UVR), but few studies have examined accumulation of UV protective compounds in marine zooplankton. Zooplankters are suggested to lack the ability to synthesize these substances and hence must accumulate them from their algal food. Here, we show that both phytoplankton (dinoflagellates) and their zooplankton grazers (copepods) respond strongly to UVR exposure by, respectively, synthesizing and accumulating the natural sunscreens mycosporine-like amino acids (MAAs). In our experiment, the MAAs content increased approximately four times in dinoflagellates exposed to UVR and PAR, as compared to non-UVR controls only receiving PAR (PAR = photosynthetically active radiation). The elevated MAAs level in the dinoflagellates was mirrored in the copepods, which accumulated more MAAs when exposed to UVR as compared to a non-UVR treatment. Overall, copepods accumulated approximately 2–5% of the total MAAs pool. Other UV protective compounds, like carotenoids, were however not accumulated by the copepods. The ability of some species to produce or accumulate photoprotective compounds may lead to increased fitness, and thus these taxa may become more dominant in plankton communities.

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1. Introduction

Organisms of several aquatic taxa can be exposed to harmful levels of ultraviolet radiation (UVR) when staying close to the surface (Bancroft et al., 2007). Stratospheric ozone depletion and the increase in ultraviolet radiation (UVR) represent an important threat to all ecosystems (Madronich et al., 1998; UNEP/WMO, 2006) but also natural levels of UVR may be harmful to organisms (Williamson et al., 1994).

Among the most abundant animals in open waters are zooplankters, especially copepods, which constitute one of the most important components of the marine food web. These organisms are known to be susceptible to UVR, which is reflected in suppressed reproduction and increased adult and juvenile mortality (Williamson et al., 1994; Zagarese et al., 1994; Leech and Williamson, 2000; Ban et al., 2007). It has, hence, been suggested that UV radiation could be structuring the zooplankton community (Williamson et al., 2001). To minimize or even completely avoid this threat, zooplankton species, however, employ a variety of defense mechanisms, such as vertical migration,

accumulation of photoprotective compounds, and photoenzymatic repair (Hairston, 1976; MacFadyen et al., 2004; Hansson and Hylander, 2009a, 2009b; Hylander et al., 2009a, 2009b). Recent studies from freshwaters have indicated that some zooplankters exhibit vertical migration in response to UVR (Rhode et al., 2001; Hansson and Hylander, 2009b; Hylander et al., 2009b). This UV avoidance is mainly observed among cladocerans, and it seems that, as an alternative UV defense mechanism, copepods instead invest in accumulation of photoprotective compounds (Bollens and Frost, 1990; Leech and Williamson, 2001; Hansson et al., 2007; Hylander et al., 2009b), primarily carotenoids and mycosporine-like amino acids (MAAs; e.g. Hairston, 1976; Sommaruga and Garcia-Pichel, 1999; Hylander et al., 2009a). Here, we focus on accumulation of these UV protective compounds among marine planktonic copepods. One of the compounds, the carotenoids, are beneficial for the organism in that they act as antioxidants that neutralize photo-produced radicals (Goodwin, 1986), but they can also be detrimental to the zooplankton because they are pigmented and thus make the animal more conspicuous to predators (Hansson, 2004). The MAAs is another group of substances with absorption in the UV wavelength range (310–360 nm), which means that they may not be perceivable in the visible light range (photosynthetically active radiation [PAR], 400–700 nm; Karentz, 2001; Shick and Dunlap, 2002). However, it has also been suggested that some fish species may utilize UV wavelengths and forage more efficiently on zooplankton with high

Abbreviations: UV, Ultraviolet radiation; MAAs, Mycosporine-like amino acids.

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amounts of UV absorbing compounds (Leech et al., 2009). MAAs are produced de novo by some bacteria and several phytoplankton species, including dinoflagellates (Wängberg et al., 1997; Bandar-anayake, 1998; Shick and Dunlap, 2002), and they act as an effective UV screen (Karentz, 2001; Shick and Dunlap, 2002; Moeller et al., 2005). A number of studies have shown that many phytoplankton species and especially dinoflagellates contain large amounts of MAAs and that UVR exposure increases the content of those substances (Wängberg et al., 1997; Hannach and Sigleo, 1998; Laurion et al., 2004). Here we feed copepods with the MAAs producing *Heterocapsa triquetra* (Ehrenberg) that increases its MAAs production upon UV exposure and, furthermore, seem to pack their MAAs around organelles in order to increase the UV protection efficiency (Wängberg et al., 1997; Laurion et al., 2004). Several dinoflagellate species have furthermore been observed to be phototactic and migrate vertically (Heaney and Furnass, 1980; Ekelund and Häder, 1988), which suggests that at times they are probably highly exposed to UVR.

In contrast to phytoplankton, animals seem unable to synthesize photoprotective compounds de novo (Karentz, 2001 and references therein), and thus they can accumulate those substances only if they are present in sufficient amounts in food sources. Freshwater copepods have been shown to accrue both carotenoids and MAAs upon exposure to UVR (Hansson, 2000; Moeller et al., 2005; Hylander et al., 2009a), but very few studies have addressed this matter in marine zooplankton. Furthermore, available results are contradictory. For example, it has been observed that Antarctic krill (*Euphausia superba*) contained high concentrations of MAAs after feeding on algae that had been grown under PAR-supplemented UVR (Newman et al., 2000), whereas in another krill species (*Nyctiphanes australis*) the MAAs content could not be correlated with changes in phytoplankton MAAs content or fluctuations in ambient UVR (Riemer et al., 2007). To our knowledge this is the first study that deals with dietary transfer of MAAs from phytoplankton to copepods in marine systems and the results are also compared with results from freshwater systems.

The overall objective of the present study was to assess underlying mechanisms of MAAs accumulation in the dinoflagellate *H. triquetra* and the transfer of these substances to the planktonic copepod *Acartia tonsa*. Both species occur in surface waters (0–10 m), southwest of Sweden (Edler, 2000), making them suitable test organisms for UV effects at temperate latitudes. We focused on the copepods, considering their feeding on the dinoflagellates and how they accumulate MAAs from that source. Carotenoids were also quantified in copepods but not in dinoflagellates. We conducted a mechanistic laboratory experiment in which we manipulated the UV exposure and measured the levels of MAAs in both the dinoflagellates and the copepods. We investigated whether the copepods accumulated MAAs upon UV exposure, and if such build up is associated with the availability of MAAs in the food source.

2. Materials and methods

2.1. Study site

A natural copepod (*A. tonsa*) community was collected on 23 April 2007 in Öresund (N 55°55'3"; E 12°43'3") southwest of Helsingborg, Sweden. The water temperature was 9 °C and the salinity varied between 15 and 18; no stable halocline or thermocline was observed. Light (PAR 400–700 nm) and UVR (UVA 315–400 nm) were measured in the air and the water at noon (some overcast) using an SUL 033 and 240 sensors connected to an IL 1400A light meter (International Light, Newburyport, MA, USA). The copepods were sampled by net hauls (mesh size 100 µm) from a depth of 10 m up to the surface, and they were kept in their natural water at 10 °C until they were acclimatized for the experiment.

2.2. Culture conditions and experimental setup

Before use in the experiment, the copepods and dinoflagellates (*H. triquetra*, GUMACC71 (Göteborg University Marine Culture)) were handled as follows. The collected copepods were allowed to gradually acclimatize to seawater with a salinity of 26 (optimal level for the dinoflagellates) and to a constant temperature of 14 °C (a regular spring temperature) over a period of two days. *A. tonsa* occur in brackish and saline water and can survive in salinities between 0 and 77 but the species does best between 15 and 22 and requires at least 10 °C for reproduction (http://www.frammandearter.se/0/2english/pdf/Acartia_tonsa.pdf). Key studies on the biology of *A. tonsa* have used similar salinities and temperatures as in this study (Kjørboe et al., 1985). The dinoflagellates were grown at 20 °C under 40 µmol quanta m⁻² s⁻¹ in *f/2* medium prepared in 26 salinity seawater (Barker, 1935), and the cultures were subsequently allowed to acclimatize to a constant temperature of 14 °C for five days, which is a regular spring temperature in their natural habitat.

In the experimental setup, 16 opaque cylindrical 4-L vessels were used to house the organisms, and the treatments consisted of exposure to either the full spectrum including both UVR and PAR wavelengths (full spectrum abbreviated as FS) or a UVR-reduced spectrum only receiving PAR wavelengths (reduced spectrum abbreviated RS). The radiation was produced by eight fluorescent lamps (UVA-340, Q-Panel, Cleveland, OH, USA) in a 16:8 light:dark cycle, giving a UVA intensity of 9.3 W m⁻², a UVB intensity of 0.05 W m⁻² and a PAR intensity of 20 µmol quanta m⁻² s⁻¹ (measured with sensors SUL 033 and SUL 240, connected to a logging meter IL 1400A, International light, Newburyport, Massachusetts, USA; for spectral composition see online supplementary material). Over a day this corresponds to 54 J cm⁻² of UVA, which is in the same order as used in previous studies (Hylander et al., 2009b). It is a well-established method to use such fluorescent lamps to test the effects of UVR on aquatic organisms (e.g., Hansson, 2004; Moeller et al., 2005). The PAR intensity used in this study is lower than in surface waters in the natural habitat, but the same intensity has been used in previous studies and dinoflagellates are known to grow under dim light conditions (Logares et al., 2007; Blanco et al., 2009). The amount of UVR reaching the water surface in the containers was controlled by the use of two different kinds of Plexiglas, one type that screens away UV and another type that is UV-transparent. Both types transmit PAR equally well (Hansson et al., 2007). Treatments that were relieved from UV were covered by UV-screening Plexiglas (Röhm GS 233, Röhm Darmstadt, Germany), effectively cutting off radiation below ~370 nm (i.e. in the UVA and UVB range, called reduced spectrum treatment, RS). Treatments receiving the full spectrum, including UV and PAR wavelengths (called full spectrum, FS), were covered by UV-transparent Plexiglas (Röhm GS 2458), which allowed penetration of both PAR and UVR. To estimate penetration of UV in the water column, absorbance at 320 nm (A_{320}) was measured (Beckman DU 800 spectrophotometer). The diffuse attenuation coefficient at 320 nm (K_{320}) was calculated for the water in the experimental vessels using the relationship between the absorption coefficient and the diffuse attenuation coefficient (Kirk, 1994; Morris et al., 1995). Total UV radiation at a certain depth in the vessel was calculated from $I_z = I_0 e^{(-K_{320} * z)}$, where I_z is the UV exposure at a depth of z m and I_0 is the UV exposure above the surface (Hansson, 2004).

At the onset of the experiment (day 0), the 16 vessels were filled with 4 L (water depth 0.13 m) of filtered seawater (salinity 26, 1 µm mesh size) and nutrients were added in concentrations similar to those present in the *f/2* medium (NO₃-NaNO₃ 0.1 mmol L⁻¹ and PO₄ 0.01 mmol L⁻¹; Barker, 1935). To eight of the 16 containers, we added 600 copepods each (separated by hand with plastic pipette (1.5 ml) and petri dish). Thereafter, to each of the 16 vessels we added an aliquot of dinoflagellate culture to an initial count of approximately 1660 cells mL⁻¹. Four of the copepod/dinoflagellate vessels and four of the vessels with dinoflagellates alone were

assigned to full spectrum treatment (FS), and the same numbers were assigned to a reduced spectrum treatment (RS). The eight vessels containing both copepods and dinoflagellates are designated C + D + FS (copepods + dinoflagellates + full spectrum; $n = 4$) and C + D + RS (copepods + dinoflagellates + reduced spectrum; $n = 4$), and, correspondingly, the eight vessels containing only dinoflagellates, which were to serve as controls for comparison with microalgal growth during exposure to full or reduced spectra without grazing, are designated D + FS (dinoflagellates + full spectrum; $n = 4$) and D + RS (dinoflagellates + reduced spectrum; $n = 4$). MAAs levels were not monitored in D + RS and D + FS treatments and they were only included for comparisons of phytoplankton growth without copepod grazers.

The actual experiment was started two days after the copepods had been collected in the wild. It was conducted over a period of eight days at 14 °C, and there were no significant differences in temperature between the experimental treatments ($t = 0.15$, $p > 0.05$, $df = 6$).

2.3. Sampling and pigment analysis

Initially and every other day (i.e., on days 0, 2, 4, 6, and 8), 30 copepods for MAAs analysis and 30 for carotenoid analysis were sampled from the experimental vessels containing copepods (i.e. C + D + FS and C + D + RS), and copepods were allowed to empty their guts for 1 h in filtered seawater (no chlorophyll peaks were observed in extracts, indicating that gut evacuation had been efficient). Only later stage copepodites and adults were sampled and all egg-bearing females were excluded. Thereafter, they were placed in a freezer at –80 °C, where they were kept until analyzed (within seven months). Samples were frozen with as little water as possible and the remaining water was eliminated before extraction by freeze drying the sample for 12 h at –60 °C (Lyolab 3000, Heto-Holten A/S, Allerød, Denmark). In parallel with the copepod sampling, we also collected 30 mL of water from C + D + FS and C + D + RS vessels for analysis of MAAs in the dinoflagellates; these samples were filtered onto GF/F filters (25 mm Whatman GF/F filters; copepods were excluded before filtering). Additional water samples (5 mL) were also taken (after gentle stirring) from all treatments to determine microalgal density (in C + D + FS; C + D + RS; D + FS and D + RS treatments); these samples were preserved with acid Lugol's solution, and the dinoflagellate cells were counted (at least 1 h sedimentation time in 250 μ L wells) in an inverted light microscope (Olympus CKX 41). From the final sampling date, 20 dinoflagellates were also measured in an inverted light microscope (Olympus CKX 41; longest possible diameter of the cell) to assess potential size differences among treatments.

MAAs were extracted in 25% methanol in water (v/v) and analyzed by conventional high-performance liquid chromatography (HPLC; Tartarotti and Sommaruga, 2002; Tartarotti et al., 2004) performed on an Agilent 1100 DAD system equipped with a standard flow cell and a 5- μ m C8 Phenosphere analytical column (ID 4.6 mm and length 0.25 m; Phenomenex, Torrance, CA, USA) protected by a RP-8 guard column (4.0 \times 3.0 mm; Phenomenex). The mobile phase consisted of 25% methanol in water (v/v) containing 0.1% acetic acid. Since there are no commercially available standards, peaks were identified by comparison with published retention times and DAD spectra (Tartarotti et al., 2001, 2004; Sommaruga et al., 2006). Copepod extracts were also spiked with extracts from *Porphyra ternera* for co-elution of the MAAs from that species. The MAAs content was calculated from acquired HPLC peak areas (Tartarotti et al., 2001 and references therein), and, for unknown MAAs we used a mean extinction coefficient of 137.1 L g⁻¹ cm⁻¹ (Laurion et al., 2003). The concentration of photoprotective compounds was normalized per cell for dinoflagellates and to dry weight for copepods (approximately 10 individuals measured per replicate and sampling occasion) based on published relationships between length and dry

weight in a similar species, *Eudiptomus gracilis* (Bottrell et al., 1976). Knowing the dinoflagellate MAAs cell content, the cell numbers, the numbers of copepods, and the MAAs content in copepods, a total population accumulation among copepods could be calculated according to the equation:

$$(MAA_{\text{cop}} \times N_{\text{cop}}) / (MAA_{\text{dino}} \times N_{\text{dino}}) \times 100,$$

where

MAA_{cop} = MAAs content per copepod (μ g)

N_{cop} = number of copepods

MAA_{dino} = MAAs content per dinoflagellate cell (μ g)

N_{dino} = number of dinoflagellate cells.

Copepod samples for carotenoid analysis were put in 25-mL glass scintillation vials containing 4 mL of 95% ethanol for 1 h. Thereafter, the tissues were disrupted for 30 s on ice using an ultrasonic converter (Heat Systems model CL4, Farmingdale, New York, USA), and extraction was performed at room temperature for 5 h in the dark (Hylander et al., 2009b). The samples were subsequently centrifuged for 5 min at 3000 rpm, and the extracted red pigment in the supernatant was quantified in a Beckman DU 800 spectrophotometer at 474 nm, which represents the absorption peak for the carotenoids commonly found in copepods (i.e., astaxanthin and its esters) (Hairston, 1976; Hansson, 2000, 2004). The samples were also scanned at 1 nm resolution from 350 to 700 nm. No peaks were observed at the absorption maxima of chlorophyll (665 nm), indicating that copepod gut evacuation had been effective, and hence the analysis was not disturbed by carotenoids originating from ingested dinoflagellates. The concentration of carotenoids was normalized to dry weight in the same manner as for MAAs.

2.4. Statistical analysis

To test for differences among treatments, repeated measures ANOVAs were carried out followed by post hoc comparisons when there were more than two levels. If the assumption of sphericity was not met, significance levels were adjusted using Greenhouse–Geisser corrections. Independent sample *t*-tests were applied when data only was available at the end of the experiment (carotenoids, mortality and size of dinoflagellates at day 8). When necessary, data were log-transformed to meet assumptions for the tests (only needed for dinoflagellate MAAs), and all analyses were performed in SPSS 15.0 for Windows.

3. Results

3.1. Field data

The field measurements showed that the 1% attenuation depth for UVA was 6.3 m. By comparison, the photosynthetic portion of the light spectrum (PAR) did not diminish as rapidly, showing a 1% attenuation depth of 23.1 m. Just below the surface, intensities of UVA radiation and PAR were 9.7 W m⁻² and 380 μ mol quanta m⁻² s⁻¹, respectively. The newly collected copepods appeared transparent and had a natural carotenoid content of 1.1 μ g DW⁻¹ and a slightly higher concentration of MAAs at 2.6 μ g mg DW⁻¹.

3.2. Experimental data

3.2.1. MAAs

After experimental exposure, including UVR and PAR wavelengths, (C + D + FS), the amount of MAAs increased in both the dinoflagellates and the copepods (Figs. 1, 2). The dinoflagellates initially had an MAAs content of 7 pg cell⁻¹ (day 0), but their MAAs levels

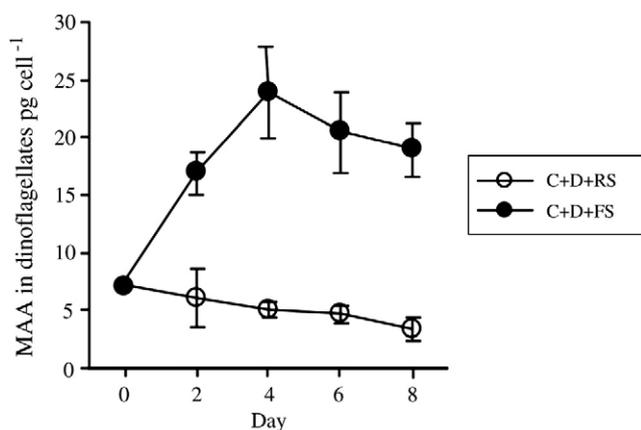


Fig. 1. MAAs content measured in dinoflagellates incubated together with copepods and exposed to a full spectrum including UVR and PAR wavelengths (C + D + FS) or a reduced spectrum only receiving PAR wavelengths (C + D + RS) during the eight-day experiment. Error bars denote ± 1 SD.

subsequently diverged markedly between treatments after only two days, and after eight days the dinoflagellates contained approximately 4 times more MAAs after exposure to UVR than after treatment with a reduced spectrum, i.e. only PAR wavelengths (C + D + FS) (Fig. 1; Table 1; treatment effect RM ANOVA $F_{1,6} = 246.9$, $p < 0.05$). The MAAs found most often in the dinoflagellates was shinorine ($R_t = 3.6$ min, $\lambda_{\max} = 333$ nm). There were also smaller amounts of porphyra ($R_t = 3.9$, $\lambda_{\max} = 334$ nm) and four unknown substances (respectively: $R_t = 4.5, 5.7, 6.5$, and 6.7 , $\lambda_{\max} = 302, 331\text{--}333, 334\text{--}336$, and $334\text{--}336$ nm), as well as traces of mycosporine-glycine ($R_t = 3.3$, $\lambda_{\max} = 308$ nm) and usujirene/palythene ($R_t = 9.1$, $\lambda_{\max} = 357\text{--}360$ nm).

On the final day of the experiment (day 8), copepods exposed to UVR and PAR contained more than twice the level of MAAs compared to those that were only exposed to PAR (compare C + D + FS and C + D + RS; Fig. 2; Table 1) and there was an overall treatment effect (Fig. 2; RM ANOVA, $F_{1,6} = 38.5$, $p < 0.05$). Of all the MAAs that were detected in the copepods, the most common one was shinorine ($R_t = 3.5$ min, $\lambda_{\max} = 333$ nm). We also detected smaller amounts of mycosporine-glycine ($R_t = 3.2$, $\lambda_{\max} = 308$ nm), porphyra ($R_t = 3.7$, $\lambda_{\max} = 334$ nm), palythine ($R_t = 6.0$, $\lambda_{\max} = 320$ nm), and three unknown substances ($R_t = 3.3, 5.9$, and 6.4 , $\lambda_{\max} = 333\text{--}335, 320\text{--}322$, and $330\text{--}340$ nm) as well as traces of usujirene/palythene ($R_t = 9.1$, $\lambda_{\max} = 357\text{--}360$ nm).

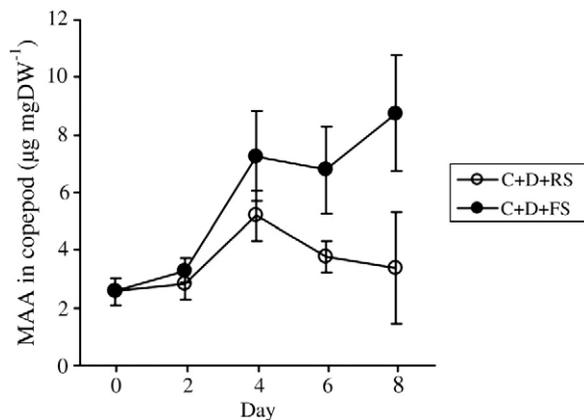


Fig. 2. MAAs content in copepods incubated together with dinoflagellates and exposed to a full spectrum including UVR and PAR wavelengths (C + D + FS) or a reduced spectrum only receiving PAR wavelengths (C + D + RS) during the eight-day experiment. Error bars denote ± 1 SD.

3.2.2. Carotenoids

The copepods were generally transparent with a few drops of carotenoids in their body tissues. UVR exposure did not lead to any visible increases in pigmentation, and the final carotenoid concentration was $0.98 (\pm 0.16)$ and $1.02 (\pm 0.24)$ $\mu\text{g mg DW}^{-1}$ in C + D + RS and C + D + FS treatments, respectively (mean \pm sd). There was no significant difference between the treatments ($t = 0.3$, $p > 0.05$, $df = 6$).

3.2.3. Mortality and cell growth

Total mortality for the copepods was estimated at the end of the experiment. Approximately 100 out of the 600 copepods that had been added to each container died in background mortality during the experiment but there were no differences in mortality between treatments (mean \pm SD), C + D + RS = 210 (± 11) and C + D + FS 200 (± 49); $t = 0.41$, $p > 0.05$, $df = 6$). Furthermore, copepod length was not affected by the treatments of UVR as indicated by the lack of significant differences between those incubated under C + D + FS and C + D + RS (RM ANOVA, $F_{1,6} = 1.6$, $p > 0.05$). On the final sampling, dinoflagellates were approximately 6.5% larger in length when treated with FS compared with RS (C + D + FS compared with C + D + RS and D + FS compared with D + RS) ($t = 2.8$, $p < 0.05$, $df = 6$ and $t = 3.1$, $p < 0.05$, $df = 6$ respectively).

The density of dinoflagellates grown without copepods at different treatments (compare D + RS and D + FS) was essentially the same regardless of the type of radiation (Table 1; Fig. 3, $p > 0.05$ according to Tukey's test). However, the dinoflagellates showed a higher level of growth when incubated alone than they did in the presence of copepods indicating that copepods were grazing on the dinoflagellates (Fig. 3; RM ANOVA, $F_{3,12} = 49.0$, $p < 0.05$, Tukey's test). Furthermore, the algal cell density was lower in the C + D + FS than in the C + D + RS vessels (Fig. 3; RM ANOVA, $F_{3,12} = 49.0$, $p < 0.05$, Tukey's test).

Knowing the dinoflagellate cell numbers and MAAs content, as well as the copepod numbers and MAAs content, a total population accumulation among copepods could be calculated. At the final sampling date (using the above mentioned parameters) the copepod population contained 2–5% of the total MAAs content in the dinoflagellate population.

4. Discussion

Both dinoflagellates and copepods responded to UVR by significantly increasing their MAAs content. The level of these substances in the copepods was twice as high after exposure to UVR as compared to non-UV treatment. The copepod population contained approximately 2–5% of the dinoflagellate population total MAAs content at the end of the experiment.

The dinoflagellate *H. triquetra* has previously been shown to contain approximately 35 pg of MAAs cell⁻¹ when grown in PAR supplemented with UVA (Laurion et al., 2004), which is in the same order of magnitude as the level found in our study (20–25 pg cell⁻¹, Fig. 1). The strain used here is different from the previously studied strains, and was first isolated in a similar area as the copepod was caught. The ability of *H. triquetra* to rapidly increase the amount of MAAs when exposed to UVR implies that the plasticity of MAAs production is important for dinoflagellate fitness. It has also previously been shown that synthesis of MAAs is stimulated by changes in the radiation regime (Carreto et al., 1990; Wångberg et al., 1997) but when exposed to high UV radiation *H. triquetra* may indeed be photosynthetically inhibited (Helbling et al., 2008). Dinoflagellates are exposed to varying light fields during their vertical migrations, and thus need to have an inducible UV defense in order to avoid the costs of unnecessary production of sunscreens. The large difference in MAAs content between full spectrum treatment including both UVR and PAR wavelengths and reduced spectrum treatment only including

Table 1

Results from RM ANOVA:s including differences in MAAs among dinoflagellates and copepods and differences in cell densities of dinoflagellates.

Test	Factors	df	Error df	F	p	Tukeys test
MAAs in dinoflagellates	Treatment	1.0	6.0	246.9	<0.001	N.A.
	Time	1.6	10.1	6.1	0.022	
	Time*treatment	1.6	10.1	6.4	0.019	
MAAs in copepods	Treatment	1.0	6.0	38.5	0.001	N.A.
	Time	1.5	8.9	9.4	0.009	
	Time*treatment	1.5	8.9	4.6	0.051	
Dinoflagellate density	Treatment	3.0	12.0	48.9	<0.001	p<0.05 for all combinations
	Time	3.0	36.0	214.5	<0.001	Apart from D + FS vs. D + RS
	Time*treatment	9.0	36.0	12.4	<0.001	

N.A. = not applicable, only two levels.

PAR wavelengths confirms that UVR exposure is a critical stimulus for generation of MAAs in *H. triquetra*. On a weight basis, the MAAs present in the dinoflagellate would account for approximately 7% of the biomass (assuming $210 \text{ pgC cell}^{-1}$ representing 50% of the cell biomass) and would, according to models, give sunscreen efficiency in the same order of magnitude as damage repair mechanisms (Garcia-Pichel, 1994). This MAAs accumulation is, however, also in the same order of magnitude as investments in total cellular nucleic acids and this raises doubts as to whether MAAs in dinoflagellates solely exhibit sunscreen properties, or if they are also associated with other functions, for example as solutes to ease salt stress as proposed by Oren and Gunde-Cimerman (2007). One should also be careful when comparing experimentally derived accumulation rates of MAAs with rates in nature, since natural populations of dinoflagellates experience lower UV to PAR ratios than in this experiment. The fluorescent lamps that were used also produce a different spectrum compared with the solar spectrum. Furthermore, the absorption spectrum of *H. triquetra* (Laurion et al., 2003) includes several areas where the lamps did not produce any emission peaks to excite photosynthesis. In all, this may affect the growth of the dinoflagellates negatively and potentially also the rates of MAAs accumulation. This potential effect may be seen in figure one where MAAs levels in the non-UV treatment (C + D + RS) decreased somewhat over time even though the dinoflagellates prior to the experiment did not receive any UV radiation. However, the important conclusion in this study is that elevated UV exposure is a crucial mechanism for inducing higher MAAs accumulation in the dinoflagellate. Among the different MAAs that were detected in *H. triquetra*, shinorine was the most abundant. Also in another strain of *H. triquetra* (CCMP449, Provasoli-Guillard National Center for Culture of Marine Phytoplankton), shinorine is the most prevalent MAAs and this strain also contains large amounts of an unknown MAAs with absorption maximum at 333 nm (Laurion et al., 2003).

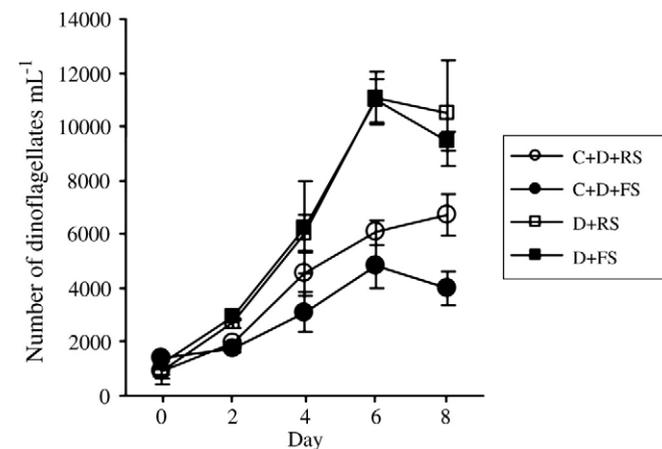


Fig. 3. Treatment effects of a full or reduced spectrum on the density (number) of dinoflagellates (*H. triquetra*) in vessels containing the algae alone (D – FS and D – RS) or together with copepods (C + D + FS and C + D + RS). Error bars denote ± 1 SD.

Even though UV treated dinoflagellates were slightly larger than those who only received PAR wavelengths, *H. triquetra* in non-copepod treatments grew equally well with similar cell densities under reduced spectrum and full spectrum treatments (see D + RS and D + FS in Fig. 3). This suggests that MAAs synthesis has no particular growth-related cost, and the exponential growth we observed indicates that there was no apparent nutrient-associated limitation in that context. Our observations also agree with a previous study of the same species (Wångberg et al., 1997), in which it was found that UVB did not affect growth but resulted in larger cell size. Also other phytoplankton species have been shown to increase their cell size which is hypothesized to be a measure to protect sensitive sites from photo-damage within the cells (Hannach and Sigleo, 1998).

The copepod *A. tonsa* accumulated approximately twice the amount of MAAs when exposed to UVR and PAR wavelengths as compared to a reduced spectrum only containing PAR wavelengths. These levels of MAAs correspond to about 0.9% of the dry weight of the animals in the C + D + FS treatment and 0.3% in the C + D + RS treatment, and those values are within the range found in a previous study of a freshwater copepod (Moeller et al., 2005). Since *H. triquetra* is present in considerable amounts in the same area as the copepod (Edler, 2000), MAAs from this species, and other MAAs producing algae, may be an important part of the copepod UV defenses. Accumulation of MAAs in copepods seems to follow a sigmoid curve, so that increasing dietary availability does not automatically lead to increased accumulation (Moeller et al., 2005). When calculated for the marine species we investigated, total accumulation was $25 \text{ ng of MAAs copepod}^{-1}$ in the C + D + FS treatment compared with $3 \text{ ng copepod}^{-1}$ in the C + D + RS treatment, which implies that the copepods in our experiment had amassed MAAs to the greatest extent possible.

When dinoflagellates were incubated with copepods there was a difference in cell density between radiation treatments indicating that grazing may have been more intense in the full spectrum treatment compared with the reduced spectrum treatment. The underlying mechanisms to an increased copepod grazing with consequent MAAs accumulation are largely unknown. We suggest that copepods that are exposed to UV increase their metabolism when subjected to a physical stress like UV and in this way increase their feeding and subsequently their contents of MAAs. This is in line with recent observations indicating that respiration rates seem to increase at moderate UV exposure which is suggested to be a reflection of energetic costs paid for zooplankton UV defenses (Fischer et al., 2006). However, another explanation could be that UV treated phytoplankton in some cases have been shown to decrease their motility (Tirlapur et al., 1993) and thus would be more susceptible to copepod predation. Apart from differences in feeding rate, the difference in dinoflagellate density may also be due to other factors. If the size of the dinoflagellates was smaller in the full spectrum treatments one would expect the feeding rate to be higher there to compensate for the lower biomass. This was however not the case and dinoflagellate size was actually slightly larger in the full spectrum treatment compared with the reduced

spectrum treatment. Furthermore, one could argue that UV avoidance in combination with biotic interactions would affect the abundances of the dinoflagellates. This is also unlikely due to small water volumes where more than 95% of the radiation at 320 nm reached the bottom of the vessels (day 0) creating a homogeneous UV environment, even though UV penetration may have been lower at the end of the experiment due to excretion from the organisms (not estimated). Finally, the rate of temporary dinoflagellate cyst formation could differ between treatments, but since we did not observe any cysts this is also an unlikely explanation. Increased feeding rate of copepods exposed to UV is hence the most probable explanation to the differences observed in dinoflagellate densities.

We also found that the copepods exposed to UVR did not accumulate carotenoids, which is in contrast to studies showing that several species of freshwater copepods do accrue those pigments when subjected to UV irradiation (Hansson, 2000; Hansson et al., 2007; Hylander et al., 2009a, 2009b). The accumulation of carotenoids in marine copepods is not very well understood, although it seems to depend on the diet and can be increased by a diversity of food sources (Andersson et al., 2003) which may imply that our single species diet was not sufficient for carotenoid accumulation (but we did not quantify carotenoids in the dinoflagellates). However, there are also other studies that show no differences in carotenoid accumulation in copepods when fed with different phytoplankton communities (Van Nieuwerburgh et al., 2005). If we compare the carotenoid levels in *A. tonsa* with freshwater copepods from the same latitude, animals initially displayed approximately the same levels (Hansson, 2004).

Many zooplankton, including *A. tonsa*, can display nocturnal migration by avoiding the surface during the day (Forward, 1988; Stearns and Forward, 1984). In this way they may efficiently avoid the UV threat when it is at its peak. Copepods indeed have deeper depth distribution when exposed to UV (Hylander et al., 2009b). We did, however, not observe any such phototactic behavior, but the experimental vessels were shallow not allowing the animals to use any depth refuge. If migration is present in the natural copepod population, which is the case for some populations of *A. tonsa* (Stearns and Forward, 1984), our experimental setup may overestimate the natural MAAs accumulation.

In conclusion, the dinoflagellates and copepods in our investigation responded strongly to UVR by, respectively, producing and accumulating MAAs. In dinoflagellates, the MAAs content was approximately four times higher after exposure to UVR and PAR than in the reduced spectrum treatment only receiving PAR, but there was no corresponding difference in growth. The copepods accumulated approximately twice the amount of MAAs when exposed to UVR as they did under non-UV treatment and cumulative accumulation estimates indicate that they amassed MAAs at their highest possible rate. How the mixture of different UV protective compounds, like carotenoids and MAAs, is regulated in marine copepods is largely unknown but this study shows that UVR is a crucial cue. Furthermore, in freshwaters it has been shown that both MAAs and carotenoid levels in copepods increase upon UV exposure and, interestingly, copepods compensate with higher carotenoid accumulation if they are fed with a MAAs-poor diet (Hylander et al., 2009a). In a broader perspective, the ongoing depletion of the ozone layer can potentially expose planktonic organisms to higher levels of detrimental radiation (Bancroft et al., 2007). Given that the organisms can respond to these changes the ability of some species to produce or accumulate photoprotective compounds may lead to higher fitness and thereby increase the occurrence of those organisms in the plankton community.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jembe.2010.03.020.

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