Functional Ecology

British Ecological Society

Functional Ecology 2014, **28**, 149–158 doi: 10.1111/1365-2435.12159

Fitness costs and benefits of ultraviolet radiation exposure in marine pelagic copepods

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Summary

1. Life-history theory predicts that organisms should allocate energy throughout their life such that they maximize their fitness. Copepod zooplankton are known to accumulate sunscreens (so-called mycosporine-like amino acids, MAAs) and antioxidant carotenoids to mitigate negative effects of ultraviolet radiation (UVR), but it is not well known how this affects their fitness.

2. We followed cohorts of the marine copepod *Acartia tonsa* and assessed how fitness was affected by UVR exposure and a diet rich in UVR-protective sunscreens.

3. Several fitness components including somatic growth, egg quality and nauplii production (larvae) were negatively affected by UVR, whereas other components such as size at maturity, survival and length of life were not. Nauplii production through low egg quality was the most influential life-history parameter that changed in response to UVR.

4. There was interaction between fitness costs and food source. If copepods were fed a diet rich in UVR-screening MAAs, they were able to maintain and even increase their fitness even though they were exposed to otherwise detrimental radiation. Levels of UVR-protective carotenoids were low in the studied species and a meta-analysis revealed that marine copepods in general have much lower – by an order of magnitude – levels of carotenoids than freshwater species, while levels of MAAs are similar between the two habitats.

5. We conclude that allocation to different fitness components to some extent is plastic although egg quality is by far the most influential factor, and this is an example of how environmental variability affects overall fitness. Fitness costs associated with UVR exposure in the absence of UVR-screening MAAs were present. Other costs such as costs for accumulating MAAs were not detected, and if present, they were outweighed by a stimulated fitness in combined UVR and MAA treatments challenging the common model that inducible defences (such as accumulation of MAAs) should come with a cost. Low levels of carotenoids in marine systems suggest high predation pressures on pigmented specimens. Accumulation of nonpigmented MAAs could hence be a key adaptation for surface-dwelling marine zooplankton to maintain or even increase their fitness when exposed to detrimental radiation.

Key-words: calanoid copepod, carotenoids, life history, mycosporine-like amino acids, phenotypic plasticity, pigments, ultraviolet radiation, zooplankton

Introduction

All organisms have to grow, survive and reproduce. The integral result of these fundamental activities is the average number of offspring that an individual delivers to the next generation, the net reproductive rate, R_0, a commonly used proxy for fitness (Stearns 1992; Kiørboe & Hirst 2008). The optimal investment allocation into different fitness components is dependent on the biotic and abiotic setting. For example, high predation pressure may warrant a higher relative investment in survival (defence), and environmental stress, like suboptimal temperature, salinity and ultraviolet radiation (UVR), may force the organism to allocate more resources to handle a challenging abiotic environment at the cost of reduced growth and/or reproduction.

An abiotic factor that has recently received attention is the threat from increasing levels of ultraviolet radiation

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(UVR) reaching Earth with consequent damages on DNA and other cellular structures (Malloy *et al*. 1997; Yasui & Sakurai 2000). UVR has been present throughout evolutionary time (Hessen 2008), and regardless of any human-induced changes, organisms have adapted to this environmental factor. Here, we focus on the potential fitness costs associated with UVR exposure in marine systems and especially in the planktonic part of the food web. UVR attenuates with depth mainly due to dissolved organic carbon (Morris *et al*. 1995), and marine systems generally have relatively low DOC contents. A significant portion of surface waters can therefore be exposed to harmful levels of radiation (Tedetti & Sempere 2006), and marine surface-dwelling zooplankton such as copepods may experience UVR-induced mortality of eggs and early life stages (Browman *et al*. 2000). But organisms, including copepods, have a number of defence mechanisms to counteract UVR damage, including accumulation of photoprotective compounds, cellular defence mechanisms and avoidance of the surface waters (Hansson & Hylander 2009; Rautio & Tartarotti 2010; Hylander *et al*. 2012). Pelagic copepods mainly accumulate carotenoids (Hairston 1979a; Hylander *et al*. 2009) and mycosporine-like amino acids (MAAs) to reduce UVR damage (Tartarotti, Laurion & Sommaruga 2001; Moeller *et al*. 2005). The carotenoids are strong antioxidants neutralizing harmful agents that are produced due to UVR exposure, while MAAs have been suggested to function as UVR filters (so-called sunscreens; Palozza & Krinsky 1992; Bandaranayake 1998). Hence, these two photoprotective substances (carotenoids and MAAs) defend organisms against UVR damages in different ways and both can be accumulated in copepods if UVR exposure is intense (Moeller *et al*. 2005; Hylander, Larsson & Hansson 2009; Hylander *et al*. 2009). Conversely, levels of carotenoids and, in some cases, MAAs, are reduced when animals are released from UVR stress (Hansson, Hylander & Sommaruga 2007), which suggests that it entails a cost for the organism to maintain high levels of UVR-protective compounds. These compounds are generally not synthesized by the zooplankton themselves and hence have to be accumulated from the algal food (Goodwin 1986; Bandaranayake 1998; Laurion, Lami & Sommaruga 2002). While this has been studied in freshwaters (Hansson & Hylander 2009), surprisingly little is known about the function of MAAs and carotenoids in marine systems. Measurements of MAAs in marine copepods are scarce (Karentz *et al*. 1991; Hylander & Jephson 2010). Carotenoids have been measured in some species (e.g. Juhl, Ohman & Goericke 1996; Lotocka, Styczynska-Jurewicz & Bledzki 2004) but the adaptive benefits of carotenoids in marine copepods are not well known. Additionally, it is not well known how UVR defences and environmental variability affect life-history parameters and ultimately fitness (Kiørboe & Hirst 2008; Hansson & Hylander 2009).

The purpose of this study is to quantify the sublethal fitness costs that are associated with different UVR defence strategies in marine zooplankton, both costs directly associated with the UVR exposure and costs for accumulating UVR-screening substances. We expose populations of the copepod *Acartia tonsa* to UVR and non-UVR treatments crossed with high or low levels of sunscreen photoprotective compounds (MAAs) in the food source. We quantify the fitness costs of UVR and test the hypothesis that fitness is reduced at high UVR exposure if animals do not have sunscreens in the food source. This potential fitness cost would be due to lower survival and/or higher allocation of resources to repair and maintenance processes.

Materials and methods

Cohorts of the calanoid copepod *Acartia tonsa* were hatched from eggs and reared until they died (*c*. 60 days) while monitoring survival, growth, egg production, nauplii production and accumulation of UVR-protective compounds. Sixteen plastic cylindrical and UVR opaque containers with open top were filled with 10 L of 0·2-μm filtered sea water with a salinity of 27, and 1026 ± 104 nauplii L^{-1} were added to each container (18 °C). Each container was illuminated with two fluorescent lamps (36 W, UVA-340, Q-Panel) mounted 0·5 m above the containers at a 18:6 light/dark cycle, producing an intensity in the UVA spectrum of 523 ± 5·6 μW cm^{-2} (UVR sensors SUL 033, 240, connected to a logging meter IL 1400A, International light, Newburyport, MA, USA). On a daily basis, this results in a UVR dose at the surface that corresponds to a summer day with some overcast in temperate systems (Hylander, Larsson & Hansson 2009). For the complete spectrum of the lamps, see Hansson, Hylander & Sommaruga (2007). In treatments not exposed to UVR, radiation was screened by a lid of Plexiglas (Röhm GS 233), effectively cutting off radiation below 370 nm, that is, in the UVA and UVB range. In treatments that were exposed to UVR, radiation was admitted by UVR-transparent Plexiglas (Röhm GS 2458). There is no difference, however, in transmittance of photosynthetically active radiation (PAR) between Plexiglas types (Hansson, Hylander & Sommaruga 2007).

Copepods were fed every day with phytoplankton (*Rhodomonas salina* and *Heterocapsa triquetra*) reared in repeated batch cultures and kept in exponential growth (18 °C; 140 μE M^{-2} s^{-1}). *H. triquetra* is known to produce photoprotective sunscreens (MAAs; Hylander & Jephson 2010), whereas *R. salina* does not (this study). These algae were not exposed to UVR before daily addition to the experimental containers. Food levels in the containers were adjusted to 1000 μg C L^{-1} every day after measurement of cell concentrations with a particle counter (Beckman Coulter Counter). Carbon contents of the phytoplankton were taken from the literature (Berggreen, Hansen & Kiørboe 1988; Hylander & Jephson 2010). Four different treatments were applied, each with four replicates: (i) P – only PAR light and fed with *R. salina*; (ii) U – UVR exposure and PAR light fed with *R. salina*; (iii) PH – PAR light and fed with 50% *R. salina* and 50% *H. triquetra* (on a carbon basis) and (iv) UH – UVR exposure and PAR light fed with 50% *R. salina* and 50% *H. triquetra*. To keep good water quality, one-third of the water volume was exchanged three times a week, and once a week, the containers were rinsed. When the copepods had reached maturity, offspring (eggs, nauplii) were removed once a week by filtering the entire population on a 200-μm net, such that only the original cohort was retained.

The density of copepods was monitored 2–3 times a week in 50–500 mL samples that were filtered onto a 40-μm net and fixed in Lugol's solution. The samples were counted, and >20 or all individuals were measured in a dissecting microscope (Leica MZ6 and MZ8 at 40–50× magnification). Egg production rates were

monitored twice a week from when the copepods reached adulthood at days 18–20. Six to ten females were incubated in 620-mL screw cap bottles in darkness on a slowly (*c*. 1 rpm) rotating plankton wheel. They were fed *R. salina* at a carbon concentration of 1000 μg C L^{-1}. Eggs were counted after 24 h and females measured (prosome length). Egg hatching success was quantified three times during the experimental period, at days 27, 35 and 42, by transferring eggs from the egg production experiments into 6-well petri dishes with filtered sea water (total volume *c*. 50 mL). The petri dishes were placed in dim light at 18 °C (no UVR), and the number of nauplii and remaining eggs were counted after 48 h.

We used estimates of the net reproductive rate,

$$R_0 = \int_{x=0}^{\infty} l_x m_x \mathrm{d}x,$$

as a proxy of Darwinian fitness (Stearns 1992; Kiørboe & Hirst 2008). By following cohorts of copepods in the different treatments, from egg to death, while monitoring survival (l_x) and nauplii production rates (m_x) as a function of time (x), we approximated the net reproductive rate of the experimental population as

$$R_0 = \sum_{x=0}^{\infty} l_x m_x.$$

By comparing R_0 between the four different treatment populations, it was hence possible to get a direct measure of the fitness cost of UVR exposure at different UVR doses and blends of protective compounds in the food of the copepods.

PHOTOPROTECTIVE COMPOUNDS

After 27 days of exposure, 20–40 adult copepods were sampled from each container. They were placed in filtered water for >2 h for gut evacuation to avoid phytoplankton pigments in the analysis. Samples were then stored at −80 °C until analysis within 1 month. Carotenoid and MAA extractions followed standard protocols (Tartarotti & Sommaruga 2002; Hylander, Larsson & Hansson 2009). In short, carotenoid samples were extracted in ethanol (95%) and quantified spectrophotometrically (Shimadzu UV-2450) at 474 nm and normalized to dry weight. This is the absorption peak of astaxanthin and its esters, which are common carotenoids in copepods (Hansson 2004). MAAs were extracted in 25% MeOH in water (Tartarotti & Sommaruga 2002) and scanned every nanometer from 250 to 800 nm (Shimadzu UV-2450). MAA concentration was estimated by the peak height at 312 nm, which was the absorbance maximum in the 300–370 nm wavelength range. MAAs were expressed as optical density (OD) normalized for dry weight. Dry weights were calculated from length to dry weight relationships (Klein Breteler, Fransz & Gonzalez 1982).

STATISTICS AND META-ANALYSIS

Amounts of photoprotective compounds and net reproductive rate (R_0) were analysed with two-way ANOVAS or *t*-tests. Survival, somatic growth, egg production and nauplii production were analysed with a linear mixed model with food (*H. triquetra* or *R. salina*), UVR and time as fixed factors and replicate as random factor. The survival data were log-transformed, and assumptions of the tests were assessed by visual inspections of residual plots. Egg hatching success and proportion of adults were analysed with generalized linear mixed models with binomial distribution (replicate as random factor for egg hatching) following Warton & Hui (2011). Data on MAA and carotenoid concentrations in marine and freshwater calanoid copepods were gathered from a database search (Web of Science) with the search word 'copepod' combined with either 'carotenoids' or 'mycosporine-like amino acids' (Kleppel *et al*. 1988 (assuming a dry weight of 210 μg ind^{-1}); Karentz *et al*. 1991; Juhl, Ohman & Goericke 1996 (assuming a dry weight of 210 μg ind^{-1}); Lotocka, Styczynska-Jurewicz & Bledzki 2004; Tartarotti *et al*. 2004; Moeller *et al*. 2005; Sommer *et al*. 2006; Hansson, Hylander & Sommaruga 2007; Persaud *et al*. 2007; Garcia *et al*. 2008; Holeton *et al*. 2009; Hylander *et al*. 2009; Hylander, Larsson & Hansson 2009; Rautio, Bonilla & Vincent 2009; Hylander & Jephson 2010; Sommaruga 2010; Zengling, Wei & Kunshan 2010; Hylander *et al*. 2012; Perez, Ferraro & Zagarese 2012; Schneider *et al*. 2012). This survey was complemented by the addition of all relevant references in the MAA database compiled by Sinha, Singh & Hader (2007) and also data from ten other marine species (S. Hylander, unpublished data). We only included studies that had starved the animals before sampling to avoid phytoplankton pigments in the analysis or when HPLC separation enabled the authors to summarize astaxanthin and its esters (the dominant pigments in copepods which are also rare in phytoplankton). If several measurements were available for the same sampling site, species and occasion, a mean value was calculated and included in the data set. Samplings ranged from polar (both Arctic and Antarctic), subarctic, temperate and a few subtropical areas as well as high-altitude lakes.

Results

PHOTOPROTECTIVE COMPOUNDS

Levels of carotenoids were *c*. 0·75 μg mg dry $weight^{-1}$ and showed little variation among treatments (Fig. 1a, Table 1). In contrast, MAA levels were nondetectable in the treatments fed only *R. salina* (P and U; no absorption peaks in the 300–360 nm wavelength range), while in the treatments that were fed with the dinoflagellate (*H. triquetra*), an absorption peak was observed at 312 nm, both in PH and UH treatments. Levels were also significantly higher in the UH compared with the PH treatment (Fig. 1b). Hence, availability of MAAs in the algal food and UVR exposure induced higher levels of MAAs in the copepod.

FITNESS

Copepods displayed rather similar survival in all treatments (Fig. 2; Table 2), and the life cycle from hatched egg to death lasted for *c*. 60 days in all treatments. There was a UV by time interaction resulting in slightly higher mortality in UVR treatments in the end of the experiment (only the last samplings). Somatic growth was negatively affected by UVR during the copepodite stages (days 7–18), especially in combination with non-MAA food source (i.e. U treatment; Fig. 3). However, final adult size did not differ among treatments (Fig. 3). The difference in growth rate materialized in a slightly but not statistically significant longer development time in the UVR treatments: 80–85% of the population had reached adulthood in the U and UH treatments on day 18, whereas *c*. 90% had reached adulthood in the non-UVR treatments (P and PH; Table 2). On day 25, all individuals in all treatments had

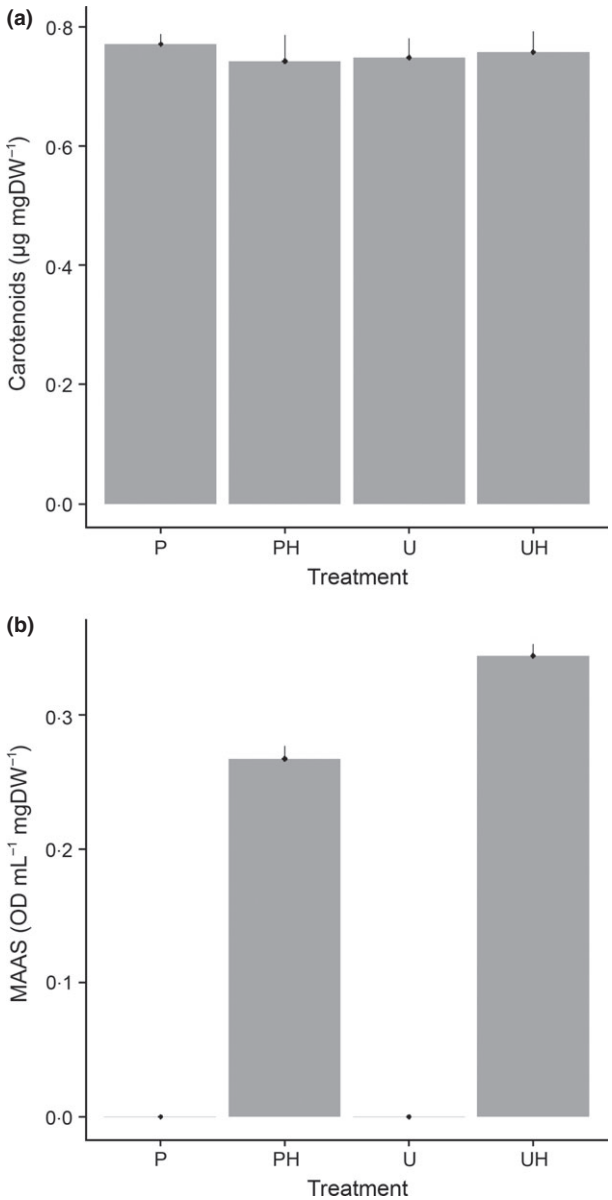

Fig. 1. Amount of photoprotective compounds in copepods after 27 days of treatment [carotenoids and mycosporine-like amino acids (MAAs) in (a,b), respectively; mean ± SE, $n = 4$ per treatment, and SE represents variation within each treatment]. Carotenoid content is expressed as amount pigment per dry weight, and amount of MAAs is expressed as the optical density at 312 nm per dry weight (which was the absorbance maxima in the MAA wavelength range). Treatments were as follows: P – non-UVR treatment and fed with *Rhodomonas salina*; PH – non-UVR treatment and fed with *R. salina* supplemented with MAA-producing *Heterocapsa triquetra*; U – UVR exposure and fed with *R. salina;* and UH – UVR exposure and fed with *R. salina* supplemented with MAA-producing *H. triquetra.*

Table 1. Statistical results on differences in amount of photoprotective compounds (carotenoids and MAAs) in copepods after 27 days of treatment. Differences in carotenoids were analysed with two-way ANOVA, and MAAs were analysed with a *t*-test between PH and UH treatments since MAAs were not detected in P and U treatments. Bold font indicates significant results.

	Statistic		
	F	d.f.	*P*
Carotenoids			
UVR	0·042	1	0·841
Food	0·003	1	0·960
UVR/food	0·453	1	0·514
Error		12	
MAAs	*t*		
PH–UH	5·9	6	**0·001**

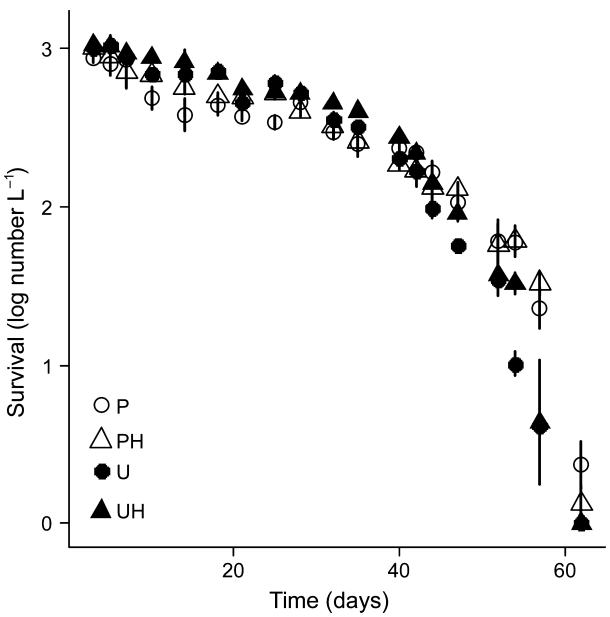

Fig. 2. Survival of copepods (log number L^{-1}, mean ± SE, $n = 4$ per treatment, and SE represents variation within each treatment) over time (days) in the four treatments: P – non-UVR treatment and fed with *Rhodomonas salina*; PH – non-UVR treatment and fed with *R. salina* supplemented with MAA-producing *Heterocapsa triquetra*; U – UVR exposure and fed with *R. salina;* and UH) UVR exposure and fed with *R. salina* supplemented with MAA-producing *H. triquetra.*

reached adulthood. Egg production was relatively similar among treatments with only a slight interaction effect between food type and UVR (Fig. 4a; Table 2). The egg production initially increased to reach a maximum at day 25 in all treatments but then decreased as the animals became older (Fig. 4a). The egg hatching success was low in the UVR treatment when the animals were fed solely *R. salina* (i.e. U treatment) where only 30–50% of the eggs hatched. The effect of UVR radiation was removed when the animals were fed MAA-containing food, and the egg hatching success was similarly high, 70–90%, in UH, PH and P treatments (Fig. 5, Table 2). The similar egg production rates but lower egg hatching success lead to a lower nauplii production rate in the U treatment compared with all other treatments (i.e. number of produced nauplii per female; Fig. 4b), and there was also a significant food by time interaction. The integral result of the different treatments is expressed in the net reproductive rate, R_0,

Table 2. Statistical results for the survival (number of copepods L^{-1}, sampled 19 times), somatic growth (prosome length days 7–18, sampled four times), proportion of the population that are adults (% days 7–18, sampled twice), egg production rate (eggs $female^{-1}$ day^{-1}, sampled 12 times), egg hatching success (%, sampled three times) and nauplii production (nauplii $female^{-1}$ day^{-1}, sampled 12 times; d.f. = degrees of freedom, num = numerator, den = denominator, resid = residual). The factor 'UV' denotes UVR or non-UVR exposure, and the factor 'food' denotes either *Rhodomonas salina* or a mixture of *R. salina* and the MAA-producing *Heterocapsa triquetra*. Replicate was added as random factor for survival, somatic growth, egg production, egg hatching success and nauplii production (16 replicates in total). Bold font indicates significant results.

	F/z	d.f. (num)	d.f. (den, resid)	*P*
Survival				
UV	3·9	1	12	0·071
Food	6·5	1	12	**0·025**
Time	368·9	18	216	**< 0·001**
UV/food	2·5	1	12	0·143
UV/time	11·3	18	216	**< 0·001**
Food/time	1·1	18	216	0·333
UV/food/time	1·3	18	216	0·221
Somatic growth (days 7–18)				
UV	9·9	1	12	**0·008**
Food	35·5	1	12	**< 0·001**
Time	445·7	3	36	**< 0·001**
UV/food	5·4	1	12	**0·039**
UV/time	2·4	3	36	0·084
Food/time	1·0	3	36	0·408
UV/food/time	0·6	3	36	0·638
Proportion adults (days 7–18)				
UV	1·2	1	12	0·247
Food	1·7	1	12	0·084
Time	−5·1	1	12	**< 0·001**
UV/food	1·7	1	12	0·089
UV/time	0·2	1	12	0·873
Food/time	−1·1	1	12	0·285
UV/food/time	−0·3	1	12	0·796
Egg production				
UV	1·9	1	12	0·197
Food	19·2	1	12	**< 0·001**
Time	44·6	11	132	**< 0·001**
UV/food	5·0	1	12	**0·045**
UV/time	1·8	11	132	0·068
Food/time	1·8	11	132	0·059
UV/food/time	1·2	11	132	0·323
Egg hatching success				
UV	5·8	1	12	**< 0·001**
Food	−2·3	1	12	**0·021**
Time	−2·0	2	24	0·050
UV/food	−4·8	1	12	**< 0·001**
UV/time	−8·4	2	24	**< 0·001**
Food/time	1·5	2	24	0·141
UV/food/time	2·7	2	24	**0·006**
Nauplii production				
UV	10·4	1	12	**0·007**
Food	100·3	1	12	**< 0·001**
Time	42·8	11	132	**< 0·001**
UV/food	30·1	1	12	**< 0·001**
UV/time	1·7	11	132	0·084
Food/time	3·6	11	132	**< 0·001**
UV/food/time	2·2	11	132	**0·019**
R_0				
UV	3·2	1	12	0·097
Food	81·2	1	12	**< 0·001**
UV/food	36·3	1	12	**< 0·001**

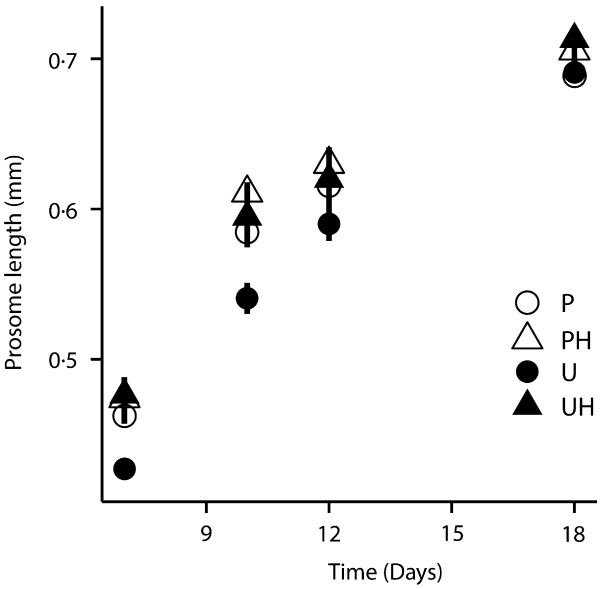

Fig. 3. Somatic growth measured as prosome length (mm; averaged from >20 measured individuals per replicate) during the copepodite stages from day 7 to 18 (mean ± SE; $n = 4$ per treatment, and SE represents variation within each treatment).

and UVR had a negative impact on R_0 when the animals were fed algae low in MAA (U vs. P treatments; Fig. 6; Table 2). MAA-containing food generally lead to higher R_0, and despite the potentially negative effects of UVR, the highest values were in the UH treatment where the copepods had the highest MAA content (Figs 1 and 6).

Discussion

Life-history theory predicts that organisms should allocate energy throughout their life such that they maximize their fitness (Stearns 1992). Fitness can be divided into several fitness components, for example, size at maturity, number and size of offspring and length of life. There are typically trade-offs between these components, so that investment in one affects the others. For example, increased investment in maintenance and repair may come at the cost of reduced growth or reproduction. In this study, we examined how copepods allocate resources into different fitness components in relation to exposure to detrimental UVR and with diets containing different amounts of UVR-screening substances. Several fitness components, including somatic growth, nauplii production (larvae) and egg quality, were negatively affected by UVR, whereas other components such as size at maturity, survival and longevity were not affected by UVR. Low nauplii production due to low egg quality was the most influential life-history parameter, and the effects on somatic growth were only present during the copepodite stages. The negative effects of UVR were efficiently countered by MAA-containing food (i.e. sunscreens), and fitness was even stimulated in the combined UVR and MAA treatments. Allocation to different fitness components is hence to some extent plastic and UVR

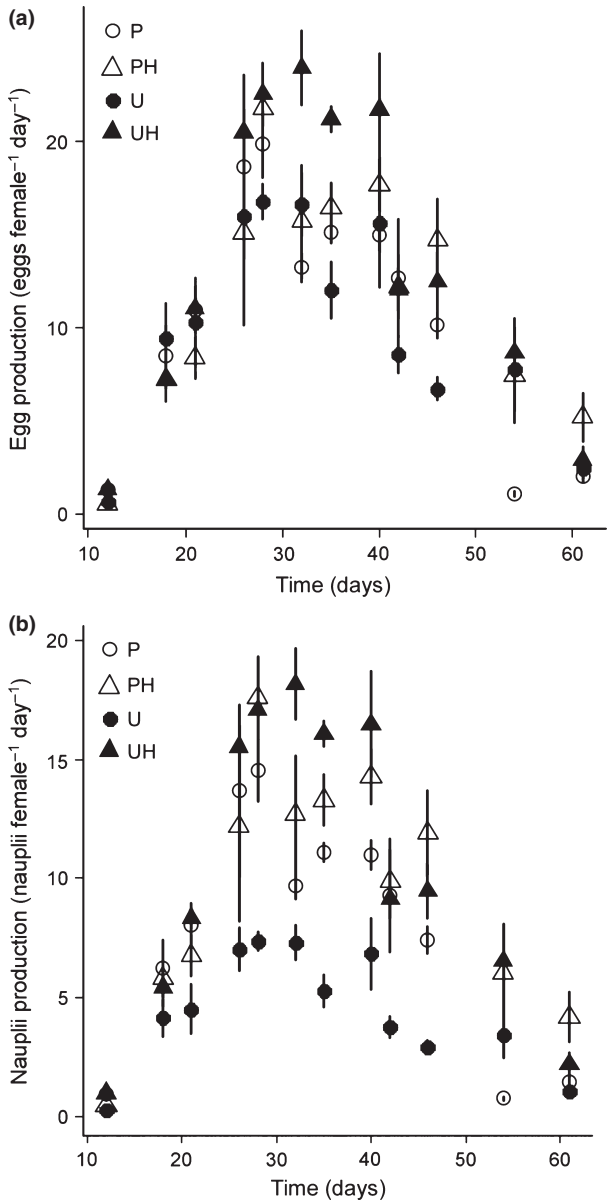

Fig. 4. Egg production rate per female (a) and nauplii production per female (b) over time (days). Treatments were as follows: P – non-UVR treatment and fed with *Rhodomonas salina*; PH – non-UVR treatment and fed with *R. salina* supplemented with MAA-producing *Heterocapsa triquetra*; U – UVR exposure and fed with *R. salina;* UH) UVR exposure and fed with *R. salina* supplemented with MAA-producing *H. triquetra* (mean ± SE, $n = 4$ per treatment, and SE represents variation within each treatment).

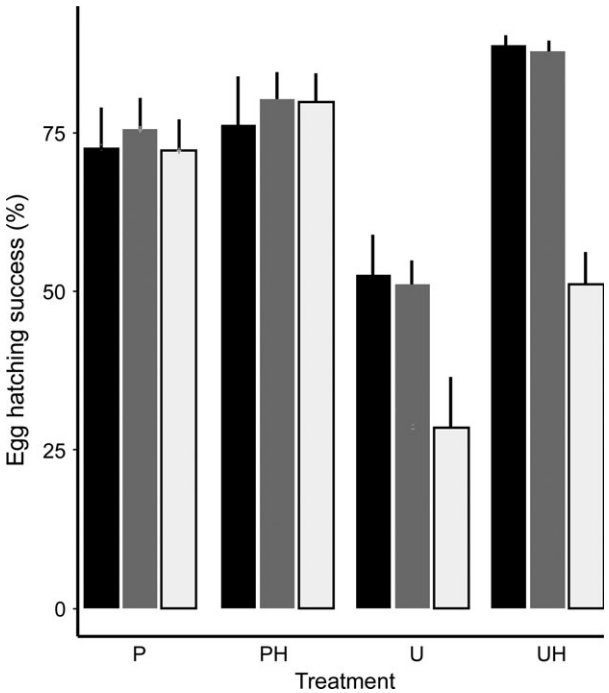

Fig. 5. Percent copepod eggs that hatched after 48 h of incubation (dim light, no UVR). Egg hatching success was measured three times at days 27, 35 and 42 (black, grey and white, respectively; mean ± SE). Eggs were from prior treatments of: P – non-UVR treatment and fed with *Rhodomonas salina*; PH – non-UVR treatment and fed with *R. salina* supplemented with MAA-producing *Heterocapsa triquetra*; U – UVR exposure and fed with *R. salina;* and UH – UVR exposure and fed with *R. salina* supplemented with MAA-producing *H. triquetra* ($n = 4$ per treatment, and SE represents variation within each treatment).

sunscreens such as MAAs enable copepods to maintain or even increase their fitness.

The direct detrimental effects of UVR are well documented, and several studies have shown that zooplankton experience DNA damage and ultimately increased mortality when exposed to radiation (Malloy *et al.* 1997; Helbling & Zagarese 2003). Little is, however, known about how sublethal doses of UVR affect fitness (Dahms, Dobretsov & Lee 2011). UVR can furthermore exert stronger effects on juvenile life stages of zooplankton compared to adults (Leech & Williamson 2000; Vega & Pizarro 2000), and the retarded growth we observed in this study indicates that the allocation of energy for growth is reduced by the UVR exposure. This retarded growth is likely the price that the organism has to pay to be able to maintain UVR protection including induction of cellular antioxidants (Borgeraas & Hessen 2002; Hylander *et al.* 2012) and cell repair systems (Sancar 1994; MacFadyen *et al.* 2004). The slow somatic growth we observed in the U treatment did, however, not lead to lower size at maturity, and when copepods eventually became mature, they had obtained the same size in all treatments.

Egg production rates were relatively similar among treatments with only a slight interaction between UV and food, but overall fitness was negatively affected by UVR observed by reduced hatching success of the eggs and hence low nauplii production (i.e. without UVR sunscreens in the food source). These results suggest that the egg production machinery is mainly affected by UVR, not in terms of the rate at which eggs are produced, but in terms of their quality. Browman *et al.* (2000) likewise observed low egg hatching success of copepod eggs when incubated in surface waters. Modelling efforts suggest that UVR-induced mortality of eggs and early life stages of surface-spawning copepods in subarctic marine systems can be as

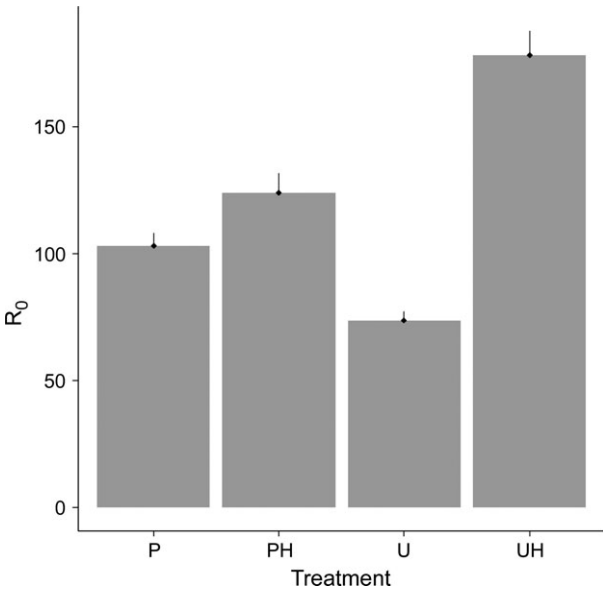

Fig. 6. The net reproductive rate (R_0), which is the average number of offspring that an individual delivers to the next generation (mean ± SE). Treatments were as follows: P – non-UVR treatment and fed with *Rhodomonas salina*; PH – non-UVR treatment and fed with *R. salina* supplemented with MAA-producing *Heterocapsa triquetra*; U – UVR exposure and fed with *R. salina;* and UH – UVR exposure and fed with *R. salina* supplemented with MAA-producing *H. triquetra* ($n = 4$ per treatment, and SE represents variation within each treatment).

high as 30%, but is usually lower and mortality rates are highly variable mainly due to cloud cover, mixing and water transparency and, to a lesser extent, ozone reductions (Browman *et al.* 2000; Kuhn *et al.* 2000).

UVR may affect the egg directly by damaging DNA or other cellular components. For example, dosimeters incubated in surface waters show significantly more DNA damage when exposed to UVR compared with controls (Cooke, Williamson & Saros 2006). In contrast to other studies that have demonstrated low egg hatching success of eggs exposed to UVR, our study shows that UVR exposure of the female is enough to induce low egg quality (the eggs in our study were not exposed to UVR after they were spawned). Similarly, Cooke, Williamson & Saros (2006) showed that nauplii production was reduced under UVR exposure but the exposure was constant throughout development (both female, egg and nauplii). Egg viability in general is variable for field populations and may range from 0 to 100% within some common copepod genera (reviewed in Ianora 1998). Apart from cannibalism and the need for mating possibilities, it has been shown that egg viability can decrease due to a nutritionally inadequate diet, for example, lack of essential fatty acids or diets containing harmful substances (Jonasdottir & Kiørboe 1996; Ianora 1998). Maternal UVR exposure can now be added to these factors affecting egg hatching success.

Sunscreens in the phytoplankton (MAAs) that were provided as food source for the copepods enabled them to maintain or even increase their fitness. The lack of MAAs in the copepods in both P and U treatments suggests that *R. salina* does not contain any MAAs and that the copepod cannot produce MAAs *de novo*. MAAs are common in Dinophyta (like *H. triquetra*) but they also occur to some extent in other groups, for example, Bacillariophyta, Cyanophyta and Haptophyta (Liu, Hader & Sommaruga 2004; Llewellyn & Airs 2010). Chlorophyta and Cryptophyta (e.g. *Rhodomonas* spp.) generally contain low or nondetectable amounts of MAAs (Llewellyn & Airs 2010). UVR-exposed copepods receiving MAAs in their food source had high egg and nauplii production (UH treatment). They were also able to maintain high egg quality, at least in the beginning of the experiment. Late in the experiment (day 42), copepods in the UH treatment also showed reduced egg hatching success, suggesting that the protection from MAAs is not complete.

Mycosporine-like amino acids (MAAs) are a group of substances that absorb in the UVR wavelength range (Sinha, Singh & Hader 2007) and are thought to screen the radiation and thus protecting zooplankton from radiation damage (Moeller *et al.* 2005). Interestingly, fitness was not only maintained but even elevated in the combined UVR and high MAA treatment (UH). MAA-producing *H. triquetra* were added to the experiment on a daily basis, and this species has been shown to increase its MAA production by more than 100% within 2 days of UVR exposure (Hylander & Jephson 2010). MAAs are rich in nitrogen and have been proposed to be a potential molecule for nitrogen storage (Oren & Gunde-Cimerman 2007), and we suggest that this additional nitrogen relieved the copepods from nitrogen limitation in the UH treatment. This also illustrates that potential costs for accumulating MAAs were of minor importance and that UVR exposure can be beneficial in some contexts. Cooke & Williamson (2006) also observed positive UVR effects in a freshwater copepod species, suggesting that indirect stimulation of phytoplankton and bacteria may have been responsible for this effect.

Carotenoids have also been shown to be accumulated by copepods (Hairston 1979a; Hansson 2000; Hylander *et al.* 2009), and pigmented individuals have a higher tolerance against UVR exposure (Ringelberg, Keyser & Flik 1984). Vividly coloured zooplankton are, however, easily detected by visual predators (mainly fish) (Zaret 1972), and pigmented specimens tend to inhabit lakes with low predation pressures (Hairston 1979a,b; Luecke & Obrien 1981; Hansson 2000). In contrast to freshwater copepods (Hansson, Hylander & Sommaruga 2007; Hylander *et al.* 2009), the studied species (*A. tonsa*) did not accumulate carotenoids upon UVR exposure. Recent literature reviews indicate that UVR penetration in marine systems ranges from close to zero up to more than 40 m (10% remaining at 340 nm; Tedetti & Sempere 2006) but UVR penetration is generally low in the neritic water where *A. tonsa* occur. The lack of carotenoid accumulation in the studied copepod species could hence be an adaptation to low UVR exposure and high predation pressure in its habitat. However, marine copepods in general have much lower – by an order of

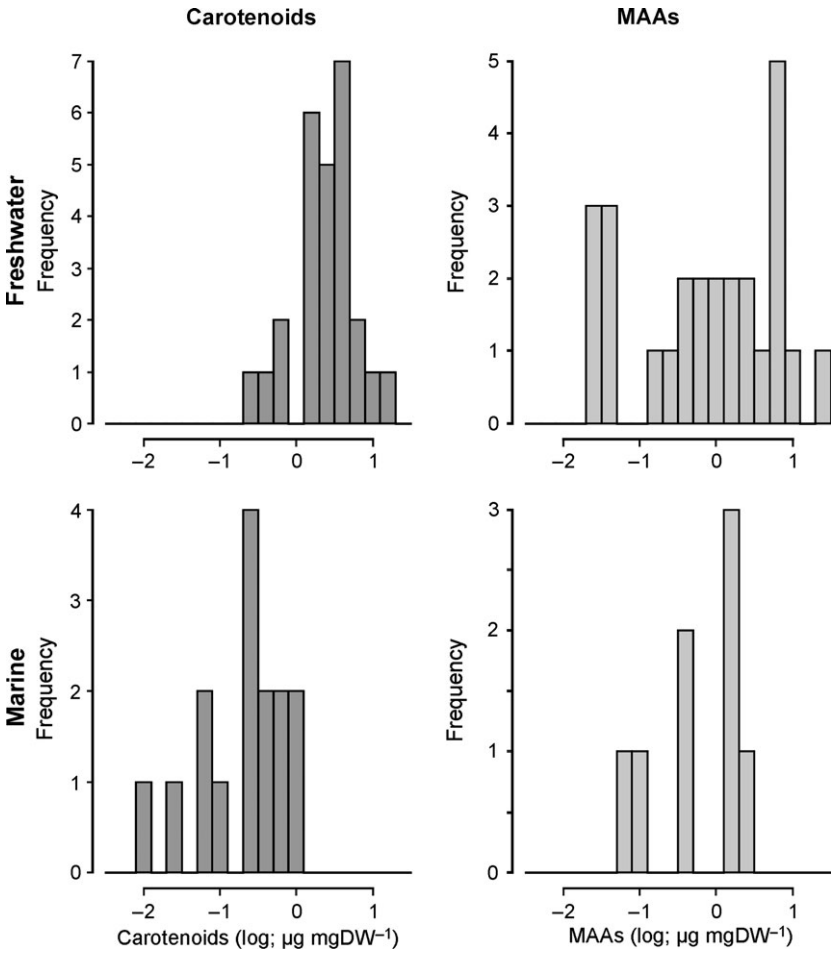

Fig. 7. Concentrations of carotenoids (astaxanthin and its esters) and MAAs in calanoid copepods in marine and freshwater systems (n = 26, 26, 15 and 8 for freshwater carotenoids, freshwater MAAs, marine carotenoids and marine MAAs, respectively). For included studies, see methods. Concentrations of carotenoids were higher in freshwaters than those in marine systems (t = 7·1; P < 0·001; d.f. = 39). There were no differences in MAAs between systems (t = 0·3; P = 0·78; d.f. = 32).

magnitude – levels of carotenoids than freshwater species, while levels of MAAs are similar between the two habitats (Fig. 7). Carotenoid pigmentation among freshwater copepods has generally been studied in rather transparent high-altitude and high-latitude lakes where pigmentation is known to be high (Hansson & Hylander 2009; Rautio & Tartarotti 2010). Lakes can also be devoid of fish but such a situation is nowhere to be found in the ocean and copepods in marine systems never display as high pigmentation as in lakes (Fig. 7), even though marine samples include copepods from several different sites in subtropical, temperate and polar seas. UVR attenuation depths are highly variable across both freshwater and marine systems (Williamson *et al.* 2011) but UVR transparency data are not available for a majority of the studies included in Fig. 7, and potential differences among systems in UVR attenuation and the consequent effects on copepod pigmentation are therefore not possible to assess from these data. However, when comparing lakes and marine systems (both with fish) at temperate latitudes and with comparable UVR transparencies (i.e. 0·1–0·2 m 1% at 320 nm), carotenoid levels are approximately six times lower in temperate marine copepods than those in copepods from lakes (freshwaters: n = 12, marine: n = 10; Hylander *et al.* 2009 and Fig. 7). Predation in the ocean is intense, and mortality rates of zooplankton are in the order of 10% per day (Kiørboe 2008; Hirst *et al.* 2010). The numerous measurements of low pigmentation in marine copepods may suggest that predation pressure is generally higher in the ocean than that in lakes, but we have been unable to identify data to test this idea. To disentangle the pigmentation differences between freshwater and marine copepods would require a more comprehensive data set with both UVR exposure and predation pressures at all sites. MAAs are nonpigmented and may provide UVR protection without increasing the predation risk (Hylander *et al.* 2009; S. Hylander, unpublished data). Low carotenoid levels and the ability to accumulate MAAs could hence be an important adaptation for surface-dwelling marine zooplankton when challenged with UVR exposure.

We conclude that copepods respond to UVR by changing the resource allocation to different fitness components resulting in reduced somatic growth, egg quality and naupliii production. Availability of sunscreens in the food source (MAAs), however, enables copepods to maintain high or even an elevated fitness when exposed to UVR. This illustrates that fitness costs of UVR are diet dependent and may be considerable if UVR sunscreens are not available in the food source. Any potential costs associated with accumulation of MAAs were not detected, and UVR exposure can actually be beneficial when combined with MAA-rich diet. Concentrations of other kinds of UVR-

protective compounds, such as carotenoids, are generally lower in marine than those in freshwater copepods, which suggest that predation is intense on pigmented specimens in the ocean. Accumulation of MAAs can hence be a key adaptation for marine copepods to cope with UVR stress in an environment with intense visual predation although the comparison between systems should be done with caution since data on differential UVR transparency and predation pressures are lacking. Zooplankton employ a variety of UVR defence mechanisms to avoid damage including cell repair systems and induction of antioxidant enzymes (for reviews, see Hansson & Hylander 2009; Rautio & Tartarotti 2010). We show that the cost to this is lowered fitness when UVR sunscreens are not available, and important life-history parameters and ultimately fitness are hence altered by environmental variability. However, the UVR cost can be avoided if sunscreens are available in the food source and these compounds even stimulate an elevated fitness. This suggests that the traditional model assuming costs associated with induced defences is not generally valid and that the induced accumulation of sunscreens even may lead to a beneficial effect of UVR exposure in zooplankton.

Acknowledgements

The study was funded by the Royal Swedish Academy of Sciences, the H.C. Ørsted postdoc programme of the Technical University of Denmark and by a grant from the Carlsberg Foundation.

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Received 15 November 2012; accepted 5 July 2013
Handling Editor: Michael Pfrender