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UV radiation changes algal stoichiometry but does not have cascading effects on a marine food chain

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Ultraviolet (UV)-B levels are still increasing at high and polar latitudes where ozone depletion continues. Changes in the quality of algae at the base of the food web can have a cascading effect on higher trophic levels. We examined whether UV radiation altered the synthesis of fatty acids (FA) and mycosporine-like amino acids (MAAs), as well as the C:N elemental stoichiometry of marine primary producers and whether these modifications transferred through a simple food chain (copepod, fish larvae). Two diatoms (*Thalassiosira weissflogii* and *Thalassiosira pseudonana*) and one flagellate (*Dunaliella tertiolecta*) were exposed to three different UV exposure treatments. Copepod nauplii were then fed with *Thalassiosira pseudonana* for 3 days. Fish larvae were, in turn, fed with these nauplii. C:N ratios and total lipid decreased with increasing UV exposure in the diatoms, but did not vary in copepods or fish. PAR-only-treated algae had more saturated FAs, myristic (14:0) and palmitic acid (16:0), while UV-treated algae had more long-chain polyunsaturated FAs. These changes did not transfer to copepods or fish larvae. No MAAs were found in either algae, copepods or fish. Our results suggest that copepods are able to compensate for lower food quality by increasing food intake.

KEYWORDS: copepod; algae; climate change, ozone reduction; fatty acid; MAA; diatom; fish larvae

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INTRODUCTION

The diatom → copepod → fish food chain is the key driver of ocean productivity. Changes in quantity and quality at the base of the food chain can have a cascading effect on food web trophodynamics including decreased food availability for marine mammals and the recruitment of commercially important fish stocks and other economically valuable species. Particularly pervasive problems can arise when essential metabolic compounds, synthesized by primary producers, decrease as a result of environmental change.

Global warming, caused by increasing greenhouse gas concentrations in the atmosphere, is contributing to stratospheric ozone loss, especially at the poles, and delaying the recovery of the ozone layer (McKenzie *et al.*, 2011). Reduction in stratospheric ozone is linked to increases in ultraviolet-B (UVB) radiation (=280–315 nm; e.g. Madronich *et al.*, 1998) and UVB levels are still increasing substantially at high and polar latitudes where ozone reduction has been greater than at mid-latitudes (WMO, 2010; McKenzie *et al.*, 2011).

High-resolution UVB spectral measurements available for a small number of systems have shown that the depth to which UVB penetrates in water depends on the optical properties of the water mass (Arts *et al.*, 2000; Kjeldstad *et al.*, 2003).

It is now well established that UVB penetrates to greater depths than had previously been accepted (Smith and Baker, 1981; Kuhn *et al.*, 1999; Croteau *et al.*, 2008). During the Norwegian spring and summer, significant levels of UVB are present as early as 05:00 h, and as late as 22:30 h (Browman, unpublished results). When superimposed upon ozone reduction-related increases in UVB, such extended daily exposures to sunlight represent an increase in the overall dose rate and hence susceptibility of aquatic organisms to UVB-induced damage.

A rapidly growing number of studies indicate that UVB, even at current levels, is harmful to aquatic organisms and may reduce the productivity of marine ecosystems (Bancroft *et al.*, 2007; Llabres *et al.*, 2013). Direct effects of UVB on phytoplankton, heterotrophic microzooplankton and larger zooplankton are well known (Karentz, 1994; Williamson *et al.*, 1994; Zellmer, 1998; Browman *et al.*, 2000; Grad *et al.*, 2001; Williamson *et al.*, 2001; Zagarese and Williamson, 2001; Kouwenberg and Lantoine, 2007; Leu *et al.*, 2007). In phytoplankton, UV radiation (UVR) has deleterious effects on cell morphology, growth rates and several biochemical pathways, including those involved in photosynthesis (Zheng, 2013), the production of essential amino acids (Goes *et al*, 1995), the synthesis of specific fatty acids (FAs) (Goes *et al.*, 1994), the production of mycosporine-like amino acids (MAAs) and nitrogen dynamics within the cells (Dohler, 1985; Dohler and Biermann, 1987; Hessen *et al.*, 1997; Skerratt *et al.*, 1998; Liang *et al.*, 2006). Direct effects of UVB on the early-life stages of marine zooplankton and marine fishes have also been documented (Kouwenberg *et al.*, 1999a,b; Browman *et al.*, 2003; Jokinen *et al.*, 2008; Hickford and Schiel, 2011).

Laboratory observations suggest that UV-induced mortality in *Calanus finmarchicus* and cod eggs is a direct result of DNA damage (Browman *et al.*, 2000; Browman and Vetter, 2002). However, direct effects are only part of the potential impact of UVR on trophic levels. Changes in the quantity and quality of many biologically important molecules such as vitamins, amino acids and FA in phytoplankton can have complex, cascading impacts on higher trophic levels (Fraser *et al.*, 1989; Graeve *et al.*, 1994; Arts *et al.*, 2009). The few studies that have investigated indirect effects illustrate how UVB-induced changes in food chain interactions can be far more significant than direct effects on individual organisms at any single trophic level (e.g. Bothwell *et al.*, 1994; Hessen *et al.*, 1997; Williamson *et al.*, 1999). For zooplankton and fish larvae, compounds such as the essential fatty acids (EFAs; Parrish, 2009) and UVR absorbing compounds that are associated with food quality are acquired by consumers through the diet (e.g. Goulden and Place, 1990; Rainuzzo *et al.*,1997; Reitan *et al.*, 1997; Sargent *et al*, 1997; Arts *et al.*, 2009). Thus, the quantity and quality of these essential molecules produced by the primary producers are a critical foundation for aquatic food webs and deficiencies can be manifested in complex ways.

In response to UV stress, microorganisms such as, fungi, bacteria, cyanobacteria, phytoplankton and macro-algae synthesize sunscreen compounds, called MAAs (Karentz *et al.*, 1991; Sommaruga and Garcia-Pichel, 1999; Shick and Dunlap, 2002; Sinha and Hader, 2002; Oren and Gunde-Cimerman, 2007). Invertebrate animals acquire MAA through their diet (Karentz *et al.*, 1991; Newman *et al.*, 2000; Helbling *et al.*, 2002; Moeller *et al.*, 2005; Hylander and Jephson, 2010). Thus, MAAs may provide protection for multiple levels within the food chain.

In this study, we examined the direct effects of UV exposure on the FA production and composition, C:N ratio and MAA production of three different species of algae. Then, we examined how the effects transferred through a simple food chain by feeding the algae to copepod nauplii, which were, in turn, fed to fish larvae to track the transfer of EFA and MAA. We hypothesized that (i) the nutritional quality of three different species of algae would be negatively affected by exposure to elevated UV versus PAR only or moderate levels of UV, (ii) and that these changes in lipid profile, C:N ratio and MAA production would transfer up the food chain, first to copepod nauplii grazing on the algae, then to cod larvae consuming these copepods.

METHOD

Study species and experimental design

We selected species that are important components of many temperate marine environments, including the North Atlantic. In the first experiment, two species of algae; a green flagellate *Dunaliella tertiolecta* and a diatom (*Thalassiosira weissflogii*) were raised under three levels of UVR. The second experiment involved a food cascade with the diatom (*Thalassiosira pseudonana*) which was raised under three levels of UVR and then fed to unexposed copepod nauplii (*Calanus finmarchicus* or *Calanus helgolandicus*) for 3 days. These copepods were then used as a food source for unexposed fish larvae (*Gadus morhua*) to track the FA and MAA signatures through the different trophic levels. Copepod nauplii constitute suitable food items for fish larvae at 19 days posthatch (e.g. Karlsen *et al.*, 2015). *Thalassiosira pseudonana* was chosen because production of MAAs has been reported in this diatom (Helbling *et al.*, 1996) and it is also eaten by naupliar stage copepods (Lonsdale *et al.*, 1996). All experiments were conducted at the Institute for Marine Research (IMR), Austevoll Research Station.

Spectral treatments

We used three spectral exposure treatments (hereafter referred to as PAR, UV and UV+): UV depleted (photosynthetically active radiation: PAR only), ambient UV and PAR and enhanced UV and PAR (UV+) produced by using, respectively, (i) four GE lamps (General Electric Polylux X2 F36W/830), (ii) four GE and one UV panel lamps (Q Lab UVA-340) and (iii) four GE and two Q-panel lamps. All lamps were aged for 100 h before the experiment began to ensure that they had reached stable spectral output.

Spectral irradiance was measured using an OL-754-O-PMT (Gooch and Housego, Orlando, FL, USA) spectroradiometer. The integrating sphere (100 mm diameter) was placed inside the water-filled culture bags (see below). Measurements were also taken in the air with the sphere placed outside of the bags to obtain transmission values through the bags. In both sets of measurements, the outer edge of the sphere was positioned 14 cm from the lamps. Irradiance values for measurements taken inside the culture bags were corrected using an immersion correction factor (ICF) for each wavelength to account for changes in optical properties of the water. The ICFs used here are those derived for this probe by the manufacturer (Petzold and Austin, 1988).

Irradiance spectra are presented in Fig. 1 and daily irradiances in the UVB (280–320), UVA (320–400 nm) and PAR (400–700 nm) per treatment are in Table I. Ambient radiation data, collected by the Norwegian UV monitoring network, were obtained from the Norwegian Radiation Protection Authority (NRPA). NRPA uses a multi-channel radiometer (305, 313, 320, 340 and 380 nm; GUV-541, Biospherical Instruments, San Diego, CA, USA) situated in Bergen (60°22′43″N, 5°20′33″E, University of Bergen), 22 km north of Austevoll. Average daily UVB irradiance measured in Bergen between 1 June and 31 July in 2008 and 2009 was ~40 kJ m^{-2}.

Algal cultures

Dunaliella tertiolecta (National Center for Marine Algae and Microbiota (NCMA: CCMP 364), *T. weissflogii* (NCMA1052) and *T. pseudonana* (CCMP 1013) were cultured on a 15:9 light:dark photoperiod in autoclaved, 0.2 μm filtered seawater enriched with sterilized F/2 medium (Guillard). Algae were reared at 19°C (± 1.5°C) in 10 L PFA Teflon® lay flat bags (thickness of the plastic wall 2 mm) from Welch Fluorcarbon in Dover, NH, USA. To determine the maximum abundance for each cell line under our specific growth conditions, cultures were grown until they reached the stationary phase (after 7 days). Once the maximum abundance was identified, cultures were maintained in exponential growth phase using semi-continuous batch cultures to keep cell counts at 40–70% maximum carrying capacity of the treatment. Algal cells were enumerated using a Beckman Z2 Coulter counter.

The diatom *T. weissflogii* was maintained in exponential growth for a total of 8 days and was sampled for FA and MAA and C:N analyses after 4 and 8 days of exposure, whereas *D. tertiolecta* was maintained in exponential growth for 20 days before it was sampled for FA and MAA to determine the effects of long-term UV exposure. *Thalassiosira pseudonana* was cultured for 12 and 17 days to assess changes in FA, MAA and C:N under UVR exposures over a longer period of time. This alga was collected after 12 and 17 days of exposure and used as food for *Calanus* nauplii.

Copepod and fish larvae rearing

The transfer of algal FA and MAA through a simple food chain was examined using *T. pseudonana*, *Calanus* spp. nauplii and larval Atlantic cod (*G. morhua*). Zooplankton were collected from the fjord outside the IMR station. Water from a 2 m depth was passed through a series of nested nets with mesh sizes of 1000, 500 and 100 μm. Nauplii between 100 and 500 μm were sorted in the laboratory and placed in 25 L tanks (five replicate tanks per treatment level staggered in time), maintained in the dark, over a period of 6 days (one tank per day) at 18°C.

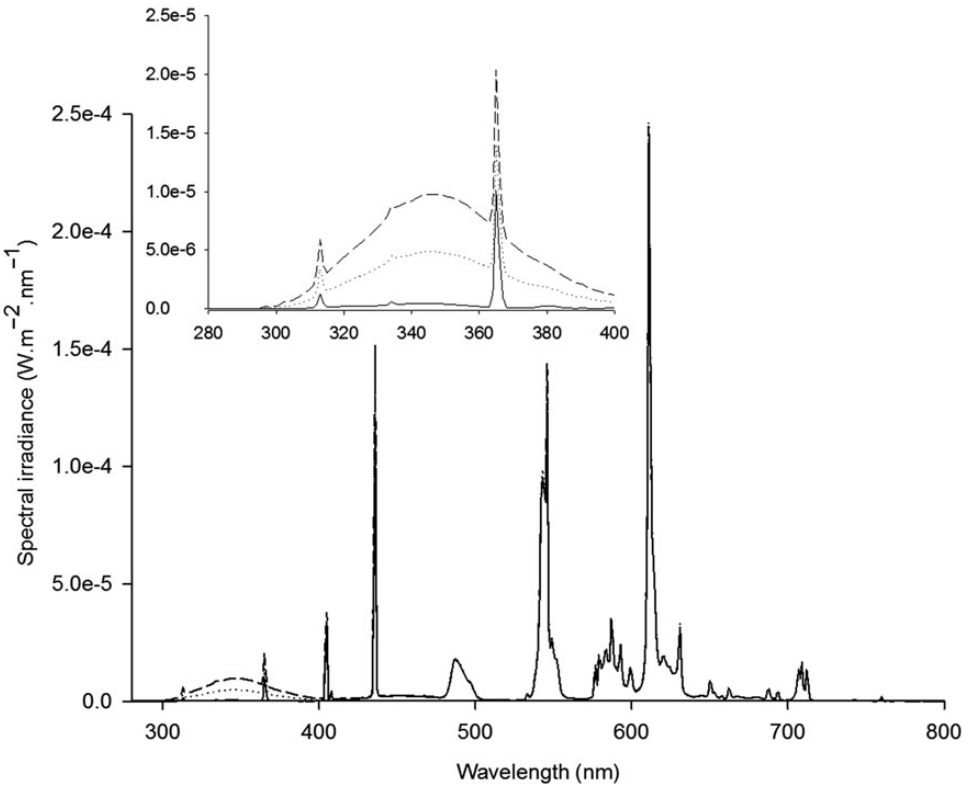

Fig. 1. Spectral characteristics of the light exposure treatments that were applied on three different types of algae. Only the amount of UV changed between treatments. Solid line: PAR only; dotted line: PAR + ambient UV; dashed line: PAR + enhanced UV.

Nauplii concentrations within the tanks ranged from 1 to 6 animals mL^{-1}. Treatment-exposed algae was provided as food for nauplii at concentrations of $4–5 \times 10^4$ cells mL^{-1}. Abundance of algae and nauplii were monitored daily. After 3 days of feeding, nauplii were harvested as a food source for the larval fish. At the end of this period, the copepods were still in the naupliar stage. Subsamples of nauplii were allowed to evacuate their guts for 3 h in filtered seawater (at the same temperature), after which they were collected for subsequent FA, MAA and CHN analyses. Since the various copepod replicate tanks were not set up on the same day, the nauplii were fed algae that received different UVR exposure durations (between 6 and 17 days).

Larval cod, 19 days posthatch (mean standard length was 7 mm), were placed in 25 L tanks (six replicate tanks per treatment level). Fish were fed with nauplii once a day for 5 days. Nauplii and fish were provided with treatment-exposed algae in these tanks as well at concentrations of 4×10^4 cells mL^{-1} (to provide food for the nauplii). Within the fish tanks, naupliar concentrations ranged from <1 to 2 animals mL^{-1} over the 5 days. Concentrations of nauplii and algae were monitored daily. Fish tanks were kept in low light, but not complete darkness. Prior to sampling for FA, MAA and CHN analysis, fish were given 6 h to clear their guts.

FA analysis

Live algae were filtered on silver membrane filters to collect enough biomass for analysis. The pellet was scraped off with a solvent-washed spatula, placed into a cryovial and frozen at $-80°$C. For *T. weissflogii*, 5 L of the culture (from the Teflon bags) was filtered at concentrations between 8×10^4 and 1.5×10^5 cells mL^{-1}. For *T. pseudonana*, between 200 mL and 1 L were filtered at concentrations between 7×10^3 and 4×10^4 cells mL^{-1}.

Copepod nauplii were collected from their holding tanks and placed in filtered seawater to clear their guts for 3 h. They were then filtered on to 53 μm Nitex screens, rinsed with distilled water and freeze-dried. Each replicate contained between 100 and 400 nauplii.

After 5 days of feeding on nauplii prey, fish larvae were collected and placed in filtered seawater for 3 h to clear their guts. Larvae were then collected on a 150 μm Nitex screen, rinsed with distilled water and freeze-dried. Each replicate contained 60 individuals. All samples were kept at $-80°$C until further analyses.

Table I: Spectral irradiances of the light exposure treatments that were applied on three different types of algae

Treatment	Waveband UVB (280–320 nm)	Waveband UVA (320–400 nm)	PAR (400–800 nm)
Spectral irradiance (W m^{-2})			
PAR			
in air	0.1	0.92	74
in bag	0.07	0.64	53
UV			
in air	0.59	6.2	76
in bag	0.43	4.7	57
UV+			
in air	1.30	13.7	82
in bag	0.87	9.2	56
Outside daylight (12:00 GMT)			
Full sunlight (in air)	1	32.8	423
Shaded (in air)	0.46	11	79
Total irradiance (kJ m^{-2}) for 15.5 h of exposure			
PAR			
in air	6	51	4108
in bag	4	36	2974
UV			
in air	33	347	4250
in bag	24	260	3156
UV+			
in air	72	762	4567
in bag	48	512	3113
June–July average in Bergen area			
2008	40	658	Not available
2009	41	684	

Only the amount of UV changed between treatments.
PAR, UV depleted (photosynthetically active radiation only); UV, UV and PAR; UV+, enhanced UV and PAR.

Prior to lipid extraction and fatty acid methyl ester (FAME) generation, algal samples were freeze-dried for ~48 h. Each sample was then weighed to the nearest microgram (Sartorius ME5 microbalance), and homogenized to extract (3×) the lipids in 2 mL of 2:1 (v/v) chloroform: methanol (Folch *et al.*, 1957). The resulting supernatants (after centrifugation to remove nonlipid containing material) were combined in a 15 mL centrifuge tube. The lipid extract was then accurately adjusted to 8 mL with 2:1 (v/v) chloroform:methanol, and 1.6 mL of a 0.9% NaCl in water solution was added. The two phases were then thoroughly mixed using a Vortex mixer and centrifuged (2000 rpm at 4°C). The upper aqueous layer was removed and discarded along with any other nonlipids leaving the solvent layer to be evaporated under nitrogen gas. The lipids were re-dissolved in 2 mL of 2:1 chloroform:methanol and, from this volume, duplicate 100 mL aliquots of sample extracts were added to pre-weighed, seamless, tin cups (Elemental Microanalysis Ltd. catalogue No. D4057). The solvent in the tin cups was then evaporated at room temperature and the remaining lipid weighed on a Sartorius ME-5 microbalance to provide a gravimetric measure of percent total lipid (on a dry weight tissue basis). Just prior to derivatizing the FAs, the remaining 1.8 mL of the lipid extract was evaporated to dryness under nitrogen. Sulfuric acid in methanol (1:99 mixture used as methylation reagent) and toluene were then added to the vials, the headspace flushed with nitrogen, the vial vortexed and then incubated (16 h) at 50°C in a water bath. After the samples cooled, potassium hydrogen carbonate, isohexane:-diethyl ether (1:1, v/v) and butylated hydroxy toluene (0.01%, w/v) were added, and the vials were vortexed and centrifuged. The upper organic layer was transferred to another centrifuge tube; isohexane:diethyl ether (1:1, v/v) was added to the original tube which was then shaken, vortexed and centrifuged. All FAME containing layers were pooled and evaporated to dryness under nitrogen. Prior to analyses, the FAMEs were dissolved in hexane and transferred to amber glass GC vials. FAMEs were separated using a Hewlett Packard 6890 GC with the following configuration: splitless injection; column = Supelco (SP-2560 column) 100 m × 0.25 mm ID × 0.20 μm thick film; oven = 140°C (hold for 5 min) to 240°C at 4°C min^{-1}, hold for 12 min; carrier gas = helium, 1.2 mL min^{-1}; detector = FID at 260°C; injector = 260°C; total run time = 42 min per sample. A 37-component FA standard (Supelco 47885-U) was used to identify FA in the samples by comparing their retention times to those of the FA standard. Quantification of individual FA components was calculated on the basis of known amounts of injected standard dilutions (1.25, 250, 500, 1000, and 2000 ng mL^{-1}) of the 37-component FA mix. In the present study, ∑SFA is used to indicate the sum of all FAs with zero double bonds,

$\sum$MUFA indicates the sum of all FAs with one double bond and $\sum$PUFA indicates the sum of all FAs with ≥ 2 double bonds.

MAA analysis

Algae, copepod nauplii and cod larvae were analyzed for MAA. Eppendorf 1.5 mL tubes were filled with algae at 70–100% maximum volume and then centrifuged 10 min at 13 200 rpm. The supernatant was removed and the pellet was placed in the freezer (−80°C). Copepod nauplii were allowed to clear their gut for 3 h, then handpicked and placed into 1.5 mL Eppendorf vials (with at least 70 nauplii) and frozen (−80°C). Cod larvae were allowed to clear their gut for 6 h, then handpicked and placed into 1.5 mL Eppendorf vials (with at least 60 larvae per replicate) and frozen (−80°C).

Extraction for MAA analysis was done following the protocol described by Tartarotti and Sommaruga (Tartarotti and Sommaruga, 2002) using HPLC-grade methanol (MeOH:Milli-Q water, 25:75, v/v). Briefly, samples were sonicated on ice at the beginning of the extraction with a tip sonicator for 1 min and 25% amplitude (UP 200S, Dr Hielscher). Then, samples were extracted in a water bath at 45°C for 2 h and stored frozen at −80°C for further analysis. After thawing the extracts, samples were centrifuged for 20 min at 16 000 *g* and 60–100 μL of the supernatant was injected into a Dionex HPLC system containing a Phenoshere 5 μm pore size C8 column (250 × 4.6 mm, Phenomenex) protected by a Guard column (Security Guard, Phenomenex) and analyzed by isocratic reverse-phase HPLC. The autosampler and column oven temperature was set to 15 and 20°C, respectively. The flow rate of the mobile phase (0.1% acetic acid in 25% aqueous MeOH, v/v) was 0.7 mL min^{-1} and the chromatogram time was set to 20 min. Spectral absorbance was measured with a diode array detector (Dionex UVD340S). Reference MAA extracted from *Porphyra* sp. was included regularly in each analysis.

C:N analysis

The nutritional quality of the organisms in the cascade was also determined by CHN analyses. One milliliter samples of algal cultures (*T. weissflogii* and *T. pseudonana*) were centrifuged as described above. The resulting pellet was placed directly into a pre-weighed tin capsule, freeze-dried and frozen at −80°C. Copepod nauplii and fish larvae were placed in filtered seawater and allowed to clear their gut (respectively 3 and 5 h). Animals were individually picked and placed in tin capsules then frozen at −80°C. Samples were analyzed for carbon and nitrogen content with a Finnigan Flash EA 1112 analyzer (Thermo Fisher Scientific) by dynamic flash combustion. The instrument was run with the configuration for CN soils analysis and it was calibrated with a four-point curve based on acetanilide.

Data analyses

The differences in the distribution of algal cell diameters between the different treatments were tested using two-sample Kolmogorov–Smirnov tests. FAs were described by the standard nomenclature of carbon chain length:number of double bonds and location ($n-x$) of the double bond nearest the terminal methyl group (Table II). Individual FAs were expressed as a percentage of total FA. We only used FA that represented >2.5% of the total mass of FA. We carried out principal component analysis (PCA) on FA proportions to look for general trends in the FA signature of the different algal species, exposed to different UV treatments. Statistical differences in the above-mentioned variables as well as total lipids (%), cell diameters and C:N ratios were tested using two-way ANOVA, followed by a Holm–Sidak multiple comparison test (with days of exposure and treatment as factors). Kruskal–Wallis tests were applied separately (for both factors) when normality assumptions and/or homogeneity of variance failed.

RESULTS

Algal cell size

The effect of UVR on cell diameter was species specific. There was no difference in the distribution of the cell

Table II: Characteristics of the FAs that were present (<2.5% of the total FA) in the algal species exposed to different PAR and UV treatments

Common name	Formula	Abbreviations
Myristic acid	14:0	MA
Palmitic acid	16:0	PAM
Palmitoleic acid	16:1n−7	PO
Stearic acid	18:0	STA
Oleic acid	18:1n−9	OLE
Linoleic acid	18:2n−6	LNA
α-Linolenic acid	18:3n−3	ALA
γ-Linolenic acid	18:3n−6	GLA
Stearidonic acid	18:4n−3	SDA
Eicosenoic acid	20:1n−9	EA
Eicosatrienoic acid	20:3n−3	ETA
Arachidonic acid	20:4n−6	ARA
Eicosapentaenoic acid	20:5n−3	EPA
Omega-3 docosapentaenoic acid	22:5n−3	n−3 DPA
Omega-6 docosapentaenoic acid	22:5n−6	n−6 DPA
Docosahexaenoic acid	22:6n−3	DHA
Nervonic acid	24:1n−9	NER

diameters ($\varnothing$) of *T. weissflogii* as a function of UVR (mean + SD: $\varnothing_{PAR} = 12.34 \pm 0.20$ μm; $\varnothing_{UV} = 12.60 \pm 0.09$ μm; $\varnothing_{UV+} = 12.36 \pm 0.15$ μm; K–S test, $P > 0.05$). However, UV treatment significantly increased cell diameters ($\varnothing_{UV+} > \varnothing_{UV} > \varnothing_{PAR}$) of *D. tertiolecta* (mean + SD: $\varnothing_{PAR} = 7.37 \pm 0.59$ μm; $\varnothing_{UV} = 7.78 \pm 0.43$ μm; $\varnothing_{UV+} = 8.15 \pm 0.43$ μm; K–S test, $P < 0.001$) after 4–13 days of exposure. On the other hand, UV treatment significantly decreased ($\varnothing_{UV+} < \varnothing_{UV} < \varnothing_{PAR}$) cell diameters of *T. pseudonana*, after 10 days of exposure (mean + SD: $\varnothing_{PAR} = 5.14 \pm 0.30$ μm; $\varnothing_{UV} = 4.71 \pm 0.58$ μm; $\varnothing_{UV+} = 4.23 \pm 0.26$ μm; K–S test, $P < 0.001$).

C:N ratio

The C:N ratio decreased significantly from PAR to the UV treatments (*T. weissflogii*: $H_2 = 11.043$, $P = 0.004$; *T. pseudonana*: $H_2 = 9.057$, $P = 0.011$) in the diatom cultures (Fig. 2a and b). There was no significant effect of spectral treatment on the C:N ratio of copepods ($F_{2,18} = 0.749$, $P = 0.487$) or of fish larvae ($F_{2,14} = 0.951$, $P = 0.410$) (Fig. 2c and d).

Percentage of total lipids

Total lipid in *T. weissflogii* decreased with UV treatment. The mean lipid percentage for the PAR treatment was

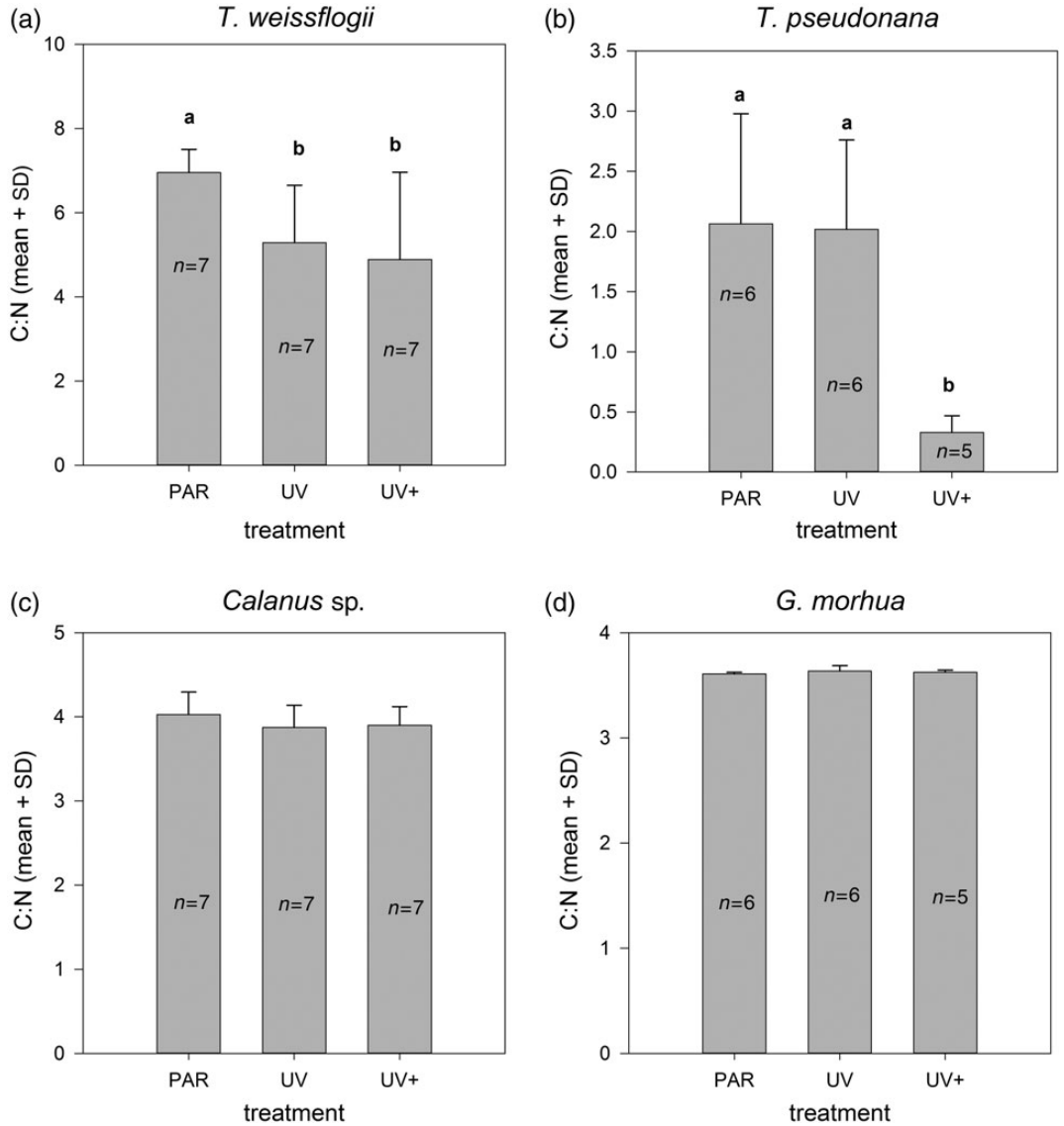

Fig. 2. Effect of three different UV exposure treatments (PAR only, PAR + ambient UV; PAR + enhanced UV) on the C:N ratio of (**a**, **b**) two species of diatoms *T. weissflogii* and *T. pseudonana* (a, b). These exposed algae were then fed to unexposed *C. finmarchicus* nauplii (**c**), which were in turn fed to *G. morhua* larva (**d**). Different letters above the bars depict significant differences in C:N ratios. *n* is the number of samples that were processed (each sample contained a bulk of algal cells, or animals). Each replicate corresponded to ~100 nauplii and 30 fish larvae.

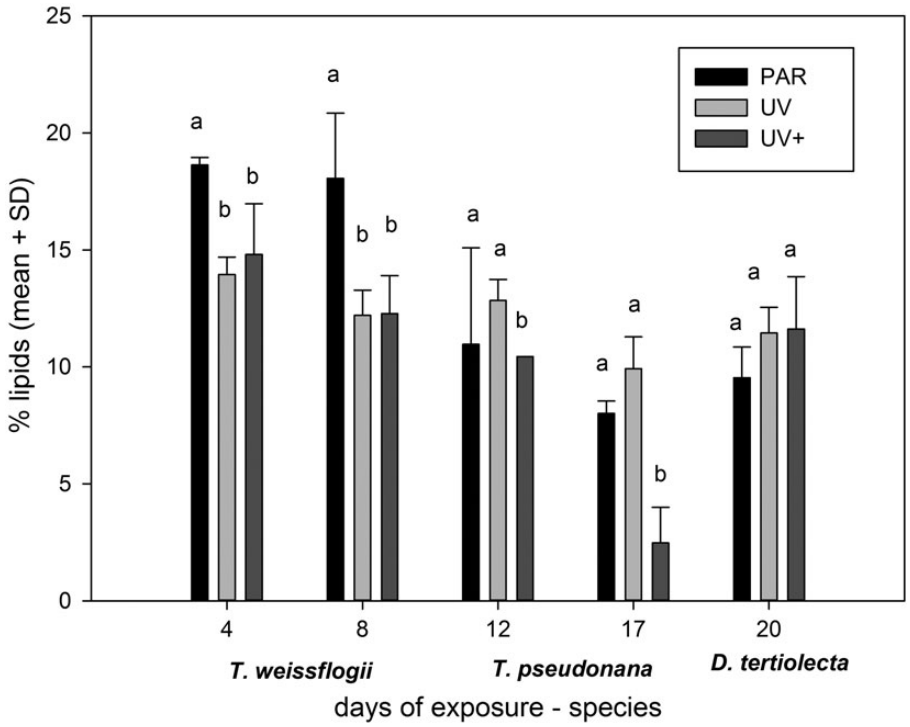

Fig. 3. Effect of three different UV exposure treatments (PAR only, PAR + ambient UV; PAR + enhanced UV) on total lipids in three algal species after 4 and 8 days of exposure (*T. weissflogii*), 12 and 17 days of exposure (*T. pseudonana*) and 20 days of exposure (*Dunaliela tertiolecta*). Letters indicate that treatments were different at a 5% significance level.

higher than in the UV and enhanced UV treatments after 4 and 8 days of exposure ($F_{2,18} = 24.1$, $P < 0.001$ and $F_{1,18} = 5.52$, $P = 0.030$; Fig. 3). In *T. pseudonana*, the enhanced UV treatment decreased total lipid content but only after 17 days of exposure ($F_{2,9} = 4.82$, $P = 0.038$ and $F_{1,9} = 14.4$, $P = 0.004$, Fig. 3).

After 20 days of exposure, lipid percentages were low in *D. tertiolecta*, and there was no effect of spectral treatment on lipid percentage ($F_{2,6} = 1.512$; $P = 0.29$, Fig. 3). There was no effect of spectral treatment on total lipid content of either copepods ($H_2 = 0.777$, $P = 0.68$) or fish larvae ($F_{2,24} = 0.209$, $P = 0.813$).

Individual FA composition

The most abundant FA in the diatoms was palmitic acid (16:0) (see Table II for nomenclature), constituting 25 and 33% of *T. pseudonana* and *T. weissflogii*, respectively. This FA represented only 0.4% in *D. tertiolecta*, while LNA was the most abundant in this alga (44%). Palmitic acid (16:0) was almost equally present in all three algae (19–22%). The other major FAs in the diatoms were EPA which represented 18% in both diatom species. Oleic acid (18:1n9c) constituted ~10% in *D. tertiolecta* and *T. weissflogii*, but only 2% of *T. pseudonana*.

Effects of UV on the FA profiles were similar in both diatoms (Figs 4–6). The first component of the PCA was positively correlated with short-chain FA (16:0 and 16:1n−7) and negatively correlated with long-chain polyunsaturated FA (LC-PUFA: EPA, ARA, DPA, DHA) (Figs 4a and 5a). Replicate samples showed homogeneous profiles as they clustered according to their exposure treatment: PAR-treated samples all had positive scores on PC 1 while UV+-treated samples had negative scores (Figs 4b,c and 5b,c). Ambient UV-treated samples had an intermediate position on the factorial plot. Thus, PAR-treated algae had more saturated fatty acids (SFAs) (14:0 and 16:0), while UV+-treated algae had more long-chain PUFA (Figs 4 and 5).

The UV exposure effects on the relative proportions of FA were stronger with increasing days of exposure (Figs 4–6). EPA significantly increased with UV in the diatoms and after 17 days of exposure, it was almost twice as high in the UV+ compared with the PAR treatment (Table III; Fig. 6). Oleic acid (18:1n−9) also increased with UV treatment in *T. weissflogii*. Stearidonic acid (18:4n3) increased with UV in *T. pseudonana*. Other FA that were present in small quantities (<5%) but nonetheless increased with UV were: LNA, ARA, DPA and DHA.

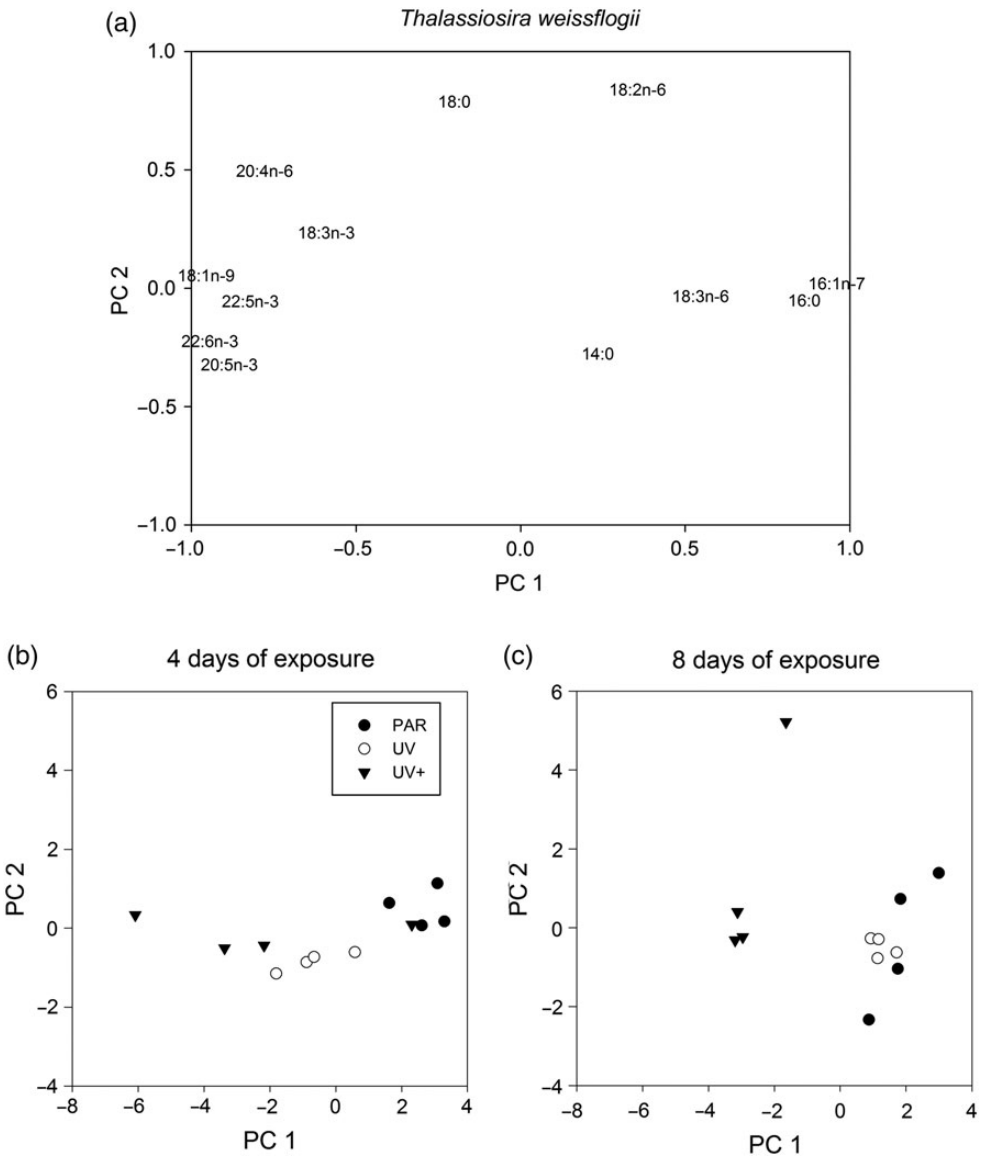

Fig. 4. Effect of UV on the FA profile of *T. weissflogii* after 4 and 8 days of exposure treatments. Coordinates were calculated using PCA. The first principal component (PC1) accounted for 53% of the total inertia, and PC2 for 16%. Correlations ($-1 < R < 1$) between FA and principal components (PC1 and PC2) are presented in (**a**). Factorial scores of each sample replicates were plotted on different figures according to days of exposure (**b**, **c**). Symbols indicate UV exposure treatments: PAR only, PAR + ambient UV; PAR + enhanced UV.

Palmitic (16:0) and palmitoleic acid (16:1n−7) decreased from the PAR to the UV+ treatment. In *T. pseudonana*, the proportion of palmitoleic acid (16:1n−7) was twice and three times lower in the UV+ treatment compared with the UV and PAR treatment, respectively (Table III, Fig. 6c and d).

Factorial scores of *D. tertiolecta* samples exposed for 20 days did not show any specific pattern relative to their exposure treatment, nor were there any effects of spectral treatment on any of the FA proportions in *D. tertiolecta* exposed for 20 days (Fig. 6d).

Copepods that fed on the exposed algae did not display any major changes in their FA profiles and differences were not statistically significant ($P > 0.05$), although similar trends in DPA, palmitic (16:0) and palmitoleic acid (16:1n−7) than those observed in the algae are visible in Fig. 7a. In fish larvae, there were no visible trends or any significant effect of spectral treatment on any of the individual FA ($P > 0.05$) (Fig. 7b). PCAs, carried out on the FA that significantly varied in the algae did not show any pattern related to the spectral treatments in the animals (copepod and fish).

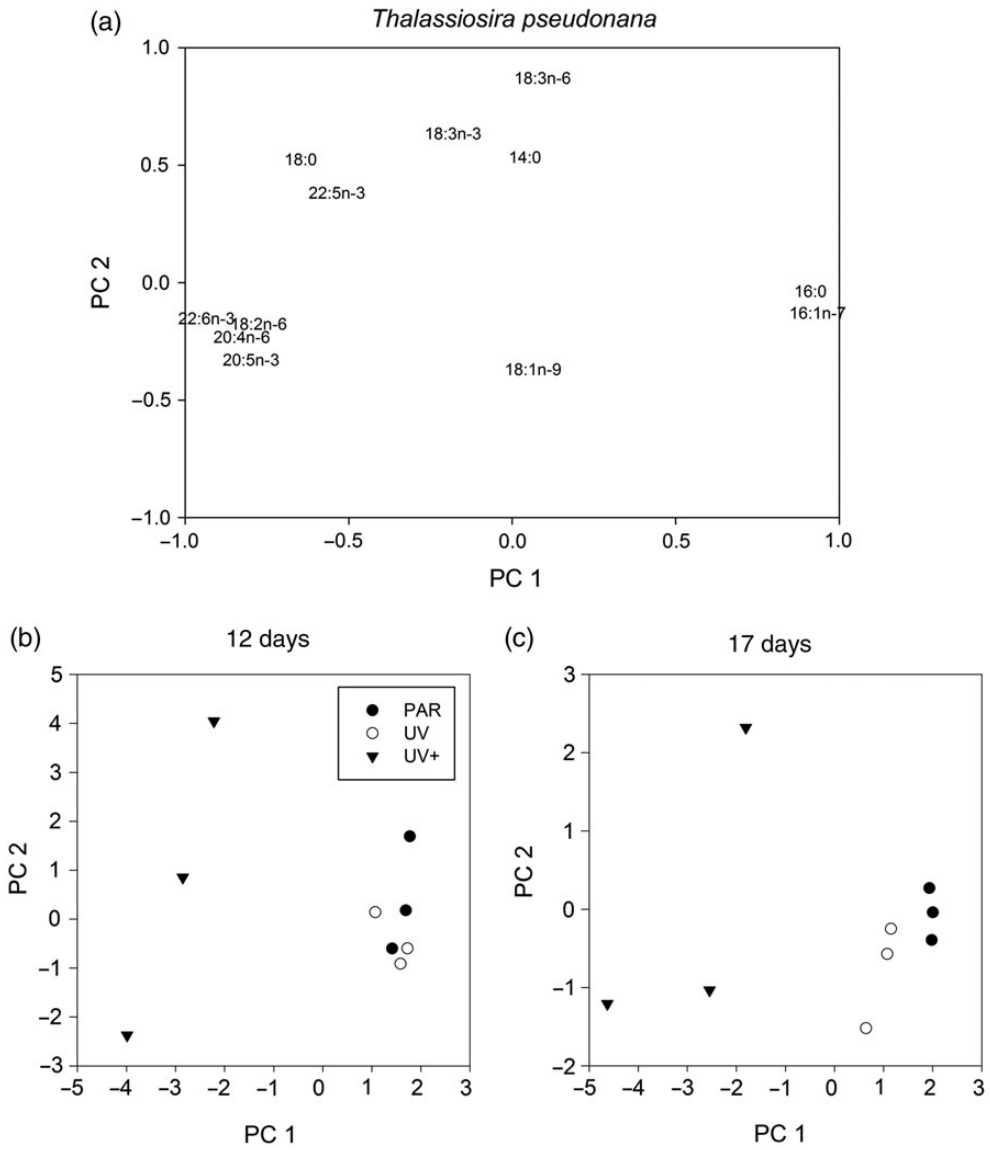

Fig. 5. Effect of UV exposure on the FA profile of *T. pseudonana* after 12 and 17 days of exposure treatments. Coordinates were calculated using PCA. The first principal component (PC1) accounted for 44% of the total inertia, and PC2 for 19%. Correlations ($-1 < R < 1$) between FA and principal components (PC1 and PC2) are presented in (**a**). Factorial scores of each sample replicates were plotted on different figures according to days of exposure (**b**, **c**). Symbols indicate UV exposure treatments: PAR only, PAR + ambient UV; PAR + enhanced UV.

Mycosporine-like amino acid

We did not detect MAA in any of the samples: algae, copepod or fish.

DISCUSSION

UVR-induced changes in the algae

We assessed the direct effects of UVR exposure on three species of algae. Some phytoplankton species are UVR tolerant as a result of protective pigmentation (Zudaire and Roy, 2001; Shick and Dunlap, 2002) and/or have efficient repair mechanisms (Sinha and Hader, 2002). *Thalassiosira weissflogii* produces UVR protective pigments, including MAAs, in response to long-term exposure (16–22 days) to UVR (Zudaire and Roy, 2001). Further, UV-absorbing compounds have been reported in both *T. pseudonana* and *D. tertiolecta*, although in relatively small amounts (Jeffrey *et al.*, 1999). However, we found no production of MAA in response to UVR exposure in any of the three algal species, even after 17 days of exposure. This suggests that the production of MAA in response to UV is not crucial for these species

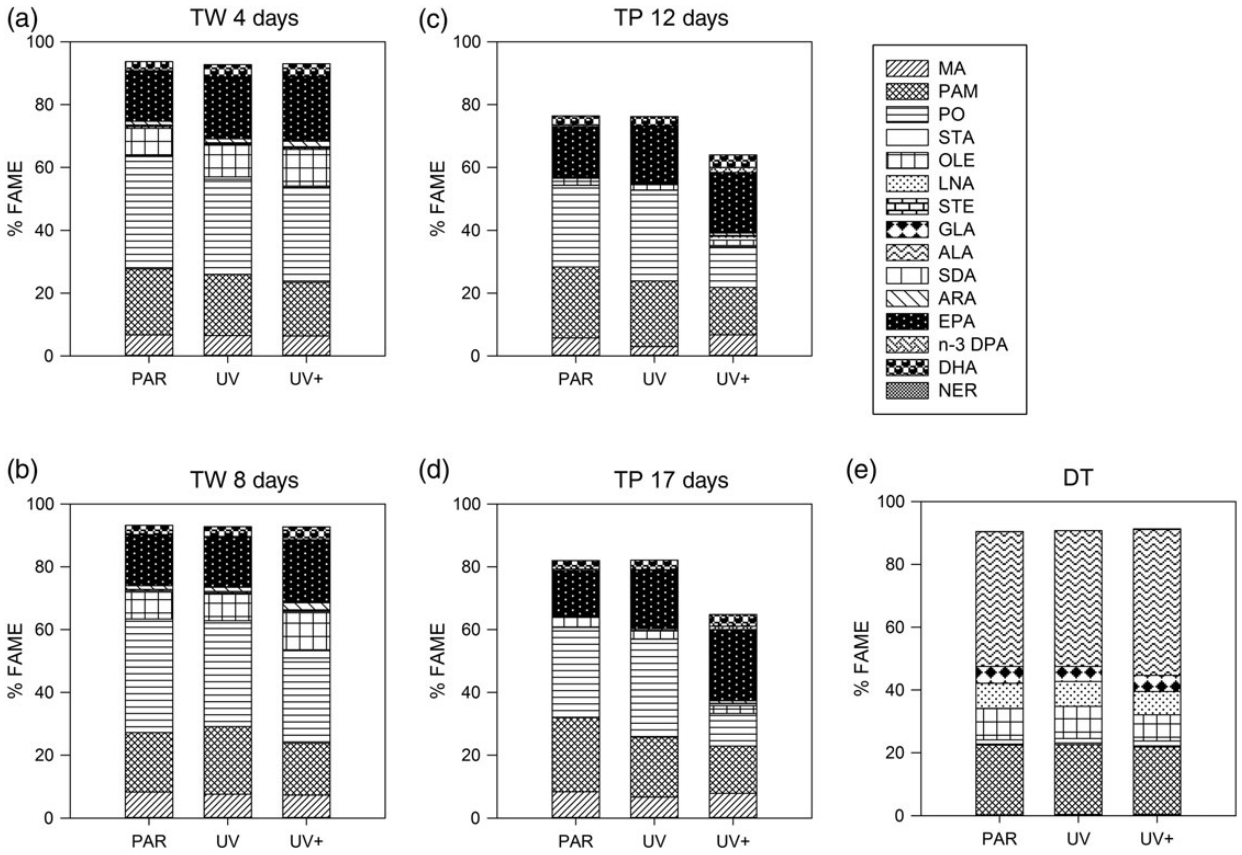

Fig. 6. Effect of UV exposure on the FA profiles of *T. weissflogii*, *T. pseudonana* and *D. tertiolecta* after several days of three different exposure treatments (PAR only, PAR + ambient UV; PAR + enhanced UV).

Table III: Effect of UV exposure on the FA profiles of T. weissflogii, T. pseudonana *and* D. tertiolecta *after several days of three different exposure treatments (PAR only, PAR + ambient UV; PAR + enhanced UV)*

FA	Algae	Days of exposure	UV treatment (pairwise multiple comparison)	*F* statistics	*P*
EPA	*T. weissflogii*	4 and 8	PAR = UV < UV+	$F_{2,18} = 8.788$	0.002
	T. pseudonana	12 and 17	PAR < UV < UV+	$F_{2,12} = 12.621$	0.001
OLE	*T. weissflogii*	4 and 8	PAR = UV < UV+	$F_{2,18} = 17.134$	<0.001
STA	*T. pseudonana*	12 and 17	PAR = UV < UV+	$F_{2,12} = 11.178$	0.002
LNA	*T. pseudonana*	12 and 17	PAR = UV < UV+	$F_{2,12} = 9.046$	0.004
ARA	*T. weissflogii*	4 and 8	PAR = UV < UV+	$F_{2,18} = 21.291$	<0.001
	T. pseudonana	12 and 17	PAR = UV < UV+	$F_{2,12} = 10.970$	0.002
n3-DPA	*T. weissflogii*	4 and 8	PAR < UV = UV+	$F_{2,18} = 24.989$	<0.001
	T. pseudonana	12 and 17	PAR = UV < UV+	$F_{2,12} = 3.946$	0.048
DHA	*T. weissflogii*	4 and 8	PAR < UV < UV	$F_{2,18} = 11.586$	<0.001
	T. pseudonana	12 and 17	PAR = UV < UV+	$F_{2,12} = 16.051$	<0.001
PAM	*T. weissflogii*	4 and 8	UV+ < UV < PAR	$F_{2,18} = 17.149$	<0.001
	T. pseudonana	12 and 17	UV+ < UV < PAR	$F_{2,12} = 85.414$	<0.001
PO	*T. weissflogii*	4 and 8	UV+ < UV < PAR	$F_{2,18} = 23.170$	<0.001
	T. pseudonana	12 and 17	UV+ < UV = PAR	$F_{2,12} = 47.819$	<0.001

Results of two-way ANOVA carried out on the relative proportions of the FA. Only significant tests are presented.

and is outweighed by the cost of synthesizing these protective screening compounds. This costly anabolic process, which requires nitrogen, might compete with other energetic demands such as growth (Shick and Dunlap, 2002). Alternatively, species kept for long periods in culture may exhibit genetic drifts that

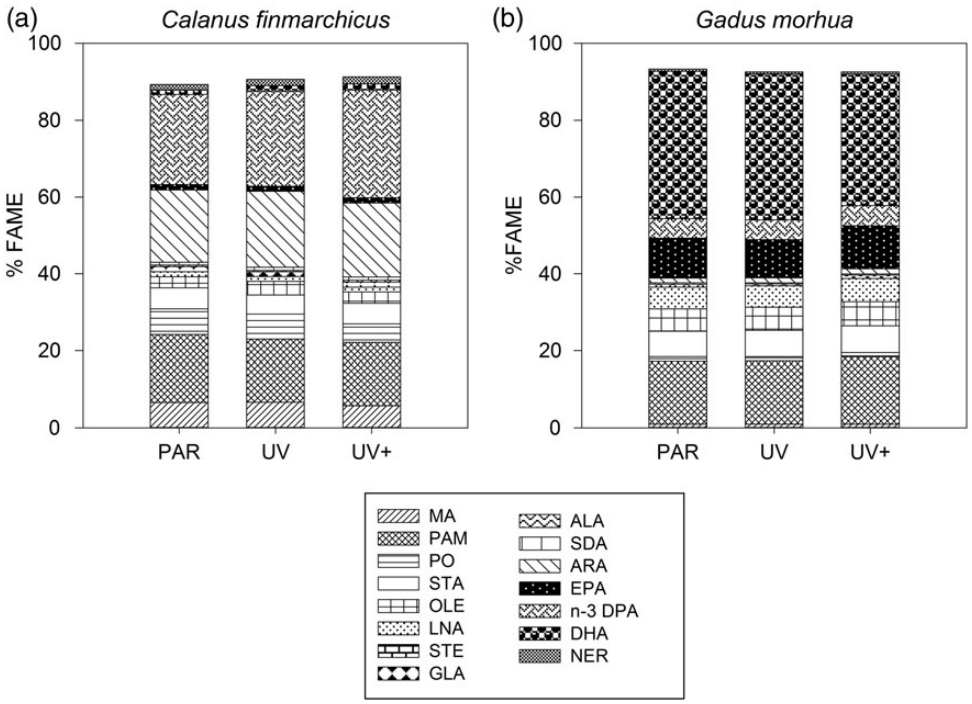

Fig. 7. Cascading effects of UV exposure treatments down a simple food chain. Algae (*T. pseudonana*) were exposed to different UV light treatments (PAR only, PAR + ambient UV; PAR + enhanced UV), then fed to unexposed copepod nauplii, which were fed to cod larvae. Results are expressed as FAMEs. Only FA that represented >2.5% were included in the figures.

compromise the synthesis of secondary metabolites such as MAA.

The percentage of lipids (on a dry weight basis) decreased substantially in the algae as a function of increasing UVR. Such a decrease in total lipid content with increasing UV exposure has been demonstrated in many studies (Wang and Chai, 1994; Arts and Rai, 1997; De Lange and Van Donk, 1997; Skerratt *et al.*, 1998; Harrison and Smith, 2009; Guihéneuf *et al*, 2010; Fricke *et al.*, 2011). However, this was only true in the diatoms (*T. weissflogii* and *T. pseudonana*). Indeed, the effects of UV on the relative proportions of FA can differ considerably depending on species. Most studies describe an overall decrease in the quality of the algae due to UVR. Even low UVB exposures (12 kJ m^{-2} day^{-1}) over short durations (4 days) altered the FA profile of a suite of marine diatoms (Goes *et al.*, 1994). At dose rates similar to our study, other researchers have demonstrated suppressed PUFA synthesis including decreases in EPA and DHA along with increasing UV (Goes *et al.*, 1994; Wang and Chai, 1994; De Lange and Van Donk, 1997; Hessen *et al.*, 1997; Liang *et al.*, 2006; Guihéneuf *et al.*, 2010; Leu *et al.*, 2010; Nahon *et al.*, 2011). *Thalassiosira pseudonana* was reported as a low tolerance species with a decrease in omega-3 FA after 4 days of exposure (Wang and Chai, 1994). In contrast, we found that, for both diatom species, LC-PUFA increased proportionally while short-chain FA and MUFA decreased with UV treatment and exposure duration. Such increases in PUFA with increasing UVR have also been reported in several species of diatoms (Skerratt *et al.*, 1998; Liang *et al.*, 2006; this study), in epilithic freshwater algae (Tank *et al.*, 2003), and in a chlorophyte, *Selenstrum capricornutum* (Leu *et al.*, 2006). As in our study, De Lange and Van Donk (De Lange and Van Donk, 1997) also observed a doubling of EPA in *Cryptomonas pyrenoidifera*. However, in our study, this increase was not linked to a decrease in the precursor DHA as appeared to be the case in the De Lange and Van Donk (De Lange and Van Donk, 1997) study.

Stoichiometry of marine phytoplankton is determined by complex interactions between biotic and abiotic factors, and reflects changes in the environment. UVR may affect cell stoichiometry of algae in different ways generally causing a simultaneous decrease in carbon (by inhibiting photosynthesis) and inhibiting nitrogen uptake (Mousseau *et al.*, 2000; Leu *et al.*, 2007), which results in only a limited impact on the overall C:N ratio. In our experiments, however, the C:N ratio in the algae decreased after UV exposure by ~25% in *T. weissflogii*, and 85% in *T. pseudonana* after exposure to enhanced UV (Fig. 2). This suggests that the algal cells became N-enriched with UVR exposure as a result of either an increase in nitrogen content or a decrease in carbon content. Increased nitrogen uptake is inconsistent with previous studies that have shown that UVR inhibits

the uptake and assimilation of nitrate and ammonium in marine phytoplankton (Goes *et al.*, 1995; Vernet, 2000; Sobrino *et al.*, 2004; Leu *et al.*, 2006). However, our results are consistent with a decrease in the FA content in UVR-exposed cells. Since FAs are carbon rich, lower fatty content within UVR-treated cells are consistent with lower C:N ratios. In addition to a decrease in the production of FA in UVR-exposed cells, there may also be a greater breakdown of carbon-rich compounds due to an increase in basal metabolic rates (respiration rates) concurrent with a decrease in the rate of photosynthesis (Beardall *et al.*, 1997). This combination would have a marked impact on the intracellular carbon content.

Trophic interactions

As mentioned previously, stoichiometric balance can be modified by abiotic factors (irradiance, temperature and pH) and biotic factors such as species and diet (Sterner and Elser, 2002; Finkel *et al.*, 2006; Fuschino *et al.*, 2011; Bradshaw *et al.*, 2012). Dietary elements are incorporated by consumers which are facing the challenge of maintaining the chemical balance required by their physiology (Sterner and Elser, 2002; Frost *et al.*, 2005). Nevertheless, we found no evidence that UVR-induced changes in *T. pseudonana* transferred in a significant way to copepod nauplii after 3 days of feeding. Decreasing C:N ratios with increasing UV treatment led to higher ingestion of C. Therefore, copepods were able to maintain stoichiometric homeostasis (in terms of carbon and nitrogen) regardless of the decrease in the quality of their algal diet. Indeed, heterotrophs are generally more homeostatic than autotrophs, as they have developed mechanisms to cope with fluctuation in food quality (Sterner and Elser, 2002; Hessen and Anderson, 2008; Malzahn *et al.*, 2010). For example, animals can modify their ingestion rate and their diet selectivity as a response to decreased food quality (Plath and Boersma, 2001; Raubenheimer and Simpson, 2004; Meunier *et al.*, 2015). In a similar trophic cascade experiment, stable isotope analyses revealed differences in the isotope composition of the consumers (copepods) which were related to their N and P depleted algal diets (Aberle and Malzahn, 2007). The fish larvae that had fed on these copepod nauplii, on the other hand, showed only slight changes in N and C isotope ratios. Consumers are able to maintain homeostasis provided that food is available in excess (Aberle and Malzahn, 2007). In contrast, the condition of lobster larvae was negatively affected after feeding on copepods raised on algae grown under N and P limitation (Schoo *et al.*, 2012). In another laboratory trophic cascade, algae and copepods raised under different pCO_2 treatments showed altered FA compositions and a decrease in EFA. However, changes alone in algal diet (irrespective of the pCO_2 treatment) did not influence the FA composition of copepods (Rossoll *et al.*, 2012). Similarly, despite UV-induced reductions in nutritional quality of *Selenestrum capricornutum*, no significant effects on *Daphnia magna* growth or reproduction were detected (Leu *et al.*, 2006). *Daphnia* fed on UV-irradiated algae showed no changes in their essential PUFA. Similarly, it seems that copepods are able to compensate for a decrease in food quality possibly by higher grazing rates (Fields *et al.*, 2011). Interestingly, the developmental rate of copepodites fed on algae grown under P limitation was significantly reduced although the algae remained rich in FA (Malzahn *et al.*, 2010). One possible explanation is that the copepods in that experiment did not respond by increasing ingestion since the FA content was sufficient for their needs.

Growth and developmental rates of zooplankton and higher trophic levels are dependent on both food quantity and food quality. To control for the effects of food quantity, the zooplankton feeding chambers were maintained at a constant algal concentration (2×10^4 cells mL^{-1}) throughout the experiment. In the present study, the copepod nauplii were fed for 3 days which was possibly insufficient to observe a response. This duration was chosen to avoid excessive mortality while still leaving some time to incorporate nutritional elements from their algal diet into their biomass; although very few studies have addressed the FA turnover issue in zooplankton. The turnover rate will depend on growth rate, temperature, life stage, food quantity and quality (Brett *et al.*, 2009). Relatively fast growing organisms, such as members of the genus *Daphnia*, are expected to rapidly incorporate FA from their diet (Brett *et al.*, 2009), more quickly than slow growing adult Arctic *Calanus*, where the complete turnover of FA took 11 days (Graeve *et al.*, 2005). In contrast, new digestive enzyme appeared in the copepod *Temora longicornis* 24 h after introduction of a new diet as well as an equally rapid (24 h) incorporation of dietary FA (Kreibich *et al.*, 2008, 2011).

In our experiment, a compensatory response (increased grazing) may have occurred on the enhanced UV-treated algae, since the FA composition remained stable in the copepods. This was possible since the animals were fed *ad libitum*. Algal cell morphology or cell fragility may affect grazing rates (Fields *et al.*, 2011). *Calanus* spp. selectively graze on larger cell sizes because they are more readily detected (Frost, 1972; DeMott and Watson, 1991; Bundy *et al.*, 1998). Because, algal cells decreased with UV treatment in our study, it is more likely that enhanced grazing was due to either changes in morphology or increased cell fragility (Donaghay and Small, 1979; Paffenhöfer, 1984). Sloppy feeding was more likely to occur on weakened UV-treated algae and the release of soluble material may have caused increased feeding activity (Gill and Poulet, 1988; Head, 1992).

Thus, copepods may have been able to counteract the low quality of the algae by increased grazing (De Lange and Lürling, 2003; Fields *et al*., 2011).

These experiments cannot include all of the complexities that exist in natural systems, such as a more diversified diet. Therefore, results must be extrapolated to natural systems with caution. Further, in their natural environment, copepods are subjected to cumulative stressors (e.g. low food, contaminants, sudden temperature variations and long-term changes such as global warming and possibly ocean acidification) for which they will have to make compensatory adjustments. Therefore, increased grazing rates may incur an ecological cost making copepods more vulnerable to predators.

CONCLUSIONS

Although UVB radiation can have negative impacts (direct effects) on crustacean zooplankton and ichthyoplankton populations, it must be viewed as only one among many environmental factors (e.g. bacterial and viral pathogens, predation, toxic algae, global warming and ocean acidification) that produce the high mortality typically observed in the planktonic early-life stages of these organismal groups. For zooplankton and fish species whose early-life stages are distributed throughout the mixed layer, UVB radiation likely represent only a minor source of direct mortality for the population, particularly at higher latitudes. For those species whose early-life stages are neustonic, there may be circumstances (albeit rare), cloudless sky, thin ozone layer, no wind, calm seas, low nutrient loading, under which the contribution of UVB radiation to the population's mortality could be much more significant.

Although UVR may induce important changes in phytoplankton, these changes seem to have limited effects on higher trophic levels under ideal conditions where food is not a limiting factor for copepods. We suggest that increased grazing may counteract the change in algal quality; however, this would only be possible if copepods are not subjected to other stressors such as the risk of predation linked to increased grazing rates. Cascading effects would also be lessened by the fact that periods of cloudless sky, thin ozone layer, calm seas and low nutrient loading would be rare at northern latitudes (Browman and Vetter, 2002; Browman, 2003).

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