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PAPER

UV radiation simultaneously affects phototrophy and phagotrophy in nanoflagellate-dominated phytoplankton from an Andean shallow lake

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Mixotrophic nanoflagellates, that combine photosynthesis and phagotrophy, are important members of planktonic food webs in many aquatic environments depending on the balance among the different carbon and energy sources. We carried out field sampling and laboratory experiments with natural nanoflagellate assemblages from an Andean North-Patagonian lake exposing them or not to UVR, and measuring photosynthetic parameters and bacterivory. The effect of different light treatments on the photosynthetic efficiency was studied by the non-invasive, pulse amplitude-modulated (PAM) fluorescence technique, and bacterivory was assessed with fluorescently labeled bacteria (FLB). Mixotrophic nanoflagellates were clearly dominant (up to 90% of total phytoplankton and 88% of total nanoflagellate abundance), and in the experiments labeled bacteria were observed in more than 75% of mixotrophic cells. These results support the idea that these phytoflagellates were never entirely photosynthetic. The high light : phosphorus ratio and the high C : N : P ratio suggest a strong nutrient limitation towards P. Our results show that both functions, photosynthesis and bacteria ingestion, were simultaneously reduced by the same level of UVR. We estimated that UVR exposure of mixotrophic nanoflagellates reduced photosystem II activity between 23% and 31% while ingestion rates were reduced between 23% and 28%. Therefore, our results suggest that the different cell functions could be concurrently impacted by UVR, implying that patterns and rates of C transfer would be substantially altered in the microbial food web.

Introduction

Nanoflagellates play a crucial role in regulating abundance, production, size distribution and composition of bacterial assemblages.[1,2] Thus, grazing activity exerts a strong influence in the pelagic microbial loop and in nutrient remineralization in the water column.[3] Mixotrophic nanoflagellates, that combine photosynthesis and phagotrophy, are included in more than one trophic level as they are able to obtain carbon as producers and as well as consumers.[4] These organisms are important members of planktonic food webs in many aquatic environments,[5,6] however their importance in planktonic communities depends on the balance among different carbon, energy, and nutrients.[7] Environments with favorable light conditions and high light : nutrient ratios are predicted to increase phosphorus (P) competition between algae and bacteria.[8,9] Because prey elemental ratios (carbon : phosphorus, C : P and carbon : nitrogen, C : N) are usually lower than in the mixotrophic nanoflagellates, bacteria cells constitute mineral nutrient sources for these organisms under nutrient-limiting conditions.[10] In that sense, the light : nutrient hypothesis[11] predicted impacts on the microbial loop and on resource limitations and C : P stoichiometry, since algae and bacteria are affected by the balance of light and nutrients.[12] Mixotrophy has evolved as an adaptive strategy for growth in adverse conditions.[13] In lake ecosystems where light or mineral nutrients could be limited, mixotrophy has an advantage since phagotrophy will provide extra C[7] or nutrients[14] through prey ingestion. In ultraoligotrophic lakes under high light : nutrient ratios, mixotrophic nanoflagellates and ciliates were observed to increase phagotrophy when light is not limiting but nutrients are scarse.[15,16]

Early studies have shown that ultraviolet radiation (UVR) reduces phagotrophy of nanoflagellates up to 70%.[17,18] In particular, studies on UVR sensitivity in *Bodo saltans* have reported DNA damage and dramatic changes in cell morphology with a concomitant reduction in motility that may cause the decrease in feeding activity.[19] In addition, the phagotrophic activity of other heterotrophic protists, like ciliates, was observed to be also negatively affected by UVR.[20] Protist sensitivity to UVR is species-specific[19] and is related to UV intensity and temperature[21] and in nature may be related to protist temporal and spatial distribution.[22] In addition, it was recently shown that mixotrophy is an adaptive strategy both to deficient environmental conditions as well as to UVR-induced stress.[23]

UVR has been recognized as an important factor causing inhibition of the photosynthetic process,[24] affecting the photosystem II (PSII)[25] and the RUBISCO enzyme.[26] Also, studies have

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reported UVR affecting phagotrophy in natural nanoflagellate assemblages.[17,27] Evidence coming from studies on summer light conditions suggest a balance between carbon acquired by photosynthesis and that derived from ingestion.[13] However, information about the effects of UVR on both photosynthesis and phagotrophy in natural assemblages of mixotrophic protists is almost lacking.

Andean North-Patagonian lakes are located near the Antarctic Polar vortex and the ozone hole,[28] with high levels of UV solar radiation.[29] Although changes would be expected in atmospheric transport, it is unlikely that ozone will stabilize at levels observed before 1980, when a decline in ozone concentrations was noted.[30] In these Andean lakes, including shallow and deep ones, previous studies reported a decrease in primary production at surface levels due the high solar irradiances, while the higher photosynthetic efficiency were obtained at 1% of surface PAR.[31,32] Planktonic communities of these Andean lakes are dominated by mixotrophic protists including nanoflagellates[32,33] and ciliates.[15] These lakes constitute an interesting scenario for the study of the effect of UVR on photosynthesis and bacterivory processes in mixotrophic nanoflagellates under high light : nutrient conditions. During lake mixing these communities are dispersed throughout the water column, so they would be potentially exposed to UVR. We have hypothesized that UVR will differentially affect photosynthesis and phagotrophy in natural mixotrophic nanoflagellate assemblages. Therefore, we carried out three experiments with natural nanoflagellate assemblages exposing them or not to UVR.

Materials and methods

Sampling

Lake Escondido is a small shallow lake (maximum depth 8 m) located at 764 m above sea level in the Northwestern Patagonia (Argentina) (41°03′29″ S and 71°34′20″ W). The climate of the region is temperate cool (mean annual temperature 8.7 °C) with an annual precipitation of 1500 mm.[34] Thermal regime is characterized by a continuous mixing with week summer stratification periods.[35] The lake is oligotrophic, with summer chlorophyll *a* concentration < 3 μg L^{-1} and total phosphorus concentration <7 μg L^{-1}.[32] In comparison with deep lakes and other shallow lakes of the area, Lake Escondido exhibits high organic carbon (~2.70 mg L^{-1}).[32,36] This higher carbon content was observed to favor bacterial production.[37]

In order to carry out the experimental program, we sampled the lake on three different occasions during 2008: summer (26 February), autumn (5 June), and spring (2 December). We performed vertical water column profiles of temperature, ultraviolet band (320 nm), and photosynthetically active radiation (PAR, 400–700 nm) with a PUV 500B submersible radiometer (Biospherical Instruments). Water samples were obtained at a central sampling station (8 m depth) with a Van Dorn bottle (2 L) at 3 m depth in three replicates. The water was placed in an acid (HCl) washed container and transferred to the laboratory within 30 min for nutrient determinations and for laboratory experiments. In addition, 100 mL of water was fixed with acid Lugol for nanoflagellates and phytoplankton determinations, and 60 mL of water were preserved with filtered (0.2 μm) formaldehyde solution (2% vol/vol final concentration) for prokaryotic and nanoflagellates enumeration.

Analysis of samples

Total phosphorus (TP) and total nitrogen (TN) were determined in unfiltered lake water digested with persulfate at 125 °C and 1.5 atm for 1 h. Phosphorus concentrations were determined by the ascorbate-reduced molybdenum method[38] and nitrogen according to Valderrama.[39] Sestonic C and N content was determined by filtering 300 mL of water through precombusted GF/F filters, and measured with a CN Thermo Finnigan EA1112 analyzer. Sestonic P was obtained by the difference between TP and TDP (total dissolved phosphorus). TDP was obtained in GF/F filtered water following the same procedures as for TP. Nutrient determinations were performed in three replicates.

Phytoplankton biomass estimated as chlorophyll *a* concentration was analyzed discriminating between the contribution of the picophytoplankton (<2 μm) and phytoplankton (>2 μm) fractions. For fraction >2 μm, up to 200 mL of sampled water were filtered through a 2.0 μm pore size polycarbonate filter (Nuclepore). Then, the filtrate was filtered through a 0.2 μm pore size polycarbonate filter (Nuclepore). Chlorophyll *a* concentration was measured by extraction with 90% ethanol following Nusch[40] and measured with a 10-AU fluorometer (Turner Design).

Phytoplankton samples were counted and measured with an inverted microscope using 50 ml Utermöhl chambers with an image analysis system (Image ProPlus 4.5; Media Cybernetics, USA). Biovolume was estimated from similar geometric models according to Sun and Liu.[41] Total bacteria were stained with the fluorochrome 4′,6-diamidino-2-phenylindole (DAPI; final concentration 2% vol/vol) according to Porter and Feig[42] and abundance determinations were performed onto 0.2 μm polycarbonate black membrane filters (Poretics, Livermore, CA, USA), at 1250× magnification using an Olympus BX50 epifluorescence microscope with UV light (U-MWU filter). A minimum of 1000 total bacteria per sample were counted and measured with an image analysis system (Image ProPlus 4.5; Media Cybernetics, USA). Nanoflagellate abundances were determined also by staining with DAPI and filtered onto 1 μm polycarbonate black membrane filters (Poretics, Livermore, CA, USA). Analysis by epifluorescence microscopy using both UV and blue filters (U-MWB filter) allowed separation of heterotrophic and phototrophic nanoflagellates. Utilizing the natural autofluorescence of chlorophyll under blue filters, the phototrophic cells were identified.[43] The abundances of nanoflagellates obtained in Lugol's preserved and formaldehyde preserved samples were similar.

Exposure experiments

We carried out three laboratory experiments in which natural lake water (3 m depth) was exposed to different light conditions in a temperature regulated incubator. In each experiment, two treatments were performed: (1) UVR + PAR (photosynthetically active radiation) and (2) PAR (>380 nm).

UVR was provided by two fluorescent tubes UVA-340 (Q-Panel Lab Products, Cleveland, OH, USA), which UV spectrum closely resembles the solar spectrum between 280 and 350 nm.[44] During the incubation, water received 35 μW cm^{-2} nm^{-1} of the 340 nm band and 7 μW cm^{-2} nm^{-1} of the 305 nm, and this irradiation level is equivalent to surface noon summer sunlight in North-Patagonian Andean lakes.[45] PAR was provided by two fluorescent tubes light-day (98 μmol photons m^{-2} s^{-1}) and this irradiance

is equivalent to mean summer light received at ~3 m depth of Lake Escondido. Previous in-lake experiments, in which primary production was measured by ^{14}C uptake, indicated that this PAR intensity was adequate to support high photosynthetic efficiency.[32] Light intensities were determined with the PUV 500B radiometer (Biospherical Instruments). However by adding only PAR light, a part of the long UVA (380–400 nm) was absent from the exposure experiments. Exposure was carried out in 250 mL quartz containers (three replicates) for all treatments and lasted 4 h. In the PAR treatment, UVR cutoff was reached using a polyethylene filter (Master Absorber UV Arcolor, Arcolor S.A., Buenos Aires, Argentina). The optical features of this polyethylene, with cutoff at 380 nm and 85% transmittance above 380 nm, were checked before the experiment using a double–beam spectrophotometer (Shimadzu UV 2450).[46] All quartz containers were previously sterilized (autoclaved for 30 min).

Quartz containers were placed in a device that half-rotates the bottles every two minutes. The device was set in an incubator with temperature adjusted to that of the lake: 21 °C in summer, 10 °C in autumn and 20 °C in spring. The experiments were started after 30 min to allow the microcommunity to acclimate to the experimental conditions.

Measurement of photosynthetic parameters

Immediately after the sample was taken in the field (T_{Field}) and after the 4 h of experimentation (T_{Exp}), 20 mL of water of each container was sampled in order to carry out the photosynthetic parameter measurements with a WATER-PAM (Heinz Walz GmbH). Rapid light curves (RLC) were obtained by exposing samples to 10 s of blue radiation at eight incremental steps of irradiance ranging from 0 to 2334 μmol photons m^{-2} s^{-1}. We determined the relative electron transport rate (rETR), an index given by the product of incidence irradiance, E, and the effective quantum yield of charge separation at PSII (ϕ_{PSII}).[47] In the case of UV inhibition the ETR measured with the PAM indicates trends in the photosynthesis effect.[48]

Maximum quantum yield of PSII ($F_v : F_m$), which is dependent on the total number and configuration of functional PSII reaction centers,[47] was also measured to determine changes in the photosynthetic efficiency.[47] The variable fluorescence F_v is the difference between the maximal fluorescence from fully reduced PSII reaction center (F_m) and the intrinsic fluorescence (F_o) from the antenna of fully oxidized PSII. Both non-photochemical quenching and photoinhibition contribute to a decrease in $F_v : F_m$ ratio.[48]

A decrease in the overall number of functional reaction centers contributes to a decrease in $F_v : F_m$, which is by definition photoinhibition.[49] Thus, an increase in photoinhibition is evidenced by a decrease in the extent of the $F_v : F_m$ chlorophyll fluorescence parameter, and changes in the latter are commonly used to assess photoinhibition *in vivo*.[47]

Rapid light curves can be effectively described and compared by parameters such as photosynthetic efficiency (α, the initial slope of the curve), irradiance of maximum photosynthesis (E_k), and maximum production rate (P_{max}) (see Data analysis).

Bacterivory experiments

At T_{Exp} we measured bacterivory in each treatment with fluorescently labeled bacteria (FLB) under the same conditions of the corresponding treatment (UVR + PAR, and PAR).

The FLB were prepared following Simek & Straskrabova.[50] After a prefiltration through 2 μm pore size filter (Osmonics Inc., MN, USA), we concentrated the bacterioplankton of Lake Escondido in a 0.22 μm pore size filter (Nucleopore). Bacterial cells were detached from the filter surface by sonication, and were heat-killed and stained with 5-[(4,6-dichlorotriazin-2-yl) amino]fluorescein (DTAF) according to Sherr *et al.*[51] FLB were stored frozen in 1 mL aliquots. The obtained FLB were coccoid-shaped of 0.76 ± 0.03 μm in diameter close to the actual size of natural bacteria (0.75 ± 0.02 μm in diameter). These values did not change significantly in the three experiments (One Way ANOVA, $P > 0.05$).

FLB were added to each quartz container at a final concentration of 20% of total bacterial abundance in each sampling occasion. Total bacterial abundance was determined within two hours before starting the experiment. Subsamples of 20 mL were taken from each container at 5, 10, 15, 20 and 40 min. Previous tests indicated that during the 40 min of the experiment, FLB exposed to UVR were not bleached. Samples were fixed sequentially with 0.5% of Lugol solution, followed by 2% formaldehyde and several drops of 3% sodium thiosulphate to clear the color.[52] Within 3 days after fixation, samples were stained with DAPI and filtered through 1 μm black polycarbonate filter (Poretics, Livermore, CA, USA). A minimum of 300 nanoflagellate cells per filter were examined for ingestion. The number of ingested prey was quantified with an epifluorescence microscope (Olympus BX50) with UV light (U-MWU) and blue light (U-MWB filter).

Uptake rates were estimated by the linear regression between time and number of bacteria ingested per cell which was calculated based on the number of FLB and the FLB: bacteria ratio in each replicate.[52]

Data analysis

Changes in the different field variables measured during the study period (nutrient and chlorophyll *a* concentrations, C : N and C : P sestonic ratios) were analyzed by a One Way ANOVA.

Data from light curves of rETR *vs* E (irradiance) were fitted to the following equation of Eilers & Peeters[53] with curve-fitting software in SigmaPlot 11 (SPSS, Inc. USA):

$$\mathrm{rETR}(E) = \frac{E}{aE^2 + bE + c}$$

Although light curves were constructed with the rETR and not with primary production we maintained the original names of the variables given by Eilers & Peeters[53] (α, P_{max} and E_k). These parameters were calculated from the constant obtained in the fitted model[53] as:

$$\alpha = \frac{1}{c}$$

$$P_{max} = \frac{1}{b + 2\sqrt{ac}}$$

$$E_k = \frac{c}{b + 2\sqrt{ac}}$$

Comparison among optimal quantum yield values ($F_v : F_m$) and photosynthetic parameters (α, P_{max}, and E_k) obtained for different treatments in the three lake conditions, were carried out through

a Two Way ANOVA, with "Treatment" and "Season" as factors. The post-hoc comparisons were carried out with the *a posteriori* Tukey test with an overall significance level of $P = 0.05$. In all cases normality and homocedasticity were verified previously.

The significance of the differences between the slopes of ingested bacteria *vs.* time, were analyzed through an ANCOVA with "Time" as covariable.

All statistical analyses were performed using SigmaStat 3.5 and Statistica 7.0.

Results

Field study

On the three sampling occasions, the lake was observed in complete mixing and the whole water column was almost included in the euphotic zone, except in autumn. However, around 10% of the water column was affected by the 320 nm wavelength (Table 1). Nevertheless, since organisms are dispersed by lake water mixing they are naturally exposed to UVR. During the sampling period, no differences were observed in total N (One Way ANOVA, $P = 0.310$) (Table 1). Total P was higher in summer, although no significant differences were observed between seasons (One Way ANOVA, $P = 0.305$). Total chlorophyll *a* concentration showed similar values during summer and autumn (One Way ANOVA, $P = 0.010$; *a posteriori* Tukey test, $P = 0.630$) with a significant decrease in spring (*a posteriori* Tukey test, $P = 0.012$ for summer *vs.* spring, and $P = 0.024$ for autumn *vs.* spring). The contribution of picophytoplankton (<2 μm) varied between 16% in summer and 22% in spring. The sestonic C : N ratio was similar on all occasions (Table 1), while sestonic C : P varied significantly between autumn and spring, with the highest ratios during autumn (One Way ANOVA, $P = 0.023$; *a posteriori* Tukey test, $P = 0.020$).

Table 1 Temperature, light parameters and chemical features of Lake Escondido. Values are expressed as mean of the three replicates ± standard error

	Summer	Autumn	Spring
T/°C 0 m	21.5	10.02	20.6
T/°C 3 m	21.4	10.01	20.5
T/°C 6 m	21.4	10.01	20.5
K_d (PAR) (m^{-1})	0.66	0.78	0.61
K_d (320 nm) (m^{-1})	8.73	7.88	9.01
$Z_{1\%}$ (m) PAR	7.01	5.90	Bottom (9.61)
$Z_{1\%}$ (m) 320 nm	0.52	0.58	0.51
TN (μg L^{-1})	201.73 ± 48.67	212.90 ± 41.41	218.48 ± 39.79
TP (μg L^{-1})	7.67 ± 0.55	6.54 ± 0.43	6.95 ± 0.50
Chlorophyll *a* (μg L^{-1})	2.10 ± 0.68	1.86 ± 0.06	0.99 ± 0.14
Sestonic C : N	11.61 ± 5.81	15.46 ± 2.63	14.71 ± 1.12
Sestonic C : P	277.16 ± 17.33	335.55 ± 21.80	185.86 ± 61.28

References: PAR: photosynthetically active radiation; K_d: diffuse attenuation coefficient; $Z_{1\%}$: depth of 1% of surface irradiance; TP: total phosphorus; TN: total nitrogen.

Phytoplankton was dominated by four species of mixotrophic nanoflagellates (Fig. 1a and b). The contribution of this assemblage varied between 85% in summer to a maximum of 97% of total phytoplankton cell abundances in autumn. Biovolume was also dominated by this fraction since the contribution of mixotrophic nanoflagellates was of 77% in autumn up to 80% in spring (Fig. 1b). *Chrysochromulina parva* was observed in all seasons with high abundances, especially in autumn when it represented 86% of total phytoplankton abundance (Fig. 1), reaching 1846 cell mL^{-1}. *Rhodomonas lacustris* and *Gymnodinium varians* were also observed on the three sampling occasions with higher abundances during spring and summer respectively. On the contrary, *Dinobryon divergens* was only observed with an important contribution in summer (Fig. 1). The remaining phytoplanktonic species (*Gymnodinium* spp., *Peridinium* sp., *Fusola viridis*, and *Chroococcus planctonicus*) became only abundant in summer (Fig. 1) though the pooled abundances of these species remained less than 211 cell mL^{-1}.

The dominance of nanoflagellates was also assessed by epifluorescence microscopy. In all cases mixotrophic nanoflagellate abundances were higher than heterotrophic ones (Fig. 2). Heterotrophic nanoflagellates reached the highest contribution (up to 800 cell mL^{-1}) in spring, and the lowest in autumn (240 cell mL^{-1}) (Fig. 2). On the other hand, total bacterial abundances were 2.9×10^6 cell mL^{-1} in summer, 1.6×10^6 cell mL^{-1} in autumn, and 2.5×10^6 cell mL^{-1} in spring.

Although the taxonomic composition of the three mixotrophic nanoflagellate assemblages was similar; we observed different photosynthetic parameters on each sampling occasion (Fig. 3).

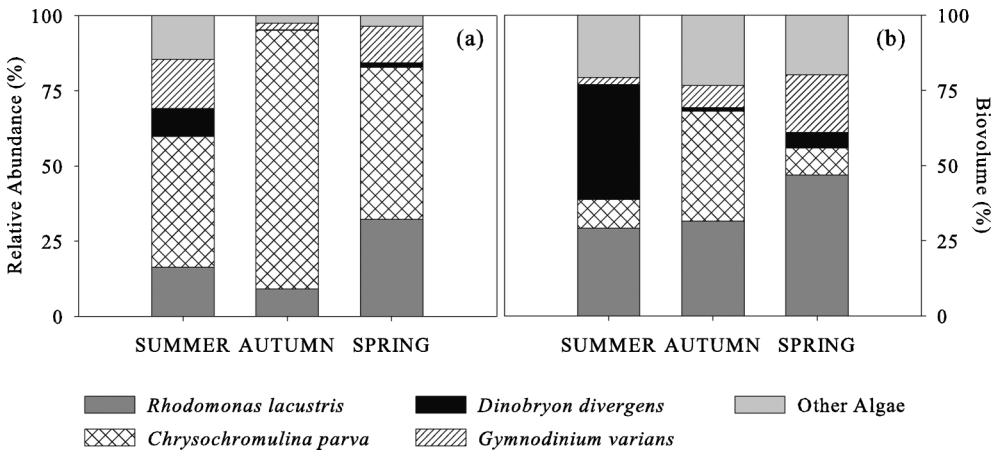

Fig. 1 Phytoplankton >2 μm of Lake Escondido. (a) Relative abundance (percentage of total phytoplankton abundance) and (b) relative biovolume of mixotrophic nanoflagellates and the remaining autotrophic planktonic fraction in the three sampling occasions.

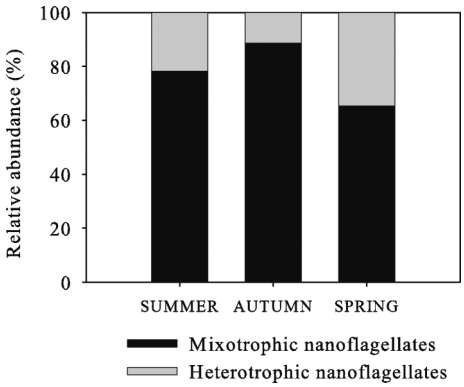

Fig. 2 Relative abundance of mixotrophic and heterotrophic nanoflagellates.

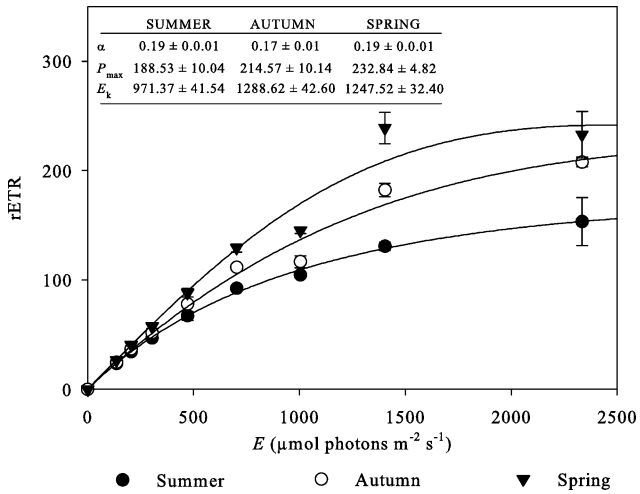

Fig. 3 Rapid light curves of rETR *vs.* irradiance (E) immediately after sampling (T_{Field}). Error bars = 1 standard error.

The autumn and spring assemblages presented similar values of maximum production rates (P_{max}) (One Way ANOVA, $P < 0.01$, *a posteriori* Tukey's test $P = 0.203$), and saturation light intensity (E_k) (One Way ANOVA, $P = 0.012$; *a posteriori* Tukey's test $P = 0.818$). No differences in photosynthetic efficiency (α) were observed among the different seasons (One Way ANOVA, $P = 0.094$) (Fig. 3).

Photosynthetic parameters in the exposure experiments

In our experiments, after 4 h of exposure (T_{Exp}), values of P_{max} and E_k were significantly higher in the PAR treatments than in the UVR + PAR on all occasions (Two-Way ANOVA, $P < 0.001$ for both parameters; *a posteriori* Tukey's test, $P < 0.001$ in all cases) (Fig. 4). In particular, higher values of P_{max} and E_k were observed in autumn and spring experiments (Two Way ANOVA, $P < 0.002$ for both parameters; a posterior Tukey's test, $P < 0.005$ for autumn/spring *vs.* summer for both parameters).

UV radiation reduced significantly the Photosystem II (PS II) maximum quantum yield ($F_v : F_m$) in all mixotrophic assemblages, indicating an inhibition effect by UVR (Two Way ANOVA, $P = 0.021$) (Fig. 5). The decrease in $F_v : F_m$ due to UVR exposure was always less than 31% and no significant differences were observed among seasons (Two Way ANOVA, $P = 0.128$) (Fig. 5). This

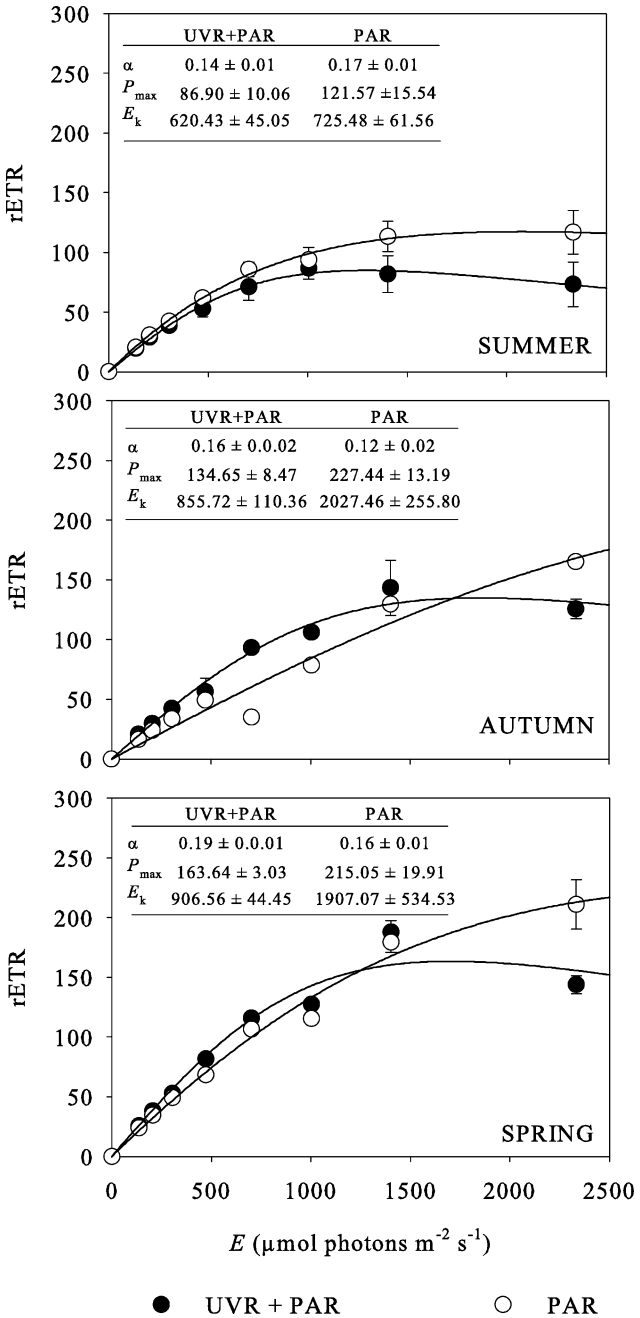

Fig. 4 Rapid light curves of rETR *vs.* irradiance (E) for UVR+PAR and PAR treatments after incubation (T_{Exp}). (a) Summer, (b) autumn, and (c) spring. Error bars = 1 standard error.

means that this photosynthetic parameter in the three natural mixotrophic assemblages was similarly affected by UVR.

Bacterivory in the exposure experiments

In the three experiments (spring, summer and autumn) we observed that more than 75% of mixotrophic nanoflagellates contained labeled prey inside the cells thus implying that these cells were never entirely photosynthetic. Mixotrophic flagellate assemblages exhibited higher ingestion rates in summer than in autumn and spring (Fig. 6a, b and c). Bacterial consumption significantly differed among light treatments in summer (ANCOVA, $P < 0.001$)

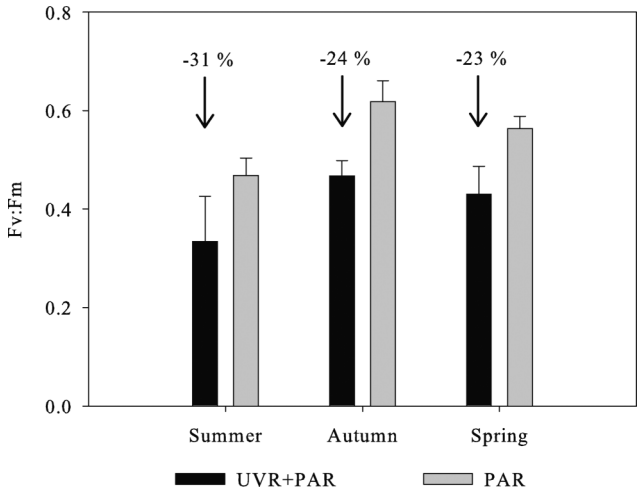

Fig. 5 Maximum quantum yield (F_v : F_m) in UVR + PAR and PAR treatment during the different sampling occasions. Percentage of F_v : F_m decrease in UVR + PAR treatment. Error bars = 1 standard error.

and spring (ANCOVA, $P = 0.025$). In both experiments in the PAR treatment, mixotrophic nanoflagellates ingested significantly more bacteria than in the UVR + PAR treatment (Fig. 6a and c) implying a reduction in bacteria consumption rate of 28% in the summer experiment and 23% in the spring one. On the contrary, in the autumn experiment we observed no significant differences in the ingestion rates (ANCOVA, $P > 0.05$) with an estimated reduction of 12% (Fig. 6 b).

Uptake rates of heterotrophic nanoflagellates were also higher in summer than spring and autumn (Fig. 6d, e and f). We observed significant differences between light treatments only in the autumn experiment (ANCOVA, $P = 0.005$) with an estimated reduction of 46% (Fig.6e). The estimated reduction in summer and spring (16 and 40% respectively) are not enough to be significant due to large dispersion among replicates (Fig.6d and f).

Discussion

In Lake Escondido, we observed that mixotrophic nanoflagellates dominate the phytoplankton community and contribute substantially (90% of total nanoflagellates) to bacteria uptake and C fixed by photosynthesis. Our experiments showed that UVR is inhibitory to both photosynthesis and phagotrophy, however future work should be evaluate to what extent these potential responses occur in the lake. Thus, photosynthesis and bacteria ingestion can be affected by the quality of the incident light and this effect could be of particular importance in shallow and relatively transparent lakes. In Lake Escondido the whole water column is illuminated (lies above 1% of surface PAR) therefore mixotrophic nanoflagellates can exploit the whole column. However, the mixing layer that also includes the whole water column, dispersing organisms up to near the surface, thus exposing them to potentially hazardous UVR, which in turn will affect both trophic modes. Nevertheless, it should be pointed out that UV transmission is low in this lake and that in our exposure experiments there were a gap between 380 and 400 nm that would imply a deficiency in photorepair mechanisms. Also the change in low PAR-UVR ratio in the laboratory experiments might have resulted in overestimation of the inhibitory results, so more experiments are needed to understand the *in situ* situation.[54]

An increase of *in vivo* photoinhibition could be evidenced by a decrease in the extent of the F_v : F_m chlorophyll fluorescence parameter[47] that would imply a decrease in the overall number of functional reaction centers.[49] In the Patagonia area, it has been indicated that natural UVR exposure has clear impacts on photosynthesis of marine phytoplankton[55] and freshwater protists.[56] Here, we also found a decrease in F_v : F_m due to UVR exposure in natural nanoflagellate assemblages. This decrease was as much as 31% and no significant differences were observed among seasons, suggesting that UVR photoinhibition in the three natural assemblages was similar. It has been indicated that UVR damage on algal photosynthetic capacities are generally higher when using the ^{14}C fixation technique than when measuring with the PAM technique,[48] so the negative effect on photosynthesis in our experiments could have been even greater; however, more work will needed to discriminate the effect in the field. UVR not only affected photosynthesis of mixotrophic assemblages but also phagotrophy. Interestingly both modes were similarly reduced in the three experiments, and in the summer experiment both feeding modes exhibited the maximum reduction (31% in photoinhibition and 28% in ingestion rate). In the three experiments, labeled particles were observed inside mixotrophic cells regardless of the previous field C : nutrient conditions. These results support the idea that these phytoflagellates were never entirely autotrophic. Prey ingestion by bacterivorous mixotrophs could be a way to obtain P[14,57] and simultaneously remove nutrient competitors (bacteria).[10] The N : P ratio (≈65) of Lake Escondido indicates a strong nutrient limitation towards P. Under such limiting conditions, mixotrophs ingest prey mainly to obtain P.[14,57] In extremely transparent lakes with very high light : nutrient ratios mixotrophy appears as a successful strategy, where organisms obtain scarce nutrients (P) through phagotrophy.[15] Under high irradiance phytoplankton can be stressed resulting in an uncoupling between photosynthesis and growing processes, particularly in environments with severe nutrient deficiency.[58] As a consequence, phytoplankton release large amounts of dissolved organic matter of high C : N and C : P elemental ratios.[59,60] These conditions favor algae with phagotrophic capabilities, as mixotrophic ones, which can incorporate important amounts of nutrients through bacteria ingestion.[61] The positive relationship between light intensity and phagotrophy has been considered as a successful strategy for the acquisition of nutrients balancing the C assimilation through the photosynthetic process.[15,23,62] Our bacterivory experiments revealed higher ingestion rates by mixotrophic nanoflagellates exposed to PAR in summer and spring.

The observed reduction of the ingestion rates due to UVR can rise from two different effects: a direct effect on the cell and an indirect effect through a negative impact on photosynthesis that would reduce the requirement of P and N *via* particle uptake. The first assumption would imply a similar effect on mixotrophic and heterotrophic nanoflagellates, whereas the second implies mixotrophic nanoflagellates should be more affected. The observed reduction in bacterivory rates showed a similar sensitivity of mixotrophic and heterotrophic nanoflagellates to low wavelength radiation suggesting that UVR has a direct effect on bacteria ingestion. The decrease in phagotrophy could be associated with alterations in cellular morphology and motility,[17,19]

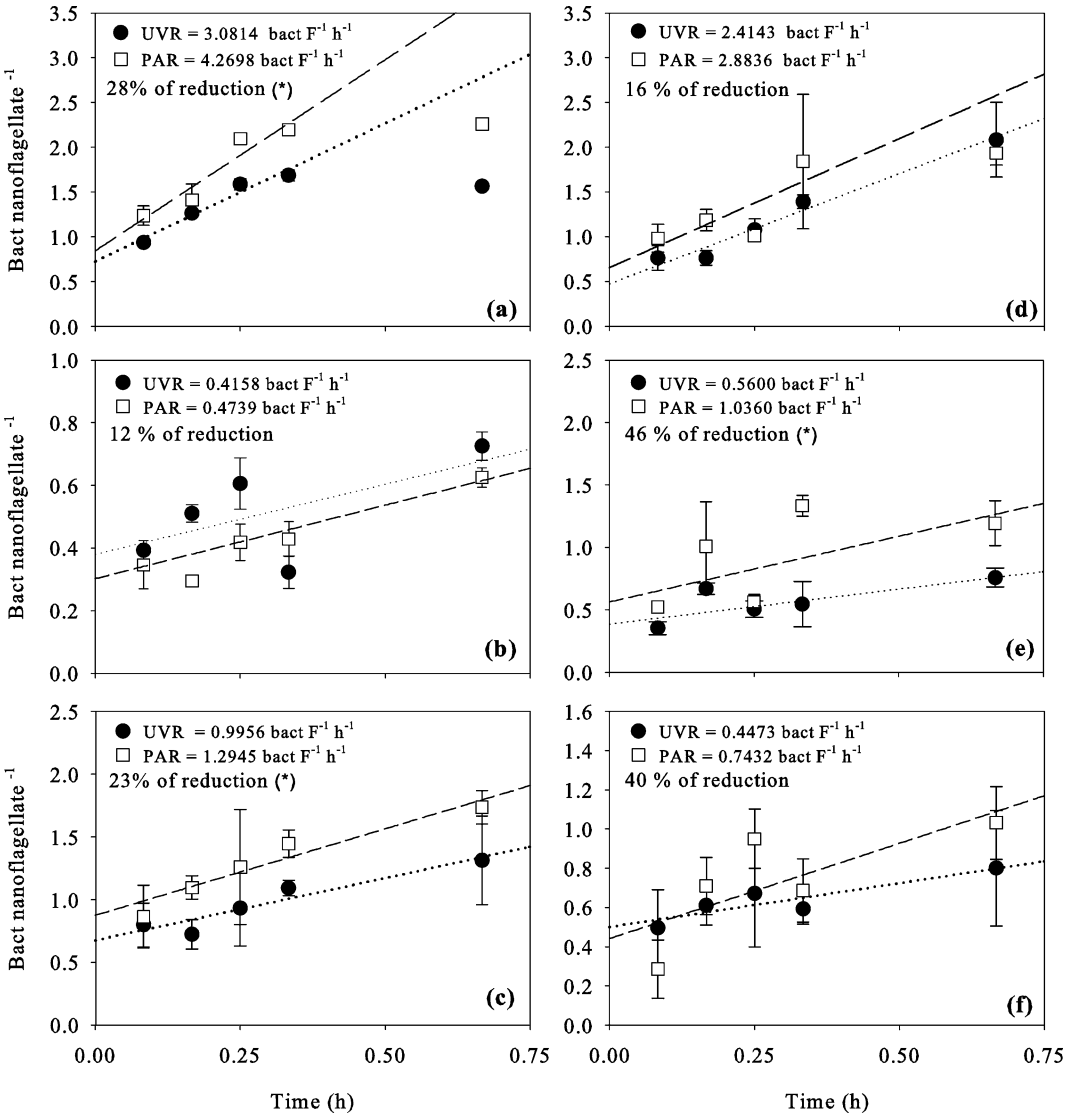

Fig. 6 Ingested bacteria by mixotrophic (a, b and c) and heterotrophic nanoflagellates (d, e and f) in bacterivory experiments under UVR + PAR, and PAR treatment. Summer (a, d), autumn (b, e), and spring (c, f). Values in each graph indicate the slope of the corresponding linear regression. Error bars = 1 standard error. Ingestion rates (bact F^{-1} h^{-1}) in each treatment and the percentage of reduction due to UVR is indicated at the top-right of the graphs. * indicates $P < 0.05$. Note: in (a), the data of 40 min were excluded from the regression analysis for the ingestion rate estimation.

since UVR was observed to induce a severe flagellar damage provoking impairment in flagellar apparatus and leading to the deflagellation of exposed cells.[63] In our experiments we did not observe deflagellation, however we showed that under radiation intensities similar to natural ones (as in our experiments) UVR can induce less damage but enough to reduce ingestion rates.

On the other hand, photosynthesis and bacterivory rates in mixotrophic nanoflagellates can be directly related,[7] so UVR can have an indirect effect on phagotrophy if it affects photosynthesis. In the three experiments we observed that the F_v : F_m ratio was negatively affected, implying a decrease in photosynthesis. This negative effect on the PSII reduces carbon fixation by photosynthesis,[25] therefore UVR lowers the C : P ratio of primary producers.[64,65] Under this scenario, the changes in the C : P of the cells would then reduce the requirement of the limiting element (P), thus decreasing the phagotrophy. In our experiments, we did observed a significant decrease in phagotrophy in summer and spring. However, similar reductions were observed in heterotrophic nanoflagellates, suggesting again a direct effect of UVR on the cells. Consequently, our results suggest that the different cell functions, though affected independently, could be similarly impacted by UVR.

In summary, we have compared through laboratory experiments the effect of summer UVR surface level and natural PAR at 3 m depth on natural nanoflagellate assemblages. In our experiments photosynthesis and ingestion were reduced by UVR; therefore, C transfer would be potentially altered in the microbial food web. The reduction in uptake rate would induce a decrease in the top-down effect of flagellates over bacteria. This may cause an increase in the P competition between bacteria and algae that will increase P limitation for algae.[66] However, the reduction in primary production due to UVR will also reduce the excreted

dissolved organic carbon and so bacteria will suffer a reduction of carbon resource.[23] These two effects (reduction in bacterivory and in dissolved organic carbon production) will be then balanced. Scenarios of increasing UVR must account for effect on the relationship between photosynthetic and phagotrophic processes of mixotrophs. This possibility of interactions between UV effects on photosynthesis and heterotrophy, will need more *in situ* experimental studies to understand exactly what interactions are taking place in the environment and their effect on C : P food web stoichiometry.

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