Are the small-sized plankton communities of oligotrophic ecosystems resilient to UVR and P pulses?

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Abstract: Evaluating how environmental stressor interactions influence ecosystem structure and functioning is critical to understanding the response of ecosystems to global change. We exposed a species-poor planktonic community to P pulses in the absence and presence of solar ultraviolet radiation (UVR). We used a field-mesocosm study in an oligotrophic mid-altitude lake to test the hypothesis that interaction between these factors affects the community's diversity and composition, but not its biomass-size spectrum, making this community resilient in terms of its C-transfer function. Our findings show that P pulses and UVR affected the relative abundance of different planktonic populations. The abundance of phytoplankton was enhanced strongly by P pulses and secondarily by UVR. The UVR × P interaction affected only the smallest autotrophic species. Chlorococcal abundance increased, whereas chroococcal cyanobacteria decreased. However, UVR had a much more pronounced effect than P on the composition of microcrustaceans, and both factors interactively affected their biomass. Hence, the biomass-size spectrum was not resilient to the UVR × P pulse interaction. The steepness of the slope increased under P-pulse conditions because zooplankton were not able to grow concomitantly with phytoplankton, as confirmed by the low zooplankton to phytoplankton biomass ratio (Z : P). The observed planktonic changes under new foreseeable conditions that include an increase in UVR fluxes and P inputs might decouple the C cycle in inland oligotrophic lakes in the Mediterranean region.

Key words: UVR, phosphorus pulse, mesocosm experiment, plankton composition, biomass size spectrum, zooplankton:phytoplankton biomass ratio, picoplankton, nanoplankton, bacteria, phytoplankton, microzooplankton

Global climate change is the result of multiple anthropic stressors that drive a cumulative negative effect on biodiversity and the functioning of ecosystems (Sala et al. 2000, Steffen et al. 2004). The rate of global change is accelerating (IPCC 2014, Williamson et al. 2014, EEA 2015), so the effects of multiple environmental drivers acting at different rates and on local (e.g., eutrophication, drought, increased ultraviolet radiation [UVR]), and global (e.g., ozone depletion, atmospheric dust, global warming) scales are receiving increasing attention. However, their interactive effects are difficult to predict because of the scarcity of multifactorial experiments (Jentsch et al. 2007, Cabrerizo et al. 2014). Thus, more research on the vulnerability of freshwater ecosystems to paired stressors is required (Jackson et al. 2016).

Information on interaction modes and target sites for most stressors is scarce, and studies are needed of complete food webs, in which ecological trade-offs (Kneitel and Chase 2004), stress-induced tolerances (Blanck 2002), and different sensitivity among trophic levels (Vinebrooke et al. 2003, Villar-Argaiz et al. 2016) can be taken into account. Phytoplankton is the base of freshwater aquatic food webs and the main source of organic C in lakes (Schindler 1997). The effects of multiple stressors associated with global change (Kennish et al. 2014) on this trophic level is worrisome and recently has been the focus of numerous studies (see Häder et al. 2015). For example, an increase in UVR harms phytoplankton and zooplankton by affecting several cellular targets (e.g., DNA, photosystems, or membranes) and metabolic processes (e.g., photosynthesis, respiration, growth) (Buma et al. 2003, Beardall and Raven 2004). In contrast, the increase in nutrient inputs in freshwater ecosystems via deposition of atmospheric dust or the intensive use of the sur-

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rounding land (IPCC 2014, EEA 2015) generally benefits primary production of microalgae (Reynolds 1984) and leads to an increase in the biomass of grazing zooplankton (Cottingham and Schindler 2000). However, UVR and nutrient changes affect the diverse planktonic populations with different intensities, thereby modifying community composition. The magnitude of UVR effects on community structure depends on the taxonomic composition of the community. For example, UVR negatively affects diatoms with more intensity than cyanobacteria and has a greater effect on cladocerans than copepods (Jiang and Qiu 2005, Hansson and Hylander 2009, Rojo et al. 2012). An increase in P concentration in aquatic ecosystems, a eutrophication process, changes community composition in ways that follow well-known patterns of substitution of some species for others (Reynolds 1984). Authors of the few studies of the combined effects of allochthonous nutrient inputs and UVR on aquatic ecosystems have reported development of blooms of UVR-tolerant phytoplankton species (Cloern 1996), a pattern of selection that decreases diversity and evenness and shapes the planktonic structure of communities (Delgado-Molina et al. 2009, Medina-Sánchez et al. 2013) and energy fluxes to higher trophic levels (Lewandowska 2011).

Global changes can be even more noticeable when the perturbations occur suddenly as short pulses (Holt 2008). The stressful effect on ecosystems of sporadic extreme weather is well known and is taken into account in reports on climatic change (e.g., Jentsch et al. 2007, IPCC 2014, EEA 2015). However, the effects of UVR and resource pulses are not mentioned in such reports, even though they are global stressors. Extremely low total ozone events of small spatial extent, which last only few days and are caused by climatic changes ('ozone mini-holes') have been reported over southwestern Spain (Antón et al. 2007). The presence of these mini-holes has been related to an increase in recorded UVR in the Mediterranean region (Antón et al. 2007, Mateos et al. 2016). In addition, a growing frequency of P pulses resulting from deposition of atmospheric dust originating from desert areas together with increased anthropogenic waste in the form of sewage (Gallisai et al. 2014, Jickells and Moore 2015) can lead to greater concentrations of limiting nutrients in clear oligotrophic lakes (Cabrerizo et al. 2017, Carrillo et al. 2017). These perturbations could affect the planktonic community structure of freshwater ecosystems in ways that vary over the short to long term (days to months) after P pulses or sudden changes in the quality of light (Rojo and Álvarez-Cobelas 1993, Álvarez-Cobelas et al. 2006a, Carrillo et al. 2008a, 2017). Therefore, the analysis of the interactive effect of UVR and P pulses on freshwater plankton is pertinent.

Body size can be used to understand mechanisms underlying planktonic compositional change because the size distribution of planktonic organisms reflects disturbances in community structure and function caused by sudden changes in environmental factors (summarized by Álvarez-Cobelas et al. 2006a, Marañon 2015). The effect of UVR on the planktonic community depends on the relative abundance of small vs large organisms in the community because small-sized microalgae can be more sensitive than larger species (Xenopoulos and Frost 2003, Häder et al. 2011). Moreover, an increase in P can be exploited more successfully by small fast-growing than by larger primary producers (Reynolds 1984). For example, Marañon (2015) reported that nanoplanktonic algae (2–20 μm) used nutrient enrichment for growth more efficiently than did larger microplanktonic species.

The response of phytoplankton to UVR and P pulses can depend on the size structure of the zooplankton in the community. Large herbivorous zooplankton exerting strong grazing control of algal growth can buffer phytoplankton responses to abiotic factors (Cottingham and Schindler 2000, Cottingham et al. 2004). Consequently, the phytoplankton and the whole planktonic community size spectra (PhSS and PSS, respectively) are sensitive to P pulses (Sprules and Munawar 1986, Álvarez-Cobelas et al. 2006a, Marañon 2015) and, presumably, to UVR changes. Productive ecosystems have large planktonic food webs with a wide range of phytoplankton body sizes and large and effective consumer zooplankton, and thus, a PSS sensitive to increasing resources (Sprules and Munawar 1986, Marañón 2015). On the other hand, the PSS of small food webs is resilient to nutrient pulses (Gaedke and Kamjunke 2006). The resilience of PSS is relevant for the overall aquatic community because changes in the slope of the PSS affect the relationship between the sizes of predators and prey and the zooplankton to phytoplankton biomass ratio (Heneghan et al. 2016). Variations in the slope of the PSS involve changes in aquatic foodweb transfer efficiency (Friedland et al. 2012, Havens and Beaver 2013) and, thus, in the effectiveness of C transfer through the food web (Sprules and Munawar 1986, Gaedke 1993, San Martin et al. 2006).

Despite the fact that the effects of UVR and P pulses on plankton are indisputable, no studies have been published in which investigators directly evaluated the effects of UVR × P on PSS. Moreover, given that the PhSS and PSS resilience to nutrient pulses and UVR changes depend on the relative proportions of small- vs large-sized microalgae and large-sized consumers in the community, a new question emerges: How do P pulses and UVR jointly affect species-poor communities composed of small organisms? Our goal in this experimental study was to test the following hypothesis: Planktonic communities in oligotrophic lakes characterized by a low-diversity food web, where both producers and consumers are small in size, respond to UVR × P pulses (UVR × P) with shifts in composition and diversity, but not their PSS, which is resilient.

Mesocosm experiments are useful for understanding natural communities (Jeppesen et al. 2000, Cottingham et al.

2004, Benton et al. 2007), so we tested our hypothesis by performing a mesocosm-based factorial experiment. We designed the experiment primarily to examine potential interactive effects of UVR and nutrients on plankton communities, i.e., to test whether plankton communities respond differently to UVR in the presence or absence of resource enrichment. We focused our study on the overall plankton community (bacteria to microcrustaceans) and quantified their response after 18 d. We conducted the mesocosm experiments in La Conceja Lake, a medium-altitude, oligotrophic lake situated in the central region of the Iberian Peninsula. This lake may receive P inputs from Saharan dust (Álvarez-Cobelas et al. 2006b) and undergoes short-term increases in UVR through ozone mini-holes (Mateos et al. 2016).

METHODS

Study site and experimental design

We carried out the experiment from 11 to 28 July 2009. This timespan is sufficient to monitor variations in growth of populations from bacteria to small microzooplankton (Gillooly 2000, Rojo et al. 2012). We decided on this duration of experiment based on a trade-off between providing enough time to allow changes in density of all populations and minimizing enclosure bias or effects caused by enclosures limitation (Stewart et al. 2013).

We placed mesocosms in La Conceja Lake in the Ruidera Lakes Natural Park in central Spain (lat 38°55′N, long 2° 48′W; 850 m asl; Fig. S1A, B). This lake has a surface area of 29 ha and a maximum depth of 14 m. The mixing layer was 2 m based on the temperature profile (Fig. S2). NO_3^- concentration is elevated and can be >40 mg/L (total N [TN] up to 16 mg N/L) because of agricultural use of the surrounding land. Nevertheless, the lake is considered oligotrophic and P-limited (total P [TP] < 20 μg P/L). Dissolved organic C (DOC) content ranges from 1.5 to 3.5 mg C/L. More information on the environmental conditions of the lake on the days of the experiment was published by Rojo et al. (2012).

We used a 2 × 2 factorial design to study the interactive effects of 2 factors (solar radiation and nutrients) on the entire planktonic community. Solar radiation had 2 levels: sunlight including ultraviolet radiation (UVR) or sunlight excluding UVR (photosynthetically active radiation [PAR] only), and nutrients had 2 levels: ambient (P-control) and P-pulse. The in situ experimental treatments consisted of 4 conditions (2 × 2 levels) × 3 replicates and was conducted in 12 mesocosms constructed from polyethylene plastic bags (each 600 L, 0.7-m diameter, 1.6 m deep) situated in the central area of the lake (Fig. S1B–D). The polyethylene plastic transmits 90% of the PAR (400–700 nm), 60% of the UVB (295–319 nm), and 75% of the UVA (320–399 nm) for the UVR treatments. A modified form of polyethylene that blocks UVR (transmitting <1% of the light ≤380 nm and 85% of the light ≥400 nm) was used for the PAR treatments (Souza et al. 2010). The optical features of this form of polyethylene were evaluated before the experiment with a double beam spectrophotometer (UV2450; Shimadzu, Kyoto, Japan).

We filled the mesocosms with water pumped from depths of 0 to 1.6 m (within the mixing layer), which is where >99% of the UVB at 305 nm is absorbed (Rojo et al. 2012). We randomly selected 3 mesocosms for initial sampling to measure initial experimental abiotic conditions and planktonic community composition. Each mesocosm was covered with a lid made from a UVR Opak 395 filter (Ultraphan; Difegra, Germany) or polyethylene for the PAR and UVR treatments, respectively. For the P-pulse treatments, we add KH_2PO_4 in 2 consecutive pulses on days 1 and 11 of the experiment at a final concentration of 30 μg P/L each to simulate the natural nutrient inputs from the dust that blows from the Sahara Desert to southern Spain (Morales-Baquero et al. 2006, Carrillo et al. 2015, Cabrerizo et al. 2017).

Sampling and measurement of physical, chemical, and biological variables

We measured vertical profiles of solar radiation and temperature with a BIC compact 4-channel underwater radiometer (Biospherical Instruments, San Diego, California). We measured light irradiance at 3 channels in the UVR range (305, 320, and 380 nm) and for PAR (400–700 nm). We calculated the vertical diffuse attenuation coefficient for downward radiation (k_d, 1/m) as the slope of the linear regression of ln(downwelling irradiance) vs depth for each region of radiation.

We sampled at the start and end of the experiment. Before sampling, we mixed the water column in the mesocosms gently. We collected integrated-depth samples with a 5-L plastic bucket to obtain a 1-L homogeneous sample of water from each mesocosm for use in all chemical and biological analyses.

In the laboratory, we measured TP and TN by treating 50-mL subsamples of each water sample with potassium persulfate at 120°C for 30 min before analysis for soluble reactive P (SRP) and NO_3^-, respectively. We also filtered 50-mL subsamples through 0.7-μm glass-fiber filters (GF/F Whatman) and measured NO_3^- (UV spectrophotometric screening), NH_4^+ (phenol–hypochlorite technique), and SRP (acid molybdate technique) (APHA 1992). To measure dissolved organic C (DOC), we filtered 25-mL subsamples through precombusted (2 h, 500°C) 0.7-μm glass-fiber filters and acidified them with 100 μL of 37% HCl before analyzing them with a total organic C analyzer (TOC-VCSH; Shimadzu, Kyoto, Japan).

We quantified the density (individuals [ind]/L), biovolume (μm^3/L), and biomass (μg C/L) of the planktonic populations in each mesocosm. We used 20-mL subsamples

to quantify picoplankton (cells between 0.2 and 2 μm), bacteria (heterotrophic picoplankton), and autotrophic picoplankton (APP). We quantified phytoplankton, heterotrophic nanoflagellates [HNF], and small ciliates in 250-mL subsamples. To quantify other zooplankton (rotifers and microcrustaceans) we filtered the remainder of the 5 L (4.6 L) through a 45-μm mesh. We preserved bacteria and APP samples in 2% neutralized formaldehyde and nanophytoplankton and zooplankton samples in 1% vol:vol alkaline Lugol's solution.

Determination of community composition

We counted bacteria, APP, phytoplankton, HNF, ciliates, and zooplankton with the aid of an inverted microscope (AX10; Carl Zeiss Microscopy GmbH, Göttingen, Germany). For bacteria, we stained 2 mL of the water sample with 4',6-diamidino-2-phenylindole (DAPI) for 20 min in the dark and counted the cells following the procedure described by Porter and Feig (1980). We counted ≥1000 cells and measured them with ImageJ image analysis software (Abràmoff et al. 2004). We calculated bacterial biomass based on biovolume estimates following the formulae published by Loferer-Krößbacher et al. (1998) and as recommended by Posch et al. (2001) for DAPI-stained samples.

We conducted APP filtration, counting, and enumeration as recommended in the review by Callieri and Stockner (2002) for *Synechococcus*-like cells. We filtered the samples onto 0.2-μm black polycarbonate filters (Millipore Corporation, Bedford, Massachusetts) and examined the filters by autofluorescence under an epifluorescent microscope (Fluovert FS; Leitz, Wetzlar, Germany). We enumerated phytoplankton, HNF, and ciliates with an inverted microscope at 100, 400, and 1000× magnification. We sedimented the 250-mL water subsamples in Utermöhl chambers and counted >400 individuals of the more abundant species in each sample with a 10% probability of error (95% confidence limit; Lund et al. 1958). We calculated individual planktonic biovolumes by the methods published by Hillebrand et al. (2002) and converted biovolumes to biomass (μg C) following recommendations by (Menden-Deuer and Lessard 2000). We multiplied the biovolume of zooplankton by 1.1 to obtain wet mass, used a factor of 0.25 to convert wet mass to dry mass, and a factor of 0.40 to transform the dry mass to C content (Reiss and Schmid-Araya 2008, Martínez-Lozano et al. 2011, Yvon-Durocher et al. 2011).

Richness and evenness calculations

Hereafter, 'bacteria' includes heterotrophic picoplankton; 'phytoplankton' includes APP and nanophytoplankton; and 'zooplankton' includes HNF, ciliates, rotifers, and microcrustaceans. We estimated richness separately for phytoplankton, zooplankton, and the full planktonic community as the sum of the constituent taxa (at the species level) found in each mesocosm. Our calculations of diversity were based on biomass instead of abundance. The use of biomass eliminates the problem of an uneven distribution of the different taxa because of the huge abundance of the smaller classes of organisms (e.g., bacteria or APP) compared to that of the larger organisms (e.g., large zooplankton) and reflects the true contribution of individual species to the phytoplankton community composition (Interlandi and Kilham 2001). We calculated Simpson's diversity index independently for phytoplankton, zooplankton, and the complete planktonic community as 1 – the Simpson concentration (Keylock 2005), taking into consideration the richness and the relative biomass of each species. We calculated evenness as the Simpson diversity index divided by the maximum potential value of the index (Smith and Wilson 1996).

Plankton community and phytoplankton assemblage size spectra

We obtained PSS and PhSS for each mesocosm based on methods published by Reuman et al. (2008) and Yvon-Durocher et al. (2011). In brief, we divided the total range of $\log_{10}$(individual biomass) (μg C) values into 10 logarithmic size classes of equal width. Next, we regressed the $\log_{10}$-transformed values of the total population abundance (ind/mL) of all organisms in each size class against the median value of the size class. From these regressions, we analyzed: 1) the slope of the linear model that defined how the abundance of individuals declined as their size increased, and 2) the intercepts at the highest and lowest values of x (corresponding with the largest- and smallest-sized organisms observed), which provided information on their abundance (Yvon-Durocher et al. 2011).

Statistical analysis

We used R (version 2.15.2; R Project for Statistical Computing, Vienna, Austria) for most analyses. The *stats* package was used to calculate the slopes and intercepts of the size spectra and for the 2-way analyses of variance (ANOVAs) used to study the combined effects of UVR and P pulses on richness, evenness, the slopes and intercepts of the biomass size spectra, and the biomass of the phytoplankton. When we found a significant interactive effect of the factors on a response variable, we used the *multcomp* package in R to perform post hoc Tukey's Honestly Significant Difference (HSD) tests to identify differences among treatment means.

We used multivariate permutational ANOVA (PERMANOVA; Bray–Curtis index and 1000 bootstrap replicates) based on the species biomass matrix to test for differences in community composition among the 4 experimental conditions. To highlight similarities among the communities, we used a multivariate ordination analysis based on a species biomass matrix (log[x + 1]-transformed values).

We based classification and ordination on their Euclidean distance measurements and the unweighted pair-group average (UPGMA) method. We performed 1000 bootstrap replicates. The percentage of replicates in which each node was supported is given on the resulting dendrogram. We ordered the treatments based on their communities, and species were assembled based on their common development in the different treatments. We used the free PAST software (version 2.17c; Hammer et al. 2001) to run PERMANOVA, classification, and ordination analyses.

We ran a redundancy analysis (RDA; *vegan* package in R) in which environmental variables were used as constraining variables to assess how they affected taxonomic composition. The selected variables were UVB (I_0; W m^{-2} nm^{-1}), TP (μg P/L), SRP (μg P/L), dissolved inorganic N (DIN; mg N L^{-1}), and the DIN : SRP ratio. Last, we analyzed the full planktonic community and the phytoplankton and zooplankton assemblages based on the biomass of each taxon identified in the mesocosms.

RESULTS

Initial abiotic conditions and planktonic community

UVR was strongly attenuated in the upper water layers of the lake, as indicated by the high k_d values (Fig. S2), and only 1% of the UVR_{305} reached a depth of ~1.5 m. In contrast, up to 2% of the surface irradiance of both UVR_{380} and PAR reached the bottom of the lake (14 m). The vertical temperature profile was stable throughout the experiment, with a thermal discontinuity at 2 to 4 m (variation ~1°C) and a thermocline at 8 to 12 m (variation >2°C; Fig. S2). At the beginning of the experiment, La Conceja lake had a high concentration of TN but a low concentration of TP, which resulted in a high DIN : TP ratio of 3221 (by mass) and a DIN : SRP (molar) ratio of 16,766, indicating that the availability of P in the lake was limited (Table S1).

The planktonic community at the beginning of the experiment was composed mostly of small organisms and included auto- and heterotrophic picoplankton, nanophytoplankton, some ciliates the size of nanophytoplankton (2–20 μm), and zooplankton <800 μm. The phytoplankton had a mean biomass (± SE) of 50 ± 8 μg C/L, whereas the average zooplankton biomass was 17 ± 2 μg C/L. Centric diatoms were dominant in the phytoplankton size range. Zooplankton biomass consisted predominantly of cladocerans and copepods (Fig. S3). HNF were not detected.

Effects of UVR and P pulses on plankton biomass

UVR, P pulse, and the UVR × P interaction did not affect bacterial biomass (ANOVA, all $p > 0.05$). Mean (±SE) bacterial biomass across all treatments was 2.32 ± 0.24 μg C/L.

The UVR × P interaction did not affect total phytoplankton biomass (Table 1, Fig. 1A). However, the UVR × P interaction exerted a positive effect on cyanobacteria (*Synechococcus* sp.) and a negative effect on chlorophytes (*Chlorocystis* sp.) (Table 1, Fig. 1B). P pulses significantly increased phytoplankton biomass (Table 1, Fig. 1A) by increasing the biomass of chlorophyceans and cyanobacteria (Table 1, Fig. 1B, C). P pulses also affected the percentage of total biomass of some phytoplanktonic groups, such as chlorophyceans (*Chlorocystis* sp.; 11%; Fig. 1E), the dinoflagellate *Peridinium umbonatum* (8%), and chrysophytes (3%) (Table 1). In contrast, diatoms (strongly dominated by small centric cells), which accounted for 97% of phytoplankton biomass in ambient nutrient treatments, decreased significantly to 74% (mean decline = 25%) in the presence of P pulses (Fig. 1D).

The UVR × P interaction significantly affected zooplankton biomass (Table 1). The lowest zooplankton biomass occurred under UVR and ambient nutrient conditions (Fig. 1F). Under these conditions, the copepod *Tropocyclops prasinus* was the dominant species (52%), followed by cladocerans (*Diaphanosoma brachyurum* [36%]), and rotifers (10%). Cladocerans made up almost 100% of the zooplankton biomass under the other 3 conditions (Fig. 1G–J). *Diaphanosoma brachyurum* was the dominant species in the PAR/P-control treatment, whereas *D. galeata* was dominant in the P-pulse treatment regardless of the light quality. Rotifers and ciliates were scarcely represented under conditions in which cladocerans dominated, and they did not differ among treatments.

Based on the data from the all mesocosms, zooplankton and phytoplankton biomasses were not correlated (Fig. 2A; $r = -0.38$, $p = 0.23$). In most cases, the zooplankton:phytoplankton (Z : P) biomass ratios were <1, and increases in phytoplankton were not followed by increases in zooplankton biomass. The effect of the UVR × P interaction on the Z : P biomass ratio (Table 1) occurred mainly because zooplankton reached their highest biomass under ambient nutrient conditions when UVR was removed (Fig. 2B).

Effects of UVR and P on plankton composition and richness

Differences in phytoplankton composition among the experimental conditions were caused exclusively by P pulses (PERMANOVA; Table 2). Zooplankton composition was sensitive to P pulses, UVR, and the UVR × P interaction (Table 2). Similar results were obtained when the entire plankton community was compared among the treatments, but the UVR effect was marginally significant (Table 2). Phytoplankton composition differed clearly between the communities inhabiting the mesocosms with and without P pulses (Fig. S4A). However, zooplankton composition clustered first based on communities in unmanipulated conditions (UVR/P-control) vs communities in other treatments, and second based on responses to P pulses (Fig. S4B). When all of the plankton populations were taken into account (Fig. S4C), the clusters with the best statistical support divided communities under ambient nutrient condi-

Table 1. Results of 2-way analyses of variance for the effects of ultraviolet radiation (UVR) and P pulses and their interaction on different features of the plankton communities in experimental mesocosms in La Conceja lake (central Spain). Treatments were sunlight including UVR or sunlight excluding UVR (photosynthetically active radiation only) with and without P supply. Only variables significant for ≥1 treatment factor are shown. Z : P = ratio between phytoplankton and zooplankton biomass, PSS = slope and intercept of full community size spectrum, PhPSS = slopes and intercepts of phytoplankton size spectra (PhPSS). Statistically significant values are shown in bold ($p \leq 0.05$).

	UVR		P pulses		Interaction	
Variable	*F*	*p*	*F*	*p*	*F*	*p*
Biomass						
Phytoplankton	0.00	0.977	6.11	**0.039**	1.44	0.265
Chlorophyceae	81.97	**<0.001**	1248.71	**<0.001**	84.77	**<0.001**
Cyanobacteria	23.45	**0.001**	101.23	**<0.001**	36.48	**<0.001**
% Bacillariophyceae	1.24	0.299	61.43	**<0.001**	1.16	0.313
% Chlorophyceae	2.28	0.169	79.08	**<0.001**	2.39	0.161
% Chrysophyceae	2.58	0.147	6.75	**0.032**	2.59	0.146
% Dinophyceae	0.53	0.487	4.93	**0.050**	0.45	0.521
Zooplankton	12.57	**0.008**	0.16	0.702	10.56	**0.012**
Cladocera	16.31	**0.004**	0.05	0.832	16.64	**0.004**
Copepoda	91.47	**<0.001**	97.64	**0.000**	117.37	**<0.001**
% Cladocera	9.18	**0.016**	6.79	**0.031**	11.96	**0.009**
% Copepoda	15.32	**0.004**	15.46	**0.004**	16.27	**0.004**
Total plankton	7.74	**0.024**	5.20	0.052	1.72	0.226
Z : P	19.07	**0.002**	10.49	**0.012**	20.43	**0.002**
Diversity						
Phytoplankton evenness	0.10	0.761	8.68	**0.019**	0.19	0.675
Zooplankton richness	0.10	0.760	2.50	0.153	16.90	**0.003**
Zooplankton evenness	3.19	0.112	0.75	0.412	7.30	**0.027**
Size spectra						
Community slope	0.81	0.393	25.86	**0.001**	4.41	0.069
Community intercept at $x = -9$	2.88	0.128	144.03	**<0.001**	3.89	0.084
Phytoplankton intercept at $x = -9$	1.12	0.318	126.48	**<0.001**	0.01	0.971
Phytoplankton intercept at $x = -2$	0.45	0.520	5.20	**0.050**	0.71	0.423

tions from communities under the P-pulse conditions and secondarily separated communities grown with or without UVR. Moreover, some clusters emerged (Fig. S4D) that could be related to the different treatments. The RDA analysis showed that the TP explained 44% of the variance in the overall plankton composition, and together with UVB, explained 57% (Table 3). Similar results were found when only the zooplankton matrix was used in the analysis. TP plus UVR explained 64% of the variance. However, when the analysis was done with the phytoplankton matrix, only the SRP was selected, and this environmental variable explained 35% of the variance (Table 3).

The only diversity measure that responded to P pulses was phytoplankton evenness (Table 1, Fig. S5B). UVR did not affect any variable related to diversity. However, the presence of UVR significantly affected the response of zooplankton diversity to P pulses (Table 1, Fig. S5C). In the presence of UVR, the zooplankton richness was significantly lower in the P-pulse than in the P-control treatment. The assemblage of *T. prasinus*, cladocerans, and rotifers in the UVR/P-control treatment was replaced almost exclusively by *D. galeata* in the UVR/P-pulse treatment. The lowest zooplankton evenness was observed when *D. brachyurum* became dominant, displacing *T. prasinus*, cladocerans, and rotifers in the UVR/P-control treatment.

Effects of UVR and P pulses on plankton size spectra

P pulses significantly affected the PSS, but UVR did not (Table 1). Small-sized plankton (bacteria and small phytoplankton; biomass range 6×10^{-9}–2×10^{-3} μg C) were relatively more abundant than zooplankton (biomass range 6×10^{-3}–2 μg C) in the P-pulse treatments (Fig. 3A). The steepness of the slope significantly increased from −0.68 ±

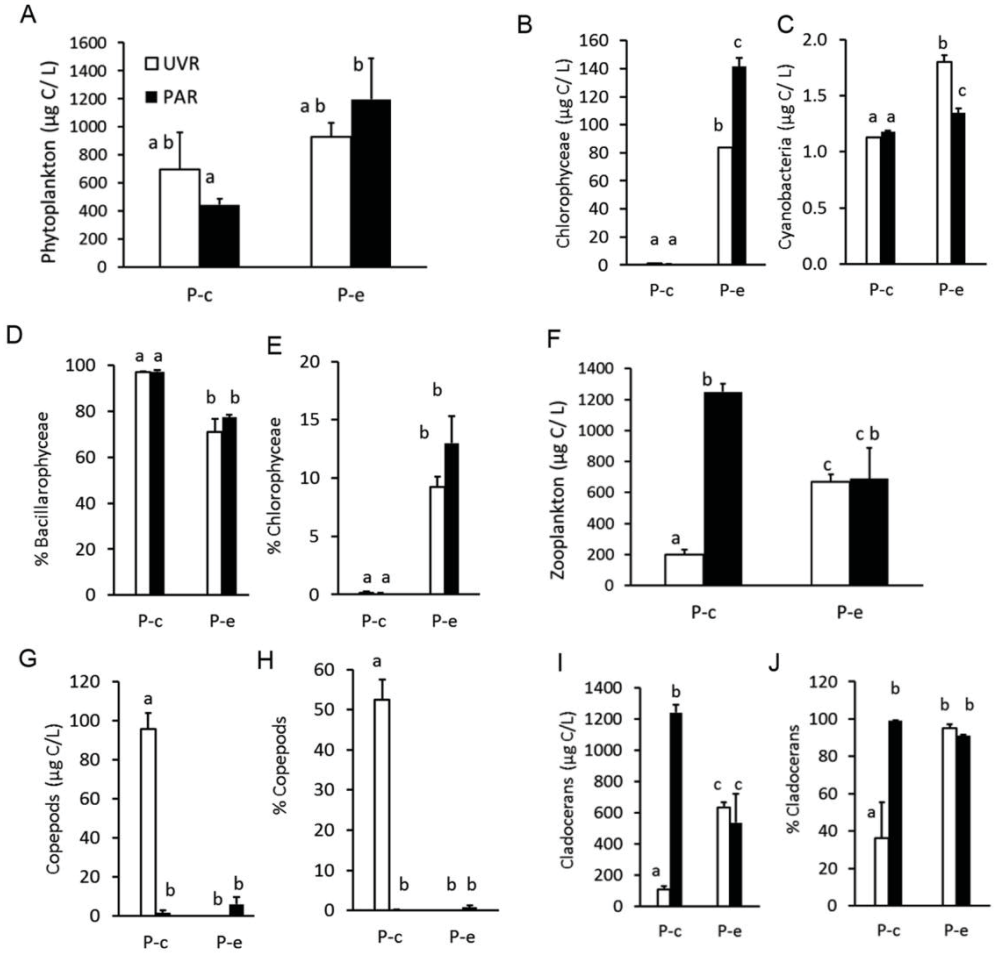

Figure 1. Mean (+SE; $n = 3$) phytoplankton (A), Chlorophyceae (B), and Cyanobacteria (C) biomass, % Bacillariophyceae (D), % Chlorophyceae (E), zooplankton (F), and copepod (G) biomass, % copepods (H), cladoceran biomass (I), and % cladoceran (J) in 4 treatments: sunlight including ultraviolet radiation (UVR) or sunlight excluding UVR (photosynthetically active radiation [PAR] only) with (P-e) and without (P-c) P supply. Bars with the same letters are not significantly different ($p > 0.05$).

0.06 in the P-control treatments to -0.81 ± 0.03 in the P-pulse treatments (Fig. 3B). Likewise, the y-intercept at $x = -9$ increased from 5.6 ± 0.3 in the P-control treatments to 6.9 ± 0.1 in the P-pulse treatments. In contrast, the intercept for the largest size fractions ($x = 1$) did not differ among treatments. The largest size fraction was represented by small copepods (<500 µm) under UVR/P-control conditions and by small cladocerans (<800 µm) under the other experimental conditions. Moreover, P pulses increased the relative abundance of the smaller size classes, altering the PSS (slope and intercept on the y-axis). We analyzed the PhSS for a more accurate description of the variation in the smaller-sized classes (Fig. 3C). Slopes did not differ among treatments, but both intercepts ($x = -9$ and $x = -2$) were significantly higher in the P-pulse treatments (Fig. 3D), reflecting a proportional increase in the biomass of all phytoplanktonic size classes. Therefore, the difference found in the slopes for the entire planktonic community was the result of a relatively minor increase in zooplankton abundance compared to the phytoplankton increase in the P-pulse treatments (Fig. 3A).

DISCUSSION

This study fills a gap in knowledge regarding how the structure, including the biomass, diversity, taxonomic composition, and full size spectra, of a species-poor planktonic community dominated by small-sized species responds to the interaction between UVR and a nutrient-pulsed resource. We are aware of the potential limitations

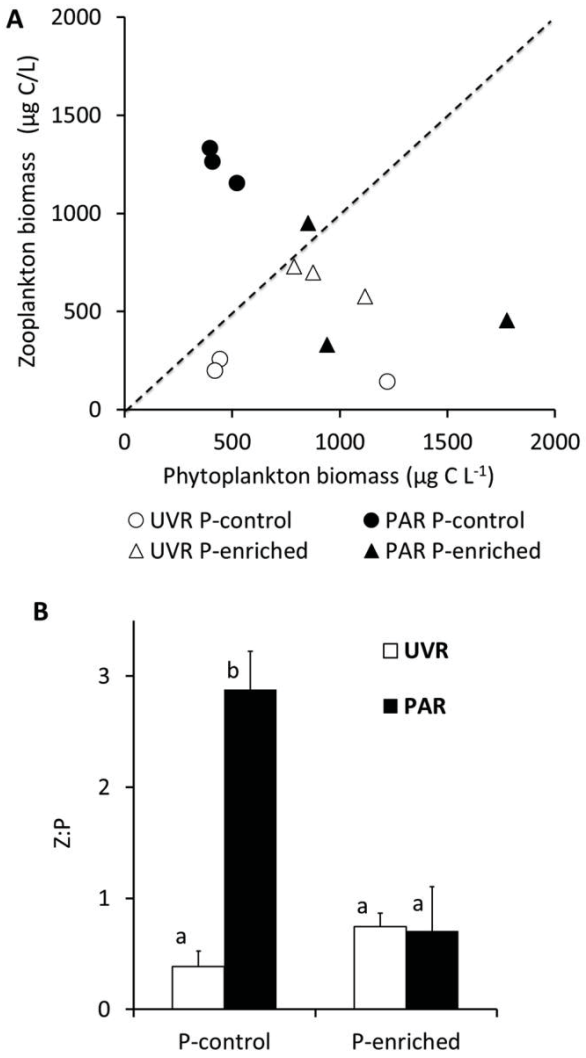

Figure 2. A.—Scatterplot showing phytoplankton vs zooplankton biomass for each mesocosm at the end of the 18-d experiment in the La Conceja lake. The dashed line marks 1 : 1 ratio. B.—Mean (+SE) ratio of zooplankton:phytoplankton biomass (Z : P) biomass ratios in each treatment. See Fig. 1 for treatment abbreviations. Bars with the same letters are not significantly different ($p > 0.05$).

of small-scale experiments when used to explain effects of global change, but they are an excellent approach to quantifying the effects of multiple stressors and their interactions and to highlighting the underlying mechanisms and processes (Spivak et al. 2010, Stewart et al. 2013). We think that our experimental approach was suitable because: 1) the temporal scale was fitted to the generation time of the small-sized planktonic species, 2) the UVR treatment reproduced the natural optical conditions in the water column, and 3) P-pulses were in the range of those measured in the southern Mediterranean area (Morales-Baquero et al. 2006, Cabrerizo et al. 2017).

Our findings did not support our initial hypothesis concerning the resilience of this community in relation to P pulses and UVR abiotic stressors because the relative abundance of the organisms of each size varied in the phytoplanktonic range, but not in the zooplankton range. Thus, UVR and P pulses generated alternative species assemblages that changed the relative proportions of the biomass, taxonomic composition, and PSS, thereby resulting in species-specific responses to P pulses and UVR global-change stressors. The RDA based on the abiotic environmental variables measured in the mesocosms indicated that primarily P availability and secondarily UVB irradiance explained the planktonic community composition. P pulses and UVR explained almost 60% of the variability in the assemblages from the different treatments. Our findings extend the ecology paradigm that light and UVR and nutrient trade-offs determine community structure (Sterner et al. 1997, Litchman and Klausmeier 2008).

UVR and P pulses did not affect the heterotrophic picoplankton. The consistency in the heterotrophic picoplankton agrees with previous studies showing no clear effect of P pulses on bacterial abundance based on experimental

Table 2. Results of 2-way permutational analysis of variance for the effects of UVR and P pulses and their interaction on the species biomass matrix in experimental mesocosms in La Conceja lake in central Spain. Analyses were done with phytoplankton data only, zooplankton data only, and overall plankton communities. Treatments were sunlight including ultraviolet radiation (UVR) or sunlight excluding UVR (photosynthetically active radiation [PAR] only) with and without P supply.

Source	SS	df	MS	*F*	*p*
Phytoplankton					
P	0.409	1	0.409	18.520	0.001
UVR	0.008	1	0.008	0.365	0.766
Interaction	0.049	1	0.049	2.208	0.114
Residual	0.177	8	0.022		
Total	0.643	11			
Zooplankton					
P	0.958	1	0.958	22.029	0.000
UVR	0.283	1	0.283	6.499	0.012
Interaction	0.334	1	0.334	7.675	0.006
Residual	0.348	8	0.043		
Total	1.923	11			
Plankton					
P	0.522	1	0.522	18.602	0.000
UVR	0.090	1	0.090	3.196	0.056
Interaction	0.120	1	0.120	4.264	0.025
Residual	0.225	8	0.028		
Total	0.956	11			

Table 3. Redundancy analysis results relating the overall plankton, zooplankton, and phytoplankton matrices to environmental physicochemical features. Cum. adjR^2 = cumulative adjusted explained variance, TP = total P, UVB = ultraviolet B irradiance, SRP = soluble reactive P.

Matrix	Cum. adjR^2	F	p
Plankton biomass			
TP	44%	9.7	0.01
UVB	57%	4.1	0.02
Zooplankton biomass			
TP	49%	11.6	0.00
UVB	64%	5.3	0.01
Phytoplankton biomass			
SRP	35%	6.6	0.03

and observational approaches (Villar-Argaiz et al. 2002, Carrillo et al. 2008b). Under the assessed conditions, a trade-off seems to have occurred between cell damage and growth because of C release by the phytoplankton under UVR (Medina-Sánchez et al. 2013). However, a UVR × P effect was observed for autotrophic picoplankton. UVR inhibited chlorococci (*Chlorocystis* sp.) and slightly favored cyanobacteria (*Synechococcus* sp.) when P pulses occurred. These results were consistent with those previously found in field and laboratory studies on the effect of UVR on the plankton community in La Conceja lake (Rojo et al. 2012). Cyanobacteria have mechanisms to cope with the stress of UVR exposure (Castenholz and Garcia-Pichel 2000), such as repairing the damage caused by UVB (Jiang and Qiu 2005, 2011). Such mechanisms are more successful when the environment is not P limited (Yang et al. 2014).

UVR irradiance did not seem to have a negative effect on phytoplankton in the subsurface mixed layer of La Conceja lake. Phytoplankton that inhabit a mixed layer where UVB varied from the maximum value 0.05 W m^{-2} nm^{-1} to undetectable can adjust physiologically to the new light environment in much less time than our experiment lasted (Xenopoulos and Schindler 2003). However, P pulses strongly stimulated phytoplankton biomass, although the increase was not related to size within the nanophytoplankton range. A mechanistic explanation is that the similar small size of all populations implies a similar P uptake rate among them, so they show weak differences in their growth after P pulses (Marañón et al. 2013). Therefore, despite the higher values observed in the intercepts after the P pulses, the steepness of the PhSS slopes remained similar, suggesting resilience of the PhSS.

In contrast, zooplankton showed clearer responses to UVR than to P pulses. The small cyclopoid copepod *T. prasinus* and the smaller filter-feeding cladoceran *D. brachyurum* (Geller and Müller 1981), which were present under initial conditions, may have been able to coexist in the presence of UVR because they selectively consume par-

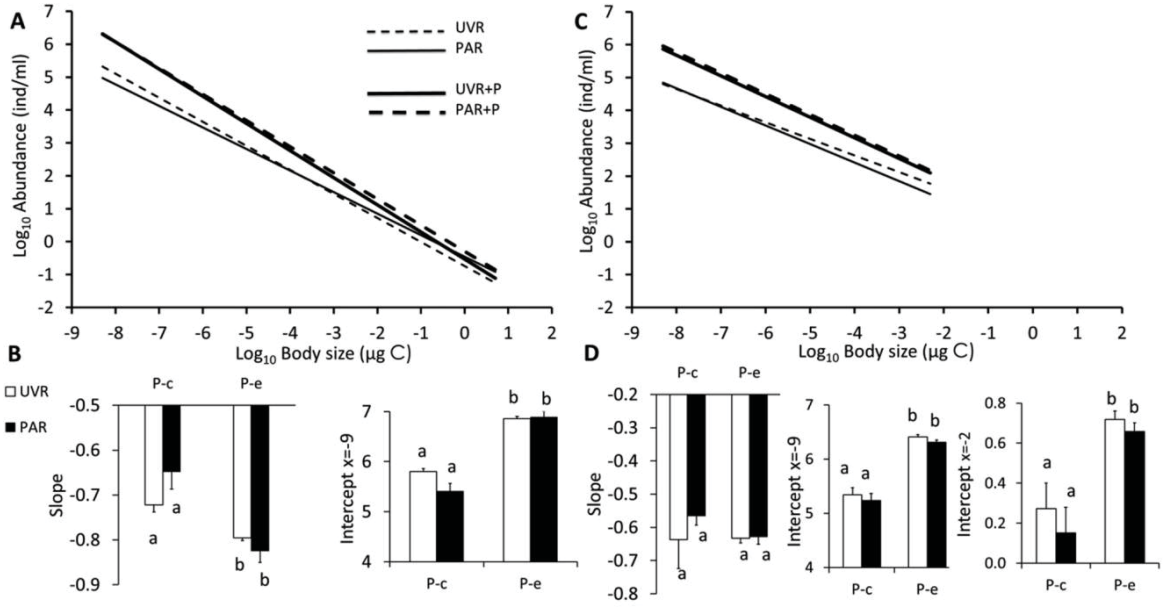

Figure 3. Regressions for plankton (A) and phytoplankon (B) body size spectra in the 4 experimental treatments, and mean (+SE) slopes and intercepts of plankton spectra at $x = -9$ (C) and phytoplanktonic spectra at $x = -2$ and -9 (D). ind = individuals. See Fig. 1 for treatment abbreviations.

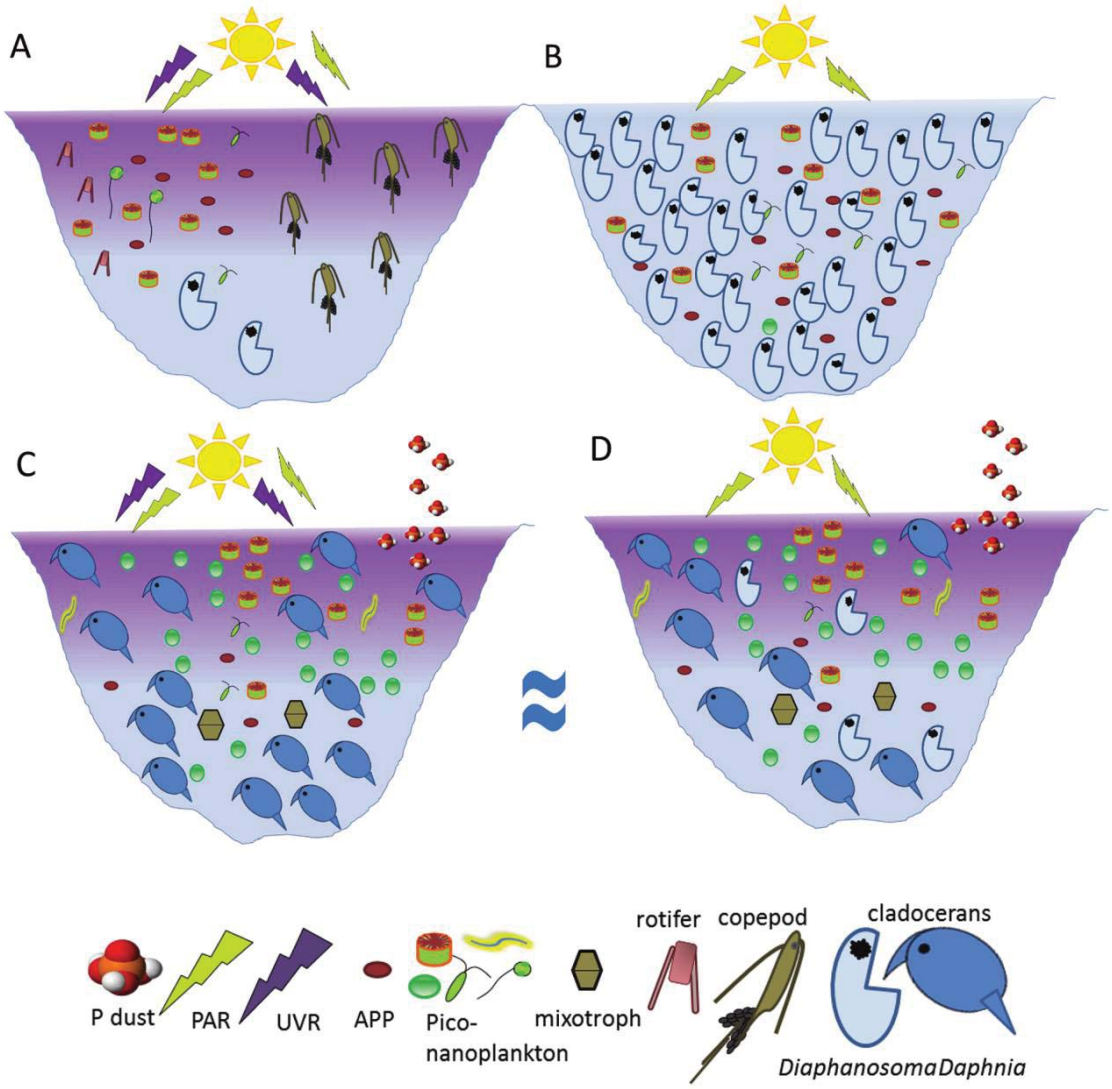

Figure 4. Four alternative communities in the upper water layer affected by ultraviolet B (UVB) radiation based on the presence or absence of ultraviolet radiation (UVR), P pulses, and UVR × P interaction. A.—Light with photosynthetically active radiation (PAR) and UVR and no P pulse with plankton dominated by copepods. B.—Light with PAR only (UVR removed) and no P pulse with *Diaphanosoma brachyurum* bloomed. C.—Light with PAR and UVR and P deposition with *Daphnia galeata* dominant. D.—Light with PAR only and P deposition with both *D. brachyurum* and *D. galeata* present. The number of drawn animals from each species is proportional to their densities in the mesocosms.

ticles of different sizes (Cottingham et al. 2004, Sommer and Sommer 2006). When UVR was excluded, cladocerans were favored and became dominant. UVR tolerance in zooplankton is related to taxonomic composition (Persaud et al. 2007) rather than to organism size (Leech and Williamson 2000). In addition, copepods have higher amounts of mycosporines and carotenoids than cladocerans, and thus have greater protection against harmful UVR effects (Tartarotti et al. 2001, Gonçalves et al. 2002, Persaud et al. 2007). After P pulses, the cladoceran *D. galeata*, a medium sized filter-feeding cladoceran (Geller and Müller 1981), was the dominant consumer replacing the copepod *T. prasinus* and the smaller sized filter-feeding cladoceran *D. brachyurum*. Cladocerans, which are more efficient filter-feeders than copepods, have an advantage when feeding is improved (e.g., smaller size and stoichiometrically better food) by P pulses (Gliwicz 1990, Elser et al. 1996). For instance, Villar-Argaiz et al. (2012) found that P-rich alloch-

thonous loads from Saharan-dust deposition facilitate the establishment of cladocerans, a result suggesting that they are more capable of exploiting the associated bloom of phytoplankton. *Diaphanosoma galeata*, which is a better competitor than the smaller filter-feeding *D. brachyurum*, can coexist with *D. brachyurum*, but alternates with it in dominance depending on the ecosystem trophic state (Geller and Müller 1981, Matveev 1991, Stich 2004). This greater ability to consume P-rich food could be explained if the P pulse offsets the possible harmful effect of UVR for *D. galeata*, which dominates in P-enriched environments regardless of the light quality. These alternative consumers, *D. brachyurum, D. galeata*, and *T. prasinus*, maintain a similar biomass under different experimental scenarios because they have similar body sizes.

Therefore, from the changes observed in the community composition promoted by UVR and P pulses, 3 alternative planktonic communities emerged (Figs 4, S6). Under ambient conditions, the phytoplankton was top-down controlled by a small copepod, *T. prasinus*, which ate larger primary producers, and the small filterer *D. brachyurum*, which grazed on bacteria and the smallest microalgae (Fig. 4A). When the community was shielded from UVR, *D. brachyurum* became dominant because it is a better competitor than copepods (Fig. 4B). When the P pulses occurred, regardless of the light conditions, *D. galeata*, a medium-sized filterer feeder and an even better competitor than *D. brachyurum*, was favored by the proliferation of microalgae of all sizes (Fig. 4C, D). This new community also included the mixotrophic species *P. umbonatum*, which showed a trade-off between UVR sensitivity and the quality of available food (Rojo et al. 2012).

In the P-pulse treatments, consumer biomass did not proportionally follow the increase in phytoplankton biomass. This uncoupling of the predator–prey relationship increased the PSS slope, thereby refuting our hypothesis. Uncoupling of the predator–prey relationship has been described in long-term nutrient enrichments in stream and lake ecosystems and reduces the overall efficiency of the food web (Davis et al. 2010, Bullejos et al. 2010). The decrease in zooplankton biomass after P pulses could be explained by a detrimental effect of food in excess derived from secretions of polysaccharides caused by an increased abundance of the small chloroccocal *Chlorocystis* sp., which collapse the filtering capacity of filter-feeder cladocerans (as observed for large inedible algae (Gliwicz 1990). An alternative explanation is a detrimental effect of food quality derived from high algal P content with a low C : P as in the stoichiometric knife-edge hypothesis (Boersma and Elser 2006, Elser et al. 2016). Moreover, UVR appears to weaken the C flow through these small-sized plankton networks under ambient nutrient conditions by selecting for copepods, which are less effective consumers than cladocerans.

From our results, we suggest that only at depths free of UVR and under conditions without P-induced microalgae blooms, would cladoceran biomass generate a high flow of C through the food web, and that rapid phytoplankton turnover rates would be required to sustain this consumer growth (Jeppesen et al. 2011). Overall, we conclude that essential ecological processes could be affected by brief (P pulses) and moderate (UVR changes) disturbances, which will occur with greater frequency under upcoming climatic changes foreseeable in the Mediterranean region and related to Saharan dust transport and ozone mini-holes (Alpert et al. 2006, Martínez-Lozano et al. 2011, Cabrerizo et al. 2017). Moreover, our results show, or suggest, that the effect on such ecological processes will be evidenced by the food webs of the smallest members of oligotrophic freshwater systems.

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