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Lessons from 30 years of multiple-stressor experiments on microbial plankton in a Spanish high-mountain lake

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ABSTRACT

Lessons from 30 years of multiple-stressor experiments on microbial plankton in a Spanish high-mountain lake

The impact of multiple environmental stressors on freshwater ecosystems has been linked to the study of global change and its effects on biotic communities since the last quarter of the 20th century. In the high mountain lakes of Sierra Nevada National Park (Spain), these studies emerged in the early 1990s, initially aiming to determine whether the most influent environmental stressors in the region (ultra-oligotrophy and high UV radiation) could explain the unique properties of the microbial communities in these systems (a simple, phytoplankton-dominated structure). Later, the focus shifted toward understanding how environmental stressors associated with global change could shape their trophic structure and functioning. In this review, we analyze the 35 experimental studies carried out in the Lakes of Sierra Nevada, with the aim of synthesizing the existing information on the combined effects of different stressors in these ecosystems. The findings highlight a predominant interest in studying the effects of nutrients and UV radiation on phytoplankton and bacterial communities. Interestingly, we report an increase in positive effects on variables such as chlorophyll-a, primary production, and bacterial production as the number of interacting stressors increases. The combination of stressors leads to a higher proportion of non-additive effects (~90%) over additive ones (~10%), with antagonistic effects predominantly affecting autotrophic variables and synergistic effects affecting heterotrophic ones. Taken together, the evidence indicates that while the microbial network structure in Sierra Nevada lakes shows a certain resistance to multiple stressors, their chronic effects may reveal an incipient shift toward a greater prominence of the bacterial compartment, ultimately transforming the microbial food web dynamics in these ecosystems.

KEY WORDS: bacteria, interactive effects, nutrients, phytoplankton, primary production, ultraviolet radiation.

RESUMEN

Lecciones de 30 años de experimentos con múltiples estresores sobre el plancton microbiano en un lago de alta montaña español

El impacto de múltiples estresores ambientales en ecosistemas acuáticos ha estado estrechamente ligado al estudio del cambio global y sus efectos sobre las comunidades bióticas desde el último cuarto del siglo XX. En las lagunas de alta montaña de Sierra Nevada estos estudios emergieron a comienzos de los años 90, primero con la intención de descubrir si los estresores ambientales más incidentes en Sierra Nevada (ultra-oligotrofia y alta radiación UV) eran capaces de explicar las propiedades únicas de la comunidad microbiana de estos sistemas (estructura trófica simple y dominada por el fitoplancton); y finalmente, *para averiguar cómo los estresores ambientales asociados al cambio global podían moldear su estructura y funcionamiento. En esta revisión, llevamos a cabo un análisis de los 35 trabajos experimentales desarrollados en las lagunas de Sierra Nevada con el objetivo de sintetizar la información existente del efecto combinado de distintos estresores en dichos ecosistemas. Los resultados revelan un mayor interés por el estudio de nutrientes y radiación UV sobre la comunidad fitoplanctónica y bacteriana. Los resultados también muestran un incremento de los efectos positivos sobre variables como la Chl-a, producción primaria y bacteriana a medida que aumenta el número de estresores. La combinación de estresores lleva a un predominio de efectos no-aditivos (~90%) sobre aditivos (~10%), y particularmente de tipo antagónico sobre las variables autotróficas y sinérgico sobre las variables heterotróficas. Este conjunto de resultados nos permite concluir que a pesar de que la estructura de la red microbiana de las lagunas de Sierra Nevada muestra una cierta resistencia al cambio bajo el efecto de múltiples estresores, su acción continuada y a largo plazo podría estar revelando un cambio incipiente hacia una mayor importancia del compartimento bacteriano y, por ende, hacia un cambio de la red microbiana de las lagunas de Sierra Nevada tal y como las conocemos.*

PALABRAS CLAVE: *bacteria, efectos interactivos, nutrientes, fitoplancton, producción primaria, radiación ultravioleta.*

IMPACT OF MULTIPLE STRESSORS ON LAKES

The study of the effects of multiple stressors on freshwater ecosystems is crucial for their conservation and management, as the growing number of environmental and anthropogenic pressures can profoundly impact their trophic structure, function, and resilience (Spears et al., 2021, Pirotta et al., 2022). Environmental stressors have been defined by Killen et al. (2013) as any intrinsic or extrinsic factor—whether abiotic or biotic—that challenges organisms and compel them to adapt their behavior or physiology to survive.

Recognition of the need for multiple stressor studies emerged in the 1990s and has since experienced huge growth (Fig. 1). Aquatic ecosystems are impacted by the combination of various climatically- and anthropogenically driven stressors that operate both synchronously and asynchronously on different response variables ranging from genes and physiological processes to community and ecosystem dynamics (Ormerod et al., 2010, Jackson et al., 2021).

Besides natural processes that expose freshwater environments to multiple stressors, current global change is introducing new biotic and abiotic impacts or altering the levels of existing stress-

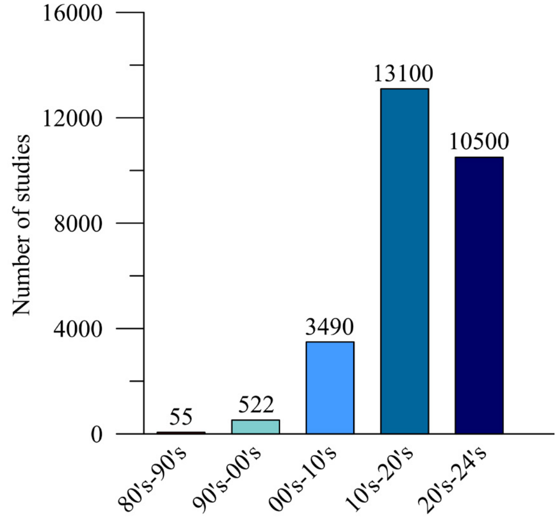

Figure 1. Global number of studies per period of years analyzing the impact of multiple stressors on lakes, including all types of lakes and locations. Keywords employed for the analysis: “Multiple stressors” AND “Lake”. Source: Google Scholar, December 2024. *Número global de estudios por periodo de años que analizan el impacto de múltiples estresores en lagos, incluyendo todo tipo de lagos y localizaciones. Las palabras clave empleadas en el análisis fueron: “Multiple stressors” y “Lake”. Fuente: Google Scholar, Diciembre 2024.*

ors that can exacerbate the response of organisms to them (Brown et al., 2013). For instance, human activity is altering aquatic environment in complex and diverse ways, such as through nutrient pollution, increased water temperature and carbon dioxide levels, and decreased water pH (Cai et al., 2021, IPCC, 2021). As consequence, the implications for ecosystems are wide, affecting community dynamics and fostering phenomena like algal blooms or hypoxia (Glibert, 2020, 2022), among others. Thus, the study of the effects of single stressors on aquatic communities has limited impact, given the wide variety of changes occurring simultaneously. Ecosystemic changes caused by multiple stressors are happening rapidly and their effects are manifesting in a non-linear way. While direct effects typically follow a dose-response pattern, multiple stressors could lead to indirect effects that result in responses different from those expected if these stressors acted independently (Pirotta et al., 2022). The terms 'additivity' and 'non-additivity' (either antagonistic or synergistic, depending on whether the interaction mitigates or enhances the effect, respectively) are commonly used to describe the combined impact of these stressors (Piggott et al., 2015, Villar-Argaiz et al., 2018, Biddanda et al., 2021).

The effects and combination of multiple stressors (e.g., solar radiation, CO_2 increase, rising temperatures, or increased nutrients concentrations associated to phenomena such as dust deposition) have been particularly studied in highly vulnerable ecosystems like high mountain lakes (Moser et al., 2019, Medina-Sánchez et al., 2022, Pastorino et al., 2024; see Fig. 2). These lakes, generally located above the tree line in remote, pristine regions near mountain peaks, are typically oligotrophic, with extended ice cover and exposed to high levels of solar radiation (Loria et al., 2020, Medina-Sánchez et al., 2022). Their relatively simple trophic structure makes them highly sensitive to global changes, leaving a distinct physicochemical imprint that impact the biological system (Weckström et al., 2016). Often regarded as sentinels of environmental change, they provide unique insights into the impacts of climate change, pollution, and human activity on aquatic ecosystems (Catalan et al., 2006, Adrian

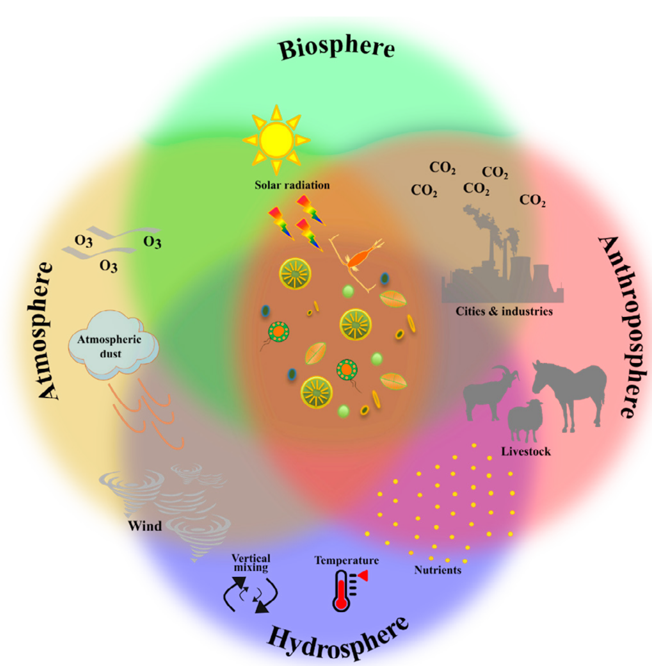

Figure 2. Graphical scheme illustrating major global change drivers, inter-related processes and associated Earth's compartments impacting plankton communities in Sierra Nevada high-mountain lakes. *Esquema gráfico que ilustra los principales factores del cambio global, procesos inter-relacionados y los compartimentos terrestres asociados a dichos procesos que impactan sobre las comunidades planctónicas en lagos de alta montaña de Sierra Nevada.*

et al., 2009, Moser et al., 2019, Pastorino et al., 2020, Pastorino & Prearo, 2020, Biddanda et al., 2022). As a result, high mountain lakes are experiencing rapid ecological changes, underscoring the need for enhanced monitoring of their biological and biogeochemistry activity under multiple anthropogenic impacts (González-Olalla et al., 2018, Oleksy et al., 2020, Lozano et al., 2022).

In this work, we review the major impacts of multiple stressors documented over 30 years of research in Lake La Caldera, a high-mountain lake in Sierra Nevada (southern Spain). To this end, we first provide an overview of the unique features that make La Caldera (37º 3' 17''N, 3º 19' 46''W) a model system for the study of environmental stressors. We then outline the rationale behind the selection of particular stressors and summarize their individual and combined effects on the main biological variables analyzed of the microbial community.

THE HIGH-MOUNTAIN LAKE OF LA CALDERA IN SIERRA NEVADA AS A MODEL ECOSYSTEM

The high mountain lakes of Sierra Nevada, with their low thermal stability, short growing season, high irradiance, simple biological communities and low nutrient concentrations, offer an ideal setting for studying the impact of multiple stressors on ecosystem dynamics. This group of over 70 small lakes, some over 3000 meters in altitude, is located in Europe's southernmost snow-capped mountain range (Morales-Baquero et al., 1992). Due to their geographical location between Europe and Africa and proximity to the Sahara Desert, they act as natural barriers capturing and depositing large amounts of Saharan dust carried by air currents during the spring and summer, supplying nutrients and alkalinity to the aquatic ecosystems (Brahney et al., 2021, Reche et al., 2022).

After the initial explorations and studies of the Sierra Nevada lakes to understand their physicochemical properties, diversity and structure of plankton communities (Martínez-Silvestre, 1975), researchers were particularly struck by their uniqueness. This was especially evident in Lake La Caldera, one of the most representative characteristic high-mountain lakes in Sierra Nevada. With an area of 2.3 ha and situated just above 3000 meters, it has a distinct oligotrophic nature. Its isolation, the complete lack of surrounding vegetation and low catchment area: lake area ratio makes its biological community largely controlled by its physical-chemical environment (Martínez, 1977, Cruz-Pizarro, 1981, Sánchez-Castillo et al., 1989). In oligotrophic ecosystems, microbial food web plays a crucial role in their trophic structure and metabolism (Biddanda et al., 2001, Cotner & Biddanda, 2002), with microbial interactions impacting processes such as energy flow and nutrient cycling (Halvorson et al., 2020, Biddanda et al., 2021). However, the microbial network of La Caldera Lake deviates from the typical high heterotrophic bacteria-to-phytoplankton biomass ratios observed in oligotrophic ecosystems (Biddanda et al., 2001). Surprisingly, bacteria in Lake La Caldera represents only a minor component of the plankton community in terms of abundance, biomass, and production. Moreover, autotrophic picoplankton is absent, and the heterotrophic microbial food web is underdeveloped compared to the grazing chain, which is dominated by calanoid copepods (mainly *Mixodiaptomus laciniatus*') supported by a phytoplankton community mainly composed of mixotrophic nanoflagellates (mainly *Chromulina nevadensis*).

To decipher this unique food web structure, early studies in the early 1990s and 2000's sought to determine how key biotic (e.g., zooplankton) and abiotic factors characteristic of high mountain environments, such as intense UV radiation and oligotrophy, could play a fundamental role in shaping the trophic structure and functioning of the lake across temporal and spatial scales (Carrillo et al., 1990, Medina-Sánchez et al., 2002, 2004, 2006). This marked the beginning of a prolific research trajectory in Lake La Caldera, which led to the finding that the microbial community is particularly simple due to factors such as: i) High UVR favors algae-bacteria coupling through increased algal excretion of organic carbon (EOC) which is readily up-taken by UVR-adapted bacteria, thereby promoting the commensalistic interaction (Carrillo et al., 2002); ii) a high abundance of mixotrophic algae that act as bacterial predators thereby replacing the niche of heterotophic nanoflagellates and simplifying the food web (Medina-Sánchez et al., 2004, Carrillo et al., 2017); "iii) Commensalism and mixotrophy foster a mutualistic (commensalistic–predatory) interaction between mixotrophic algae and bacteria, creating a C-flux bypass that restricts the development of a more complex microbial network (Medina-Sánchez et al., 2004, Carrillo et al., 2006); and iv) pulsed P-inputs that enhance algae-bacteria mutualistic interaction and the persistence of the mixotrophic algae (Medina-Sánchez et al., 2006, 2013, Cabrerizo et al., 2017).

Upon confirming that environmental stressors play a key role in shaping La Caldera's trophic network, interest grew in exploring how changes in these and other factors (e.g., temperature, CO_2 or vertical mixing effect; Helbling et al., 2013), in the context of global change, could influence its balance and functioning in the future. In the following sections, we will analyze the studies conducted on the Sierra Nevada lakes to condense all available information and attempt to answer how cumulative impact of multiple environmental stressors has disturbed the microbial community.

MAIN STRESSORS AND BIOLOGICAL VARIABLES STUDIED IN SIERRA NEVADA LAKES

To date, all studies on multiple stressors affecting the microbial community of Sierra Nevada lakes have been conducted within the Functional Ecology research group at the University of Granada. Accordingly, a total of 35 scientific articles on this topic were compiled, authored by different researchers who have worked within the group (see Supplementary Information, Table S1, available at https://www.limnetica.com/en/limnetica). Most of these studies, conducted since the early 2000s, focused on Lake La Caldera (100% of the articles included data from this lake). However, experiments were also carried out in Lake Las Yeguas (~10%), Aguas Verdes, and Lake Río Seco (~3%). All studies examined the effect of at least one biotic or abiotic stressor, with 25 exploring second-level combinations, 5 third-level combinations, and 1 examining four-level combinations, reflecting the complexity of the experimental designs (Fig. 3A).

The most studied stressors were radiation, particularly solar ultraviolet radiation (UVR; 28 articles), and nutrient addition, especially phosphorus (P; 27 articles). To a lesser extent, the impact of zooplankton on the phytoplankton community (5 articles) and vertical mixing and temperature (4 articles each) were investigated. The most frequently explored combination was UVR×Nutrients, with a total of 23 articles, followed by UVR×Vertical mixing, covered in 4 articles (Fig. 3B).

Regarding biological variables, research has mainly focused on abundance and biomass variables (e.g., cell and organism abundance, chlorophyll *a*) and metabolic variables (primarily PP and HBP), with 32 and 28 studies, respectively. Stoichiometric variables (carbon (C), nitrogen (N) and phosphorus (P) seston) have also been extensively studied in 19 articles. In contrast, physiological variables (such as enzymatic activities or photosynthetic efficiency) and studies on nucleic acids (DNA or RNA) were less explored, with only 3 and 1 article, respectively (Fig. 3C).

Research on the impact of multiple stressors on the biological communities of Sierra Nevada lakes has primarily focused on the phytoplankton community, with 34 studies analyzing at least one variable related to photosynthetic eukaryotic organisms. The bacterial and zooplankton communities have also been extensively studied, with 17 and 14 articles, respectively (Fig. 3D).

Given the extensive range of stressors and the diversity of communities analyzed, this article focuses on the main environmental stressors examined over the past decades in Sierra Nevada lakes (namely, nutrient inputs, temperature increase, UVR and vertical mixing effect). Particular attention is given to the microbial plankton community (bacteria and phytoplankton) of Lake La Caldera, given its prominence (stressors, community and lake) in the reviewed articles.

DATA ANALYSIS OF 30 YEARS OF EXPERIMENTAL STUDIES

To determine the single and combined effects of environmental stressors on the microbial community of Lake La Caldera in Sierra Nevada, we fo-

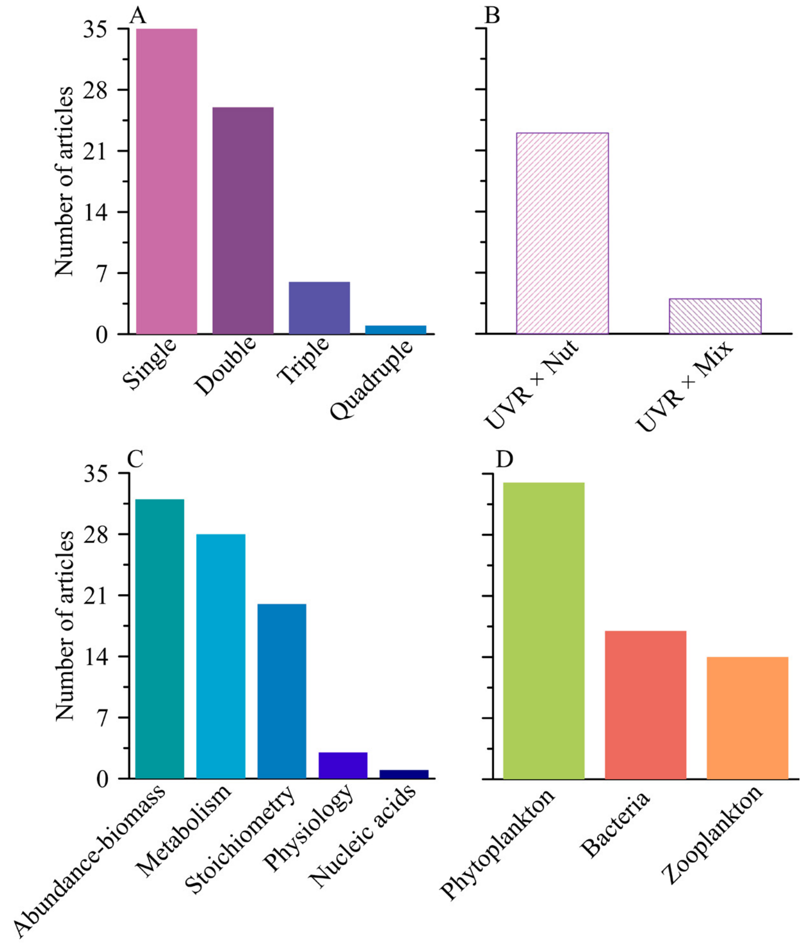

Figure 3. Number of studies in Lake La Caldera that have analyzed (A) the effect of single, double, triple, or quadruple stressors, and more specifically (B) the dual combination of UV radiation (UVR) and nutrients (Nut) or vertical mixing (Mix). Number of studies examining the effects of multiple stressors on (C) the abundance and biomass, metabolism, stoichiometry, physiology, or nucleic acids of the planktonic community, as well as (D) the different studied communities: phytoplankton, bacteria, or zooplankton, are also represented. *Número de estudios en el lago de La Caldera que han analizado (A) el efecto de estresores individuales, dobles, triples o cuádruples, y más específicamente (B) la combinación dual entre radiación UV (UVR) y nutrientes (Nut) o mezcla vertical (Mix). También aparecen representados el número de estudios que examinan el efecto de múltiples estresores sobre (C) la biomasa y abundancia, metabolismo, estequiometría, fisiología, o ácidos nucleicos de la comunidad planctónica, así como también (D) las diferentes comunidades estudiadas: fitoplancton, bacterias, o zooplancton.*

cused on the variables most frequently analyzed since the early studies. Accordingly, chlorophyll-*a* (Chl-*a*; a proxy for phytoplankton biomass) and PP and HBP (metabolic variables) were selected as the main response variables in our study. In total, 24 of the 35 reviewed articles were included in the analysis of multiple stressor effects, rendering 108 observations of single effects and 82 observations of combined effects. We analyzed the effects of environmental stressors both individually and in combination, with particular focus on the two key stressors most frequently reported in studies conducted on the lakes of Sierra Nevada: UVR and nutrients (P was added in all 24 studies, N in 4 of them, and C in only 3).

Since some articles included in our dataset only reported results as ‘effect size’ for some of the variables of interest, we calculated the effect size of specific stressors on biological variables when they were not provided in the original articles. This allowed us to work with a uniform dataset. The formula used for calculating the effect size of each stressor has been widely applied in other research articles on the effects of environmental stressors (e.g., Helbling et al., 2015, Gao et al., 2007) as well as in studies conducted in Lake La Caldera and included in our analysis (e.g., Durán-Romero et al., 2020) (Eq. 1).

Eq. 1 $$Effect\ size\ of\ each\ stressor = \frac{Single\ or\ combined\ stressor - Control}{Control} \times 100$$

On one hand, we determined the significance of the effect (positive or negative) depending on whether the single stressor or combination of stressors increased (positive effect) or decreased (negative effect) the value of the analyzed response variable (Chl-*a*, PP, or HBP) compared to the control treatment.

On the other hand, we also determined the type of combined effect between the stressors following Piggott et al., (2015) and classifying the effects as additive, when the effect size was similar to the sum of the individual effects (applying a 95% confidence interval calculated around each effect); synergistic, when the combined effect size exceeded the individual or the sum of the individual effects; or antagonistic, when the effect size of the combination was smaller than the individual or sum of the individual effect sizes.

When the analyzed articles included multiple levels for the same factor (e.g., increasing concentrations of nutrients from 0 to 60 µg P L^{-1}), for simplicity, we focused on the data obtained for the highest level (i.e., the highest concentration of nutrients added) for our study, following Gutiérrez-Cánovas et al., (2022). If there were different measurements over time for the same article and analyzed variable, we selected the time point where the responses to the stressor or stressors showed the greatest magnitude for our analysis. We are aware that this analysis might overlook non-linear and/or adaptive responses of the biological community to one or more stressors. However, we aim to prevent a single study from being overrepresented in the extracted dataset. Besides, comparing different studies with varying temporal scales requires homogenizing the information to facilitate the interpretation of the results. Finally, higher-magnitude responses to a set of stressors could reveal synergistic and antagonistic effects that might be masked over longer temporal scales.

EFFECTS OF COMBINED ENVIRONMENTAL STRESSORS ON PHYTOPLANKTON AND BACTERIA IN LAKE LA CALDERA

The results show that the set of single stressors addressed had an overall positive effect on Chl-*a* (70% of the total cases, n=23) but a general negative effect on PP and HBP (63% [n=49] and 56% [n=36] of the total cases, respectively; Fig. 4). When analyzing the results for each stressor individually, they reveal that UVR had a slightly positive effect on Chl-*a* (60% of the total cases, n=10) but a general negative effect on PP (80% of the total cases, n=24) and HBP (85% of the total cases, n=15) (Fig. 4; right panel). In contrast, Nutrients, the second most studied stressor in Lake La Caldera, showed predominantly positive effects on all three variables (92% [n=12], 56 [n=16] and 77% [n=13] of the total cases for Chl *a*, PP and HBP, respectively). Increased temperature negatively affected Chl-*a* (100%, n=1) and HBP (75% of the total cases, n=4), whereas it had a positive effect on PP (75% of the total cases, n=4). Regarding the Vertical Mixing effect, it negatively impacted PP in 100% of the cases (n=3) and did not exert a clear effect on HBP (n=2; Fig. 4).

Interestingly, when stressors interacted, their combined effects became positive, especially for Chl-*a* (92% of the total cases [n=19], Fig. 5). When broken down by the number of combined stressors, it is noteworthy that the predominance of positive effects on autotrophic variables (i.e., Chl-*a* and PP) increased as the number of combined stressors did from two to three (from 92% [n=14] to 100% [n=5] of the total cases for Chl-*a*, and from 55% [n=33] to 91% [n=12] of the total cases for PP; Fig. 5). Specifically, the UVR×Nu-

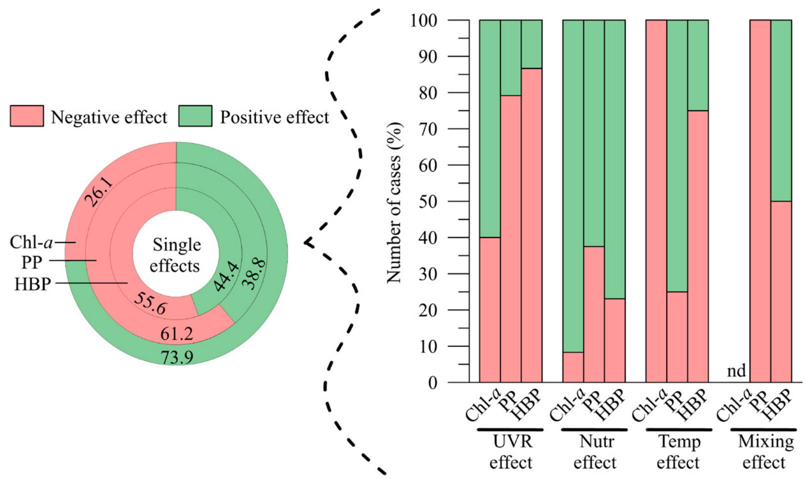

Figure 4. Percentage of cases showing a positive or negative effect for the set of all the single stressors (circular figure), and more specifically (bars figure), UV radiation (UVR effect), nutrients (Nutr effect), temperature (Temp effect), or vertical mixing (Mixing effect) on chlorophyll *a* (Chl-*a*), primary production (PP), or heterotrophic bacterial production (HBP) in Lake La Caldera (Sierra Nevada, Spain). The sample size (n) of the studies analyzed is reported in the text. *nd: no data. *Porcentaje de casos que muestran un efecto positivo o negativo para el conjunto de todos los estresores individuales (figura circular), y más específicamente (figura de barras), para la radiación UV (efecto UVR), nutrientes (efecto Nutr), temperatura (efecto Temp), o mezcla vertical (efecto Mezcla), sobre la clorofila-a (Chl-*a*), producción primaria (PP), o producción bacteriana heterotrófica (HBP) en el Lago de La Caldera (Sierra Nevada, España). El tamaño de la muestra (n) de los estudios analizados aparece representado en el texto. *nd= sin datos.*

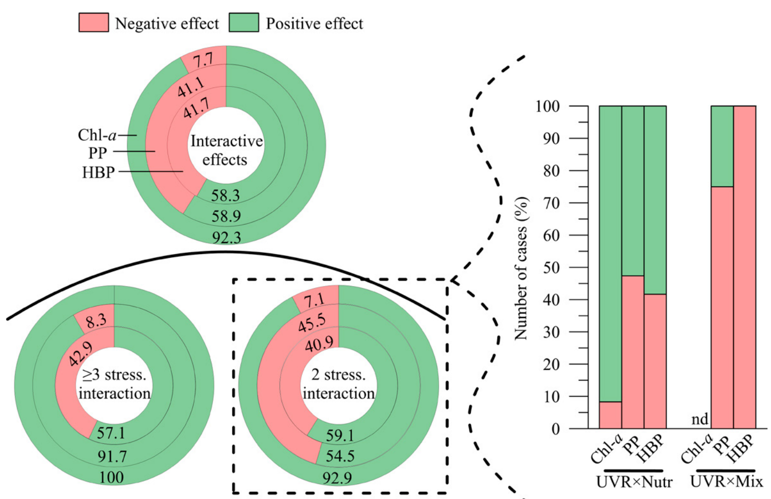

Figure 5. Percentage of cases showing a positive or negative effect of stressors combination on chlorophyll a (Chl-*a*), primary production (PP), or heterotrophic bacterial production (HBP) in Lake La Caldera (Sierra Nevada, Spain), and more specifically, for 2 stressors combination, and 3 or more stressors combination. The effect of the most studied combined pairs in La Caldera Lake (i.e., UV radiation and nutrient combination [UVR×Nutr], and UVR and vertical mixing combination [UVR×Mix]) are also represented. The sample size (n) of the studies analyzed is reported in the text. *Porcentaje de casos que muestran un efecto positivo o negativo de la combinación de estresores sobre la clorofila-a (Chl-*a*), producción primaria (PP), o producción bacteriana heterotrófica (HBP) en el Lago de La Caldera (Sierra Nevada, España), y más específicamente, para la combinación de 2 estresores, y 3 o más estresores. También se muestra el efecto de los pares combinados más estudiados el Lago de La Caldera (es decir, combinación de radiación UV y nutrientes [UVR×Nutr], y combinación de UVR y mezcla vertical [UVR×Mix]). El tamaño de la muestra (n) de los estudios analizados aparece representado en el texto.*

trient combination showed values consistent with the overall set of combinations, with positive effects, particularly on Chl-*a* (92% of the total cases, n=12). However, the UVR×Vertical Mixing combination exhibited more negative than positive effects on PP (75% of negative effects [n=4]) and HBP (100% of negative effects [n=2]) (Fig. 5; right panel).

Finally, the results indicate a prevalence of non-additive effects (antagonistic or synergistic) regardless of the level of combined stressors in Lake La Caldera. Antagonistic effects predominated over synergistic ones for autotrophic variables (84.6% vs. 7.7% of the total cases [n=13] for Chl-*a* and 71.8% vs. 15.4% of the total cases for PP [n=39]), while synergistic effects were more common for HBP (52% of the total cases [n=25]) (Fig. 6). Comparing combinations involving 2 or 3+ stressors reveals that as the number of stressors increases, there is a rise of antagonistic effects on autotrophic variables and an increase in synergistic effects on the heterotrophic variable (i.e., HBP). Specifically, for the UVR×Nutrient and UVR×Vertical Mixing combinations, antagonistic effects were dominant for all three variables analyzed (except in HBP for UVR×Nutrient combination, where percentage of antagonistic and synergistic effects was similar, 44.4% of the total cases [n=9]), with greater prevalence for autotrophic variables (Fig. 6).

NEW INSIGHT INTO THE NATURE OF MULTIPLE STRESSORS COMBINATION ON PHYTOPLANKTON AND BACTERIA IN LAKE LA CALDERA

Our analysis of studies conducted over 30 years on multiple stressors, involving over 35 independent micro- and mesocosm experiments in a high-mountain lake of Sierra Nevada, has revealed crucial insights into how microbial communities respond to multiple and combined environmental stressors. Interestingly, our analysis indicates a slight predominance of negative effects on PP and HBP when exposed to a single stressor. In contrast, an increase in the number of combined stressors is associated with a shift toward more positive effects on Chl-*a* and PP, and to a lesser extent on HBP. A likely explanation for this response involves the two primary stressors most intensively examined in Lake La Caldera: ultraviolet radiation (UVR) and nutrients. As a single stressor, UVR (including UV-A and UV-B) has consistently shown negative impact on all three studied variables, particularly on metabolic

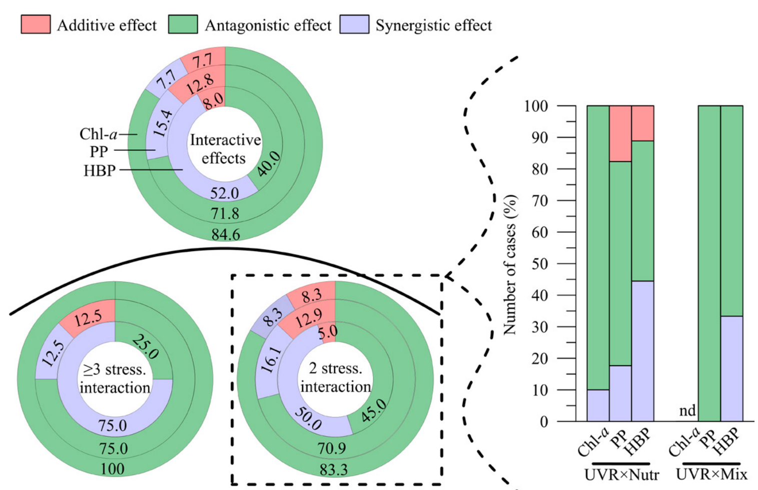

Figure 6. Percentage of cases showing additive, synergistic or antagonistic effects when stressors interacted on chlorophyll a (Chl-a), primary production (PP), and heterotrophic bacterial production (HBP), and more specifically, for 2 stressors combination, and 3 or more stressors combination. The effect of the most studied combination pairs in La Caldera Lake (i.e., UV radiation and nutrient combination [UVR×Nutr], and UVR and vertical mixing combination [UVR×Mix]) are also represented. The sample size (n) of the studies analyzed is reported in the text. *Porcentaje de casos que muestran un efecto aditivo, sinérgico o antagónico de la combinación de estresores sobre la clorofila-a (Chl-a), producción primaria (PP), o producción bacteriana heterotrófica (HBP) en el Lago de La Caldera (Sierra Nevada, España), y más específicamente, para la combinación de 2 estresores, y 3 o más estresores. También se muestra el efecto de los pares combinados más estudiados (es decir, combinación de radiación UV y nutrientes [UVR×Nutr], y combinación de UVR y mezcla vertical [UVR×Mix]). El tamaño de la muestra (n) de los estudios analizados aparece representado en el texto.*

processes (PP and HBP), according to previous findings on UVR's harmful effects on various biological targets and processes, such as photosynthesis, nutrient uptake, primary production, and heterotrophic bacterial production (Häder et al., 2014). It is important to emphasize that these results primarily highlight the harmful effect of UVR in comparison to UVR-free treatments, but do not exclude the high adaptive capacity and resilience to chronic UV stress observed in high-altitude aquatic microorganisms (Medina-Sánchez et al., 2004, Korbee et al., 2012). In contrast, nutrients (primarily P) as a single stressor exhibited a clearly positive effect, enhancing Chl-*a* levels as well as primary and bacterial production. This is in line with findings by Smith & Prairie (2004) and Jia et al. (2024), and reflects the pronounced P-limitation characteristic of Sierra Nevada high-mountain lakes, especially Lake La Caldera (Morales-Baquero et al., 1999).

Positive vs. negative effects of multiple stressors

Our analysis of stressor combinations revealed two interesting points:

i) The combination of two stressors led to a higher proportion of positive effects on the three analyzed variables, particularly in Chl-*a*. This was largely driven by the UVR×Nutrient combination, the most frequently examined in Lake La Caldera. Despite previous conflicting reports on whether nutrients mitigate or amplify UV-induced damage to autotrophic and heterotrophic microbiota (Pausz & Herndl, 2002, Ogbebo & Ochs, 2008, Villafañe et al., 2017), our results support an antagonistic interaction, with nutrients serving as a buffer against UV stress. The role of nutrients as an overriding stressor has previously been confirmed by Birk et al. (2020) in a comprehensive study analyzing experimental data and watershed studies across European lakes and rivers. Consistent with this, Morris et al. (2022) suggest that the impact of multiple stressors can be predicted by focusing on the strongest stressor, in this case, nutrients. The antagonistic interaction of Nutrients×UVR may reflect the buffering effect of nutrient enrichment against UVR exposure. At the cellular level, the addition of P can increase RNA production (a P-rich biomolecule) involved in the activation of genes and the production of protective metabolites against UVR (Heraud et al., 2005). In addition, P addition may enhance phytoplankton biomass (Quinlan et al., 2021), partially compensating for UVR-driven reductions in carbon fixation (Villafañe et al., 2003). Finally, P enrichment may promote the release of EOC into the water (Pearce et al., 2021), which can absorb harmful UV radiation (UV-B), providing an effective shield for plankton (Schindler et al., 1996). This antagonistic effect suggests that nutrient enrichment induces compensatory mechanisms against UVR, from cellular level to food web structure; ii) As the number of combined stressors increases (≥3), the proportion of positive effects on Chl-*a* and PP also rises. As we mentioned previously, nutrients may exert a dominant effect in multi-stressor scenarios, and their positive effect could be reinforced by additional factors (Brennan & Collins, 2015) studied in Sierra Nevada. For instance, the addition of third and fourth stressors, such as CO_2, increased temperature, or vertical mixing, may enhance autotrophic activity by stimulating phototrophic metabolism (Savage et al., 2004, Giordano et al., 2005) or by reducing exposure to harmful UV radiation. The latter could promote photorepair mechanisms under fluctuating light conditions caused by vertical mixing, as opposed to constant UV exposure in static systems (Köhler et al., 2001). However, the impact of other stressors, including emerging contaminants (Godoy et al., 2022) and unexamined traditional stressors in Lake La Caldera cannot be dismissed, as they may shift the balance toward more negative community-level effects.

Additive vs Non-additive effects of multiple stressors

Besides the generally positive effects of stressor combinations on studied variables in Lake La Caldera, our results reveal a predominance of non-additive interactions—both synergistic and antagonistic. This pattern reflects the growing incidence of "ecological surprises" in freshwater ecosystems during the Anthropocene (Huang et al., 2022) and aligns with findings by Villar-Argáiz et al. (2018), who observed non-additive effects dominating across autotrophic plankton globally. However, contrasting evidence from Bao et al. (2024), who found mostly additive responses in macroinvertebrates, highlights the importance of taxon-specific traits and ecological roles in shaping stressor responses.

In our studies, antagonistic effects were more frequent in autotrophic variables. This response may stem from freshwater systems' high environmental variability promoting acclimation to multiple stressors (Jackson et al., 2016), or the presence of dominant stressors, such as nutrients, driving asymmetrical responses (Folt et al., 1999, Côté et al., 2016, Tekin et al., 2020, Morris et al., 2022). By contrast, synergistic interactions – 77% of which were positive, were more common for HBP. The prevalence of positive synergistic effects could be linked to their rapid adaptation and reproduction rates. Supporting this, synergistic dominance in bacterial communities has also been reported by Jackson et al. (2016), while Lange et al. (2018) and Orr et al. (2022) highlight how rapid adaptation and longer experiment durations, respectively, can promote such outcomes.

Finally, it is also necessary to remark that 35 studies analyzed in this work (showing a prevalence of non-additive responses) are all manipulative, whereas observational studies assessing the effects of multiple stressors have reported a greater predominance of additive effects (see Gutiérrez-Cánovas et al., 2022). This discrepancy may be explained by differences in temporal scale: manipulative experiments are typically conducted over shorter time scales than observational studies and may therefore overestimate the prevalence of non-additive effects (Villar-Argaiz et al., 2018). Therefore, they may fail to capture the adaptive or recovery capacity that organisms can exhibit under natural conditions (Jackson et al., 2021).

High-order combined effects of multiple stressors

Studies analyzing the effects of higher-order combinations (i.e., three or more stressors) are scarce in field experiments, despite the fact that such combinations may be the most common in ecological systems (Levine et al., 2017, Orr et al., 2024) and can give rise to emergent responses that may alter the nature or direction of combinations (Diamant et al., 2023). Our findings on higher-order combinations reinforced the trends observed under two-stressor conditions, showing an increase in positive and antagonistic effects on autotrophic variables (i.e., Chl-*a* and PP) and a higher proportion of synergistic effects on bacteria. Despite the general pattern, some paired combinations—like UVR×P, UVR×Temp, and Temp×P—shifted when all three stressors acted together, resulting in additive effects on PP and antagonistic effects on HBP (Durán et al., 2016). These findings emphasize the need to assess higher-order combinations to effectively manage ecological stressors (Brown et al., 2013, Piggott et al., 2015).

CONCLUDING REMARKS

The results discussed above highlight distinct responses of microbial compartments to multiple stressors, with a greater positive impact on Chl-*a* and PP than on HBP. This suggests that increasing stressor intensity and combination could further reinforce the unique phytoplankton-to-bacteria biomass ratio observed in Lake La Caldera. Supporting this, an observational study in a subset of Sierra Nevada Lakes, including Lake La Caldera (see González-Olalla et al., 2018), showed that after ten years of global change impacts, bacterial production remained relatively stable despite increases in PP and Chl-*a*. However, this status quo in Sierra Nevada lakes may shift, increasing the importance of bacteria within the community. This is supported by (i) frequent occurrence of positive synergistic effects on bacterial production under the combination of multiple stressors, and (ii) several observational and experimental studies conducted in recent years that indicate a shift in the phytoplankton community composition –from mixotrophy to strict autotrophy– driven by pulsed nutrient inputs (see Cabrerizo et al., 2017, Carrillo et al., 2017, González-Olalla et al., 2018). A decline in bacterivory by mixotrophs, combined with increased excretion of labile organic C by phytoplankton, could further strengthen positive effects of multiple environmental stressors on the heterotrophic compartment.

These findings underscore the need to enhance our understanding of the long-term effects of multiple stressors, as microbial trade-offs and adaptation mechanisms may buffer short-term impacts on different biotic compartments.

DATA AVAILABILITY

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.15384306, reference number 15384306.

COMPETING INTEREST STATEMENT

The authors declare that they have no competing interests to declare.

AUTHOR'S CONTRIBUTION

J.M.G.O.: Conceptualization, Data curation, formal analysis, Methodology, Visualization, Writing-original draft. M.J.C.: Conceptualization, Visualization, Writing-review & editing. J.M.M.S.: Conceptualization, Funding-acquisition, Writing-review & editing. M.V.A.: Conceptualization, Funding-acquisition, Writing-review & editing. P.C.L.: Conceptualization, Funding-acquisition, Writing-review & editing.

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REFERENCES

Adrian, R., O'Reilly, C. M., Zagarese, H., Baines, S. B., Hessen, D. O., Keller, W., Livingstone, D. M., Sommaruga, R., Straile, D., Van Donk, E., Weyhenmeyer, G. A., & Winder, M. (2009). Lakes as sentinels of climate change. *Limnology and Oceanography*, 54(6part2), 2283–2297. DOI: 10.4319/lo.2009.54.6_part_2.2283

Bao, S., Heino, J., Xiong, H., & Wang, J. (2024). Disentangling the effects of multiple stressors on freshwater macroinvertebrates: A quantitative analysis of experimental studies. *Ecological Indicators*, 169, 112880. DOI: 10.1016/j.ecolind.2024.112880

Biddanda, B., Dila, D., Weinke, A., Mancuso, J., Villar-Argaiz, M., Medina-Sánchez, J. M., González-Olalla, J. M., & Carrillo, P. (2021). Housekeeping in the Hydrosphere: Microbial Cooking, Cleaning, and Control under Stress. *Life*, 11(2), 152. DOI: 10.3390/life11020152

Biddanda, B., Ogdahl, M., & Cotner, J. (2001). Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. *Limnology and Oceanography*, 46(3), 730–739. DOI: 10.4319/lo.2001.46.3.0730

Biddanda, B., Villar-Argaiz, M., & Medina-Sánchez, J. (2022). Protecting the Mountain Water Towers of Spain's Sierra Nevada.

Eos, 103. DOI: 10.1029/2022EO220448

Birk, S., Chapman, D., Carvalho, L., Spears, B. M., Andersen, H. E., Argillier, C., Auer, S., Baattrup-Pedersen, A., Banin, L., Beklioğlu, M., Bondar-Kunze, E., Borja, A., Branco, P., Bucak, T., Buijse, A. D., Cardoso, A. C., Couture, R. M., Cremona, F., de Zwart, D., … Hering, D. (2020). Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nature Ecology and Evolution*, 4(8), 1060–1068. DOI: 10.1038/s41559-020-1216-4

Brahney, J., Weathers, K., & Reche, I. (2021). *Dust and Fog effects on Inland Waters*. In K. Tockner, G. Lickens, & T. Mehner (Eds.), Encyclopedia of Inland Waters. Elsevier.

Brennan, G., & Collins, S. (2015). Growth responses of a green alga to multiple environmental drivers. *Nature Climate Change*, 5(9), 892–897. DOI: 10.1038/nclimate2682

Brown, C. J., Saunders, M. I., Possingham, H. P., & Richardson, A. J. (2013). Managing for Interactions between Local and Global Stressors of Ecosystems. *PLoS ONE*, 8(6), e65765. DOI: 10.1371/journal.pone.0065765

Cabrerizo, M. J., Medina-Sánchez, J. M., Dorado-García, I., Villar-Argaiz, M., & Carrillo, P. (2017). Rising nutrient-pulse frequency and high UVR strengthen microbial interactions. *Scientific Reports*, 7(1), 43615. DOI: 10.1038/srep43615

Cai, W.-J., Feely, R. A., Testa, J. M., Li, M., Evans, W., Alin, S. R., Xu, Y.-Y., Pelletier, G., Ahmed, A., Greeley, D. J., Newton, J. A., & Bednaršek, N. (2021). Natural and Anthropogenic Drivers of Acidification in Large Estuaries. *Annual Review of Marine Science*, 13(1), 23–55. DOI: 10.1146/annurev-marine-010419-011004

Carrillo, P., Cruz-Pizarro, L., & Sanchez-Castillo, P. (1990). Analysis of phytoplankton-zooplankton relationships in an oligotrophic lake under natural and manipulated conditions. In: *Biomanipulation Tool for Water Management* (pp. 49–58). Springer Netherlands. DOI: 10.1007/978-94-017-0924-8_5

Carrillo, P., Medina-Sánchez, J.M., Villar-Argaiz, M., Delgado-Molina, J. A., & Bullejos, F. J. (2006). Complex interactions in microbial food webs: Stoichiometric and functional approaches. *Limnetica*, 25(1), 189–204. DOI: 10.23818/limn.25.15

Carrillo, P., Medina-Sánchez, J. M., & Villar-Argaiz, M. (2002). The interaction of phytoplankton and bacteria in a high mountain lake: Importance of the spectral composition of solar radiation. *Limnology and Oceanography*, 47(5), 1294–1306. DOI: 10.4319/lo.2002.47.5.1294

Carrillo, P., Medina-Sánchez, J. M., Villar-Argaiz, M., Bullejos, F. J., Durán, C., Bastidas-Navarro, M., Souza, M. S., Balseiro, E. G., & Modenutti, B. E. (2017). Vulnerability of mixotrophic algae to nutrient pulses and UVR in an oligotrophic Southern and Northern Hemisphere lake. *Scientific Reports*, 7(1), 6333. DOI: 10.1038/s41598-017-06279-9

Catalán, J., Camarero, L., Felip, M., Pla, S., Ventura, M., Buchaca, T., Bartumeus, F., de Mendoza, G., & Miró, A. (2006). High mountain lakes: extreme habitats and witnesses of environmental changes. *Limnetica*, 25(1), 551–584. DOI: 10.23818/limn.25.38

Côté, I. M., Darling, E. S., & Brown, C. J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B: Biological Sciences*, 283(1824), 20152592. DOI: 10.1098/rspb.2015.2592

Cotner, J. B., & Biddanda, B. A. (2002). Small Players, Large Role: Microbial Influence on Biogeochemical Processes in Pelagic Aquatic Ecosystems. *Ecosystems*, 5(2), 105–121. DOI: 10.1007/s10021-001-0059-3

Cruz-Pizarro, L. (1981). *Estudio de la comunidad zooplanctónica de un lago de alta montaña (La Caldera, Sierra Nevada, Granada)*. University of Granada.

Diamant, E. S., Boyd, S., Lozano-Huntelman, N. A., Enriquez, V., Kim, A. R., Savage, V. M., & Yeh, P. J. (2023). Meta-analysis of three-stressor combinations on population-level fitness reveal substantial higher-order interactions. *Science of The Total Environment*, 864, 161163. DOI: 10.1016/j.scitotenv.2022.161163

Durán-Romero, C., Medina-Sánchez, J. M., & Carrillo, P. (2020). Uncoupled phytoplankton-bacterioplankton relationship by multiple drivers interacting at different temporal scales in a high-mountain Mediterranean lake. *Scientific Reports*, 10(1), 350. DOI: 10.1038/s41598-019-57269-y

Durán-Romero, C., Medina-Sánchez, J. M., Herrera, G., & Carrillo, P. (2016). Changes in the phytoplankton-bacteria coupling triggered by joint action of UVR, nutrients, and warming in Mediterranean high-mountain lakes. *Limnology and Oceanography*, 61(2), 413–429. DOI: 10.1002/lno.10204

Folt, C. L., Chen, C. Y., Moore, M. V., & Burnaford, J. (1999). Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, 44(3part2), 864–877. DOI: 10.4319/lo.1999.44.3_part_2.0864

Gao, K., Li, G., Helbling, E. W., & Villafañe, V. E. (2007). Variability of UVR Effects on Photosynthesis of Summer Phytoplankton Assemblages from a Tropical Coastal Area of the South China Sea †. Photochemistry and *Photobiology*, 83(4), 802–809. DOI: 10.1111/j.1751-1097.2007.00154.x

Giordano, M., Beardall, J., & Raven, J. A. (2005). CO_2 CONCENTRATING MECHANISMS IN ALGAE: Mechanisms, Environmental Modulation, and Evolution. *Annual Review of Plant Biology*, 56(1), 99–131. DOI: 10.1146/annurev.arplant.56.032604.144052

Glibert, P. M. (2020). Harmful algae at the complex nexus of eutrophication and climate change. *Harmful Algae*, 91, 101583. DOI: 10.1016/j.hal.2019.03.001

Glibert, P. M., Cai, W.-J., Hall, E. R., Li, M., Main, K. L., Rose, K. A., Testa, J. M., & Vidyarathna, N. K. (2022). Stressing over the Complexities of Multiple Stressors in Marine and Estuarine Systems. *Ocean-Land-Atmosphere Research*, 2022. DOI: 10.34133/2022/9787258

Godoy, V., Calero, M., González-Olalla, J. M., Martín-Lara, M. A., Olea, N., Ruiz-Gutierrez, A., & Villar-Argaiz, M. (2022). The human connection: First evidence of microplastics in remote high mountain lakes of Sierra Nevada, Spain. *Environmental Pollution*, 311, 119922. DOI: 10.1016/j.envpol.2022.119922

González-Olalla, J. M., Medina-Sánchez, J. M., Lozano, I. L., Villar-Argaiz, M., & Carrillo, P. (2018). Climate-driven shifts in algal-bacterial interaction of high-mountain lakes in two years spanning a decade. *Scientific Reports*, 8(1), 1–12. DOI: 10.1038/s41598-018-28543-2

Gutiérrez-Cánovas, C., Arias-Real, R., Bruno, D., Cabrerizo, M. J., González-Olalla, J. M., Picazo, F., Romero, F., Sánchez-Fernández, D., & Pallarés, S. (2022). Multiple-stressors effects on Iberian freshwaters: A review of current knowledge and future research priorities. *Limnetica*, 41(2), 1. DOI: 10.23818/limn.41.15

Häder, D.-P., Williamson, C. E., Wängberg, S.-Å., Rautio, M., Rose, K. C., Gao, K., Helbling, E. W., Sinha, R. P., & Worrest, R. (2014). Effects of UV radiation on aquatic ecosystems and interactions with other environmental factors. *Photochemical & Photobiological Sciences*, 14(1), 108–126. DOI: 10.1039/c4pp90035a

Halvorson, H. M., Wyatt, K. H., & Kuehn, K. A. (2020). Ecological significance of autotroph–heterotroph microbial interactions in freshwaters. *Freshwater Biology*, 65(7), 1183–1188. DOI: 10.1111/fwb.13530

Helbling, W. E., Banaszak, A. T., & Villafañe, V. E. (2015). Global change feed-back inhibits cyanobacterial photosynthesis. *Scientific Reports*, 5(1), 14514. https://doi.org/10.1038/srep14514

Helbling, W. E., Carrillo, P., Medina-Sánchez, J. M., Durán, C., Herrera, G., Villar-Argaiz, M., & Villafañe, V. E. (2013). Interactive effects of vertical mixing, nutrients and ultraviolet radiation: in situ photosynthetic responses of phytoplankton from high mountain lakes in Southern Europe. *Biogeosciences*, 10(2), 1037–1050. DOI: 10.5194/bg-10-1037-2013

Heraud, P., Roberts, S., Shelly, K., & Beardall, J. (2005). Interactions between UV-B exposure and phosphorus nutrition. II. Effects on rates of damage and repair. *Journal of Phycology*, 41, 1212–1218.

Huang, S., Zhang, K., Lin, Q., Liu, J., & Shen, J. (2022). Abrupt ecological shifts of lakes during the Anthropocene. *Earth-Science Reviews*, 227, 103981. DOI: 10.1016/j.earscirev.2022.103981

IPCC. (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the*

Intergovernmental Panel on Climate Change (V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou, Eds.). Cambridge University Press.

Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Global Change Biology*, 22(1), 180–189. DOI: 10.1111/gcb.13028

Jackson, M. C., Pawar, S., & Woodward, G. (2021). The temporal dynamics of multiple stressor effects: from individuals to ecosystems. *Trends in Ecology & Evolution*, 36(5), 402-410. DOI: 10.1016/j.tree.2021.01.005

Jia, J., Dungait, J. A. J., Yu, G., Cui, T., & Gao, Y. (2024). Nutrient enrichment and climate warming drive carbon production of global lake ecosystems. *Earth-Science Reviews*, 258, 104968. DOI: 10.1016/j.earscirev.2024.104968

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J., & Domenici, P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution*, 28(11), 651–658. DOI: 10.1016/j.tree.2013.05.005

Köhler, J., Schmitt, M., Krumbeck, H., Kapfer, M., Litchman, E., & Neale, P. J. (2001). Effects of UV on carbon assimilation of phytoplankton in a mixed water column. *Aquatic Sciences*, 63(3), 294–309. DOI: 10.1007/PL00001356

Korbee, N., Carrillo, P., Mata, M. T., Rosillo, S., Medina-Sánchez, J. M., & Figueroa, F. L. (2012). Effects of ultraviolet radiation and nutrients on the structure-function of phytoplankton in a high mountain lake. *Photochemical & Photobiological Sciences*, 11(6), 1087–1098. DOI: 10.1039/c2pp05336e

Lange, K., Bruder, A., Matthaei, C. D., Brodersen, J., & Paterson, R. A. (2018). Multiple-stressor effects on freshwater fish: Importance of taxonomy and life stage. *Fish and Fisheries*, 19(6), 974–983. DOI: 10.1111/faf.12305

Levine, J. M., Bascompte, J., Adler, P. B., & Allesina, S. (2017). Beyond pairwise mechanisms of species coexistence in complex communities. *Nature*, 546(7656), 56–64. DOI: 10.1038/nature22898

Loria, K. A., McKnight, D., Ragar, D. M., & Johnson, P. T. J. (2020). The life aquatic in high relief: shifts in the physical and biological characteristics of alpine lakes along an elevation gradient in the Rocky Mountains, USA. *Aquatic Sciences*, 82(1), 11. DOI: 10.1007/s00027-019-0684-6

Lozano, I., González-Olalla, J.M., & Medina-Sánchez, J.M. (2023). New Insights for the Renewed Phytoplankton-Bacteria Coupling Concept: the Role of the Trophic Web. *Microbial Ecology*, 86(2), 810–824. DOI: 10.1007/s00248-022-02159-6

Martínez, R. (1977). Phytoplankton species, biomass and diversity in Lake La Caldera (Sierra Nevada, Granada, Spain). *Acta Hydrobiologica*, 19, 95–107.

Martínez-Silvestre, R. (1975). First report on the limnology of the alpine lake La Caldera, in the Penibetic Mountains (Sierra Nevada, Granada, Spain). *SIL Proceedings*, 1922-2010, 19(2), 1133–1139. DOI: 10.1080/03680770.1974.11896165

Medina-Sánchez, J. M., Cabrerizo, M. J., González-Olalla, J. M., Villar-Argaiz, M., & Carrillo, P. (2022). High Mountain Lakes as Remote Sensors of Global Change. In *The Landscape of the Sierra Nevada* (pp. 261–278). Springer International Publishing. DOI: 10.1007/978-3-030-94219-9_16

Medina-Sánchez, J. M., Delgado-Molina, J. A., Bratbak, G., Bullejos, F. J., Villar-Argaiz, M., & Carrillo, P. (2013). Maximum in the Middle: Nonlinear Response of Microbial Plankton to Ultraviolet Radiation and Phosphorus. *PLoS ONE*, 8(4), e60223. DOI: 10.1371/journal.pone.0060223

Medina-Sánchez, J. M., Villar-Argaiz, M., & Carrillo, P. (2002). Modulation of the bacterial response to spectral solar radiation by algae and limiting nutrients. *Freshwater Biology*, 47(11), 2191–2204. DOI: 10.1046/j.1365-2427.2002.00969.x

Medina-Sánchez, J. M., Villar-Argaiz, M., & Carrillo, P. (2004). Neither with nor without you: A complex algal control on bacterioplankton in a high mountain lake. *Limnology and Oceanography*, 49(5), 1722–1733. DOI: 10.4319/lo.2004.49.5.1722

Medina-Sánchez, J. M., Villar-Argaiz, M., & Carrillo, P. (2006). Solar radiation – nutrient interaction enhances the resource and predation algal control on bacterioplankton : A short-term experimental study. *Limnology and Oceanography*, 51(2), 913–924.

Morales-Baquero, R., Carrillo, P., Cruz-Pizarro, L., & Sánchez-Castillo, P. (1992). Southernmost high mountain lakes in Europe (Sierra Nevada) as reference sites for pollution and climate change monitoring. *Limnetica*, 8(1), 39–47.

Morales-Baquero, R., Carrillo, P., Reche, I., & Sánchez-Castillo, P. M. (1999). Nitrogen–phosphorus relationship in high mountain lakes: effects of the size of catchment basins. *Canadian Journal of Fisheries and Aquatic Sciences*, 56, 1809–1817.

Morris, O. F., Loewen, C. J. G., Woodward, G., Schäfer, R. B., Piggott, J. J., Vinebrooke, R. D., & Jackson, M. C. (2022). Local stressors mask the effects of warming in freshwater ecosystems. *Ecology Letters*, 25(11), 2540–2551. DOI: 10.1111/ele.14108

Moser, K. A., Baron, J. S., Brahney, J., Oleksy, I. A., Saros, J. E., Hundey, E. J., ... Smol, J. P. (2019). Mountain lakes: Eyes on global environmental change. *Global and Planetary Change*, 178, 77–95. DOI: 10.1016/j.gloplacha.2019.04.001

Ogbebo, F. E., & Ochs, C. (2008). Bacterioplankton and phytoplankton production rates compared at different levels of solar ultraviolet radiation and limiting nutrient ratios. *Journal of Plankton Research*, 30(11), 1271–1284. DOI: 10.1093/plankt/fbn083

Oleksy, I. A., Baron, J. S., Leavitt, P. R., & Spaulding, S. A. (2020). Nutrients and warming interact to force mountain lakes into unprecedented ecological states. *Proceedings of the Royal Society B: Biological Sciences*, 287(1930), 20200304. DOI: 10.1098/rspb.2020.0304

Ormerod, S. J., Dobson, M., Hildrew, A. G., & Townsend, C. R. (2010). Multiple stressors in freshwater ecosystems. *Freshwater Biology*, 55(s1), 1–4. DOI: 10.1111/j.1365-2427.2009.02395.x

Orr, J. A., Luijckx, P., Arnoldi, J., Jackson, A. L., & Piggott, J. J. (2022). Rapid evolution generates synergism between multiple stressors: Linking theory and an evolution experiment. *Global Change Biology*, 28(5), 1740–1752. DOI: 10.1111/gcb.15633

Orr, J. A., Macaulay, S. J., Mordente, A., Burgess, B., Albini, D., Hunn, J. G., Restrepo-Sulez, K., …. Jackson, M. C. (2024). Studying interactions among anthropogenic stressors in freshwater ecosystems: A systematic review of 2396 multiple-stressor experiments. *Ecology Letters*, 27(6). DOI: 10.1111/ele.14463

Pastorino, P., Elia, A. C., Pizzul, E., Bertoli, M., Renzi, M., & Prearo, M. (2024). The old and the new on threats to high-mountain lakes in the Alps: A comprehensive examination with future research directions. *Ecological Indicators*, 160, 111812. DOI: 10.1016/j.ecolind.2024.111812

Pastorino, P., Pizzul, E., Bertoli, M., Perilli, S., Brizio, P., Salvi, G., Esposito, G., Abete, M. C., Prearo, M., & Squadrone, S. (2020). Macrobenthic invertebrates as bioindicators of trace elements in high-mountain lakes. *Environmental Science and Pollution Research*, 27(6), 5958–5970. DOI: 10.1007/s11356-019-07325-x

Pastorino, P., & Prearo, M. (2020). High-Mountain Lakes, Indicators of Global Change: Ecological Characterization and Environmental Pressures. *Diversity*, 12(6), 260. DOI: 10.3390/d12060260

Pausz, C., & Herndl, G. (2002). Role of nitrogen versus phosphorus availability on the effect of UV radiation on bacterioplankton and their recovery from previous UV stress. *Aquatic Microbial Ecology*, 29, 89–95. DOI: 10.3354/ame029089

Pearce, N. J. T., Larson, J. H., Evans, M. A., Frost, P. C., & Xenopoulos, M. A. (2021). Episodic Nutrient Addition Affects Water Column Nutrient Processing Rates in River-to-Lake Transitional Zones. *Journal of Geophysical Research: Biogeosciences*, 126(11). DOI: 10.1029/2021JG006374

Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2015). Reconceptualizing synergism and antagonism among multiple stressors. *Ecology and Evolution*, 5(7), 1538–1547. DOI: 10.1002/ece3.1465

Pirotta, E., Thomas, L., Costa, D. P., Hall, A. J., Harris, C. M., Harwood, J., Kraus, S. D., ... Tyack, P. L. (2022). Understanding the combined effects of multiple stressors: A new perspective on a longstanding challenge. *Science of The Total Environment*, 821, 153322. DOI: 10.1016/j.scitotenv.2022.153322

Quinlan, R., Filazzola, A., Mahdiyan, O., Shuvo, A., Blagrave, K., Ewins, C., Moslenko, L., ... Sharma, S. (2021). Relationships of total phosphorus and chlorophyll in lakes worldwide. *Limnology and Oceanography*, 66(2), 392–404. DOI: 10.1002/lno.11611

Reche, I., Mladenov, N., Pulido-Villena, E., & Morales-Baquero, R. (2022). Atmospheric Inputs and Biogeochemical Consequences in High-Mountain Lakes. In *The Landscape of the Sierra Nevada* (pp. 293–306). Springer International Publishing. DOI: 10.1007/978-3-030-94219-9_18

Sánchez-Castillo, P., Cruz-Pizarro, L., & Carrillo, P. (1989). Caracterización del fitoplancton de las lagunas de alta montaña de Sierra Nevada (Granada, España) en relación con las características físico-químicas del medio. *Limnetica*, 5, 37–50.

Savage, V. M., Gillooly, J. F., Brown, J. H., West, G. B., & Charnov, E. L. (2004). Effects of Body Size and Temperature on Population Growth. *The American Naturalist*, 163(3), 429–441. DOI: 10.1086/381872

Schindler, D. W., Curtis, P. J., Parker, B. R., & Stainton, M. P. (1996). Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes. *Nature*, 379(6567), 705–708. DOI: 10.1038/379705a0

Smith, E. M., & Prairie, Y. T. (2004). Bacterial metabolism and growth efficiency in lakes: The importance of phosphorus availability. *Limnology and Oceanography*, 49(1), 137–147. DOI: 10.4319/lo.2004.49.1.0137

Spears, B. M., Chapman, D. S., Carvalho, L., Feld, C. K., Gessner, M. O., Piggott, J. J., Banin, L. F., ... Birk, S. (2021). Making waves. Bridging theory and practice towards multiple stressor management in freshwater ecosystems. *Water Research*, 196, 116981. DOI: 10.1016/j.watres.2021.116981

Tekin, E., Diamant, E. S., Cruz-Loya, M., Enriquez, V., Singh, N., Savage, V. M., & Yeh, P. J. (2020). Using a newly introduced framework to measure ecological stressor interactions. *Ecology Letters*, 23(9), 1391–1403. DOI: 10.1111/ele.13533

Villafañe, V. E., Cabrerizo, M. J., Erzinger, G. S., Bermejo, P., Strauch, S. M., Valiñas, M. S., & Helbling, E. W. (2017). Photosynthesis and Growth of Temperate and Sub-Tropical Estuarine Phytoplankton in a Scenario of Nutrient Enrichment under Solar Ultraviolet Radiation Exposure. *Estuaries and Coasts*, 40(3), 842–855. DOI: 10.1007/s12237-016-0176-z

Villafañe, V. E., Sundbäck, K., Figueroa, F. L., & Helbling, E. W. (2003). Photosynthesis in the aquatic environment as affected by UVR. In E. W. Helbling & H. E. Zagarese (Eds.), *UV Effects in Aquatic Organisms and Ecosystems* (pp. 357–397). Royal Society of Chemistry.

Villar-Argaiz, M., Medina-Sánchez, J. M., Biddanda, B. A., & Carrillo, P. (2018). Predominant non-additive effects of multiple stressors on autotroph C:N:P Ratios propagate in freshwater and marine food webs. *Frontiers in Microbiology*, 9(JAN), 1–13. DOI: 10.3389/fmicb.2018.00069

Weckström, K., Weckström, J., Huber, K., Kamenik, C., Schmidt, R., Salvenmoser, W., Rieradevall, M., Weisse, T., Psenner, R., & Kurmayer, R. (2016). Impacts of Climate Warming on Alpine Lake Biota Over the Past Decade. *Arctic, Antarctic, and Alpine Research*, 48(2), 361–376. DOI: 10.1657/AAAR0015-058