Multiple stressors acting on the algal-bacterial interaction in Mediterranean oligotrophic systems

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MULTIPLE STRESSORS ACTING ON THE ALGAL-BACTERIAL INTERACTION IN MEDITERRANEAN OLIGOTROPHIC SYSTEMS

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Granada, de 2014

Doctorando

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Introducción y objetivos

0.1. Cambio global en la región Mediterránea: Factores de cambio global

El concepto de cambio global define el conjunto de cambios ambientales afectados por la actividad humana, con especial referencia a cambios en los procesos que determinan el funcionamiento del sistema Tierra (Duarte *et al.*, 2006; Stocker *et al.*, 2013). Desde el punto de vista climático, la región Mediterránea se sitúa en una zona de transición entre el clima Paleotropical y el clima Templado eurosiberiano, por lo que se ve afectado por la interacción de procesos meteorológicos (ej. olas de frío y calor, tormentas, sequías) de latitudes medias y tropicales (Giorgi y Lionello, 2008).

Por sus particulares características climatológicas, es una de las regiones del mundo especialmente sensible a los efectos del cambio global y el cambio climático (Giorgi, 2006; Giorgi y Lionello, 2008; Stocker *et al.*, 2013; Garcia-Herrera *et al.*, 2014), de forma que pequeños cambios en los modelos de circulación general pueden resultar en cambios importantes en dicha climatología (Fig. 1). Existen predicciones muy consistentes de un incremento de la temperatura media global (1.2°C en el periodo 2001-2020 y hasta 4.6°C en el periodo 2081-2100) y una reducción de las precipitaciones medias (desde un 7 % en 2001-2020 a un 28 % en 2081-2100) (Giorgi y Lionello, 2008). Tales cambios pueden tener un gran impacto sobre las propiedades térmicas de las masas de agua, modificando la duración e intensidad de los periodos de mezcla y estratificación, con consecuencias sobre el

reciclado de nutrientes y el funcionamiento de la red trófica (Sahoo *et al.*, 2010).

En la región Mediterránea se espera una mayor frecuencia de eventos climáticos extremos, tales como lluvias torrenciales y olas de calor o frío, que alterarán espacial y temporalmente el régimen de precipitaciones dando lugar a mayor frecuencia de sequías e incrementarían el riesgo de desertificación (Stocker *et al.*, 2013; Linares *et al.*, 2011; Garcia-Herrera *et al.*, 2014). Esto último, junto con otros procesos asociados con el cambio global tales como los cambios de uso del suelo, promoverán mayor entrada de nutrientes orgánicos e inorgánicos en los ecosistemas acuáticos (Moss, 2012; Spyropoulou *et al.*, 2012; Evans *et al.*, 2006). Adicionalmente, una importante vía de entrada de nutrientes en los ecosistemas Mediterráneos es a través de aerosoles atmosféricos, principalmente en forma de polvo procedente del desierto del Sahara Morales-Baquero2006, los cuales contienen una alta concentración de macro (P) y micronutrientes (Fe y Ca, Morales-baquero *et al.*, 2006; Pulido-Villena *et al.*, 2006).

Además, el incremento previsto de temperatura afectará de forma directa a los procesos metabólicos de los organismos, tal y como describe la ecuación de Arrhenius que representa la base de la teoría metabólica en ecología (Brown *et al.*, 2004). Así, la mencionada teoría describe el papel de la temperatura como reguladora del metabolismo de los ecosistemas de forma que las tasas metabólicas de los organismos controlan los procesos ecológicos en todos los niveles de organización (Gillooly *et al.*, 2001; Brown *et al.*, 2004). Por tanto, alteraciones de la temperatura tendrán efectos directos sobre el metabolismo de los organismos pero a su vez importantes consecuencias a escala ecosistémica y global.

Por otra parte, un incremento de temperatura tenderá a incrementar la estabilidad térmica de las masas de agua, acentuando su estratificación (De Senerpont Domis *et al.*, 2013). La disminución de la profundidad del epilimnion como consecuencia de la estratificación térmica intensificará la exposición de los organismos planctónicos a mayores intensidades de radiación fotosintéticamente activa (PAR), pero también de radiación ultravioleta (UVR, Schindler, 2009), a la vez que incrementará el gradiente de nutrientes reduciendo el intercambio de nutrientes entre la capa profunda y

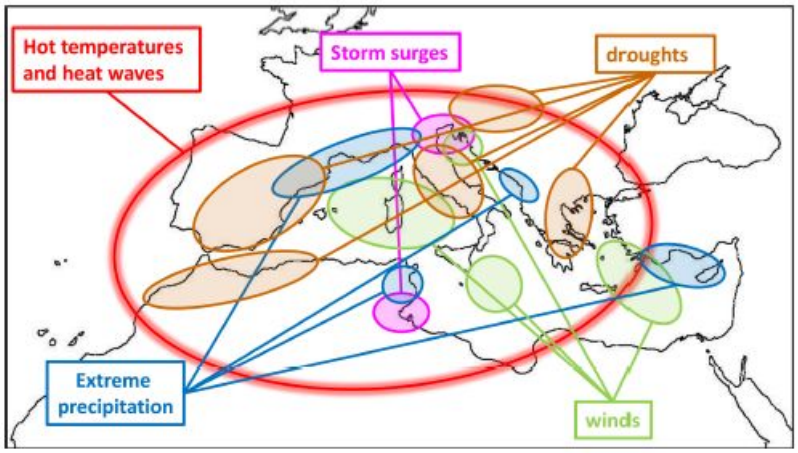

Figura 1: Ilustración simplificada que muestra las zonas de la región mediterránea específicamente afectadas por determinados eventos climáticos extremos derivados del cambio global (Garcia-Herrera *et al.*, 2014, Fig.2)

epilimnética (Sarmento *et al.*, 2010). Un incremento en la exposición a altos flujos de radiación solar puede tener importantes consecuencias negativas para los organismos. Estos efectos indirectos de la temperatura podrían ser contrarrestados por episodios de vientos fuertes que actuarían favoreciendo la mezcla dentro de la columna de agua e incrementando la profundidad del epilimnion (Helbling *et al.*, 2013). Sin embargo, incluso bajo un régimen de mezcla de la columna de agua podría existir limitación por nutrientes en capas superficiales si la profundidad de la capa de mezcla no alcanza la nutriclina (Fouilland *et al.*, 2007).

Puesto que todas estas alteraciones ambientales no actuarán de forma aislada sobre los ecosistemas Mediterráneos, es necesario el desarrollo de estudios y aproximaciones experimentales considerando la interacción de diversos factores. Sólo así podremos obtener conclusiones más realistas de la respuesta de los organismos y por ende de los ecosistemas ante el cambio global (Crain *et al.*, 2008; Christensen *et al.*, 2006; Stewart *et al.*, 2013).

0.1.1. Efecto de UVR en ecosistemas acuáticos y su interacción con factores de cambio global.

La UVR es la región del espectro de radiación solar que tiene mayor actividad fotoquímica y actúa produciendo efectos a nivel genómico, citotóxico y ontogénico sobre bacterioplancton (Ruiz-González *et al.*, 2013) y fitoplancton (Pessoa, 2012). Entre las respuestas de los organismos a UVR han sido descritas tanto la inhibición (Ej.: Morán *et al.*, 2001; Carrillo *et al.*, 2002; Helbling *et al.*, 2003a; Hernández *et al.*, 2006; Conan *et al.*, 2008) como el estímulo del crecimiento (Ej.: Church *et al.*, 2004; Pakulski *et al.*, 2008; Lionard *et al.*, 2012). Así mismo, la intensidad de los efectos varían dependiendo del metabolismo de los organismos objeto de estudio (Jeffrey *et al.*, 1996; Ogbebo y Ochs, 2008). Esta variabilidad de respuestas podría estar justificada por la existencia de efectos indirectos de UVR (Ruiz-González *et al.*, 2013), mediatizados por interacciones tróficas así como por la interacción de UVR con otros factores abióticos (Harrison y Smith, 2009; Tucker y Williamson, 2011) como la disponibilidad de nutrientes inorgánicos, concentración de carbono orgánico disuelto (DOC

Williamson y Rose, 2010), temperatura (Bullock y Jeffrey, 2010) o régimen de mezcla de la columna de agua (Helbling *et al.*, 2008) (Fig. 2).

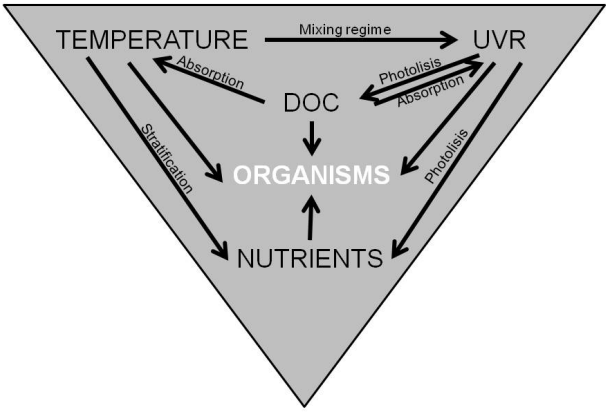

Figura 2: Interacción de factores abióticos en ecosistemas mediterráneos.

La temperatura, tanto por sus efectos directos sobre el metabolismo individual (Sarmento *et al.*, 2010) como por sus efectos indirectos sobre la estructura térmica y régimen de mezcla de las masas de agua (Saros *et al.*, 2012), se ha mostrado como un importante modulador de los efectos de UVR (Doyle *et al.*, 2005; Bullock y Jeffrey, 2010; Halac *et al.*, 2010, 2013). Del mismo modo, puesto que muchos de los mecanismos de aclimatación o adaptación a UVR, así como los de reparación (mecanismos de reparación en oscuridad), son dependientes de la disponibilidad de nutrientes (Kaiser y Herndl, 1997; Medina-Sánchez *et al.*, 2006; Carrillo *et al.*, 2008a), éstos, junto a la temperatura, son factores claves en la respuesta de los organismos a UVR. A pesar de que ha sido estudiado el efecto interactivo entre UVR y nutrientes, UVR y temperatura o UVR y régimen de mezcla sobre fitoplancton (Ej.: Carrillo *et al.*, 2008a; Marcoval *et al.*, 2008; Helbling *et al.*, 2008) y bacterioplancton (Ej.: Medina-Sánchez *et al.*, 2006; Bullock y Jef-

frey, 2010; Bertoni *et al.*, 2011) o sobre las redes tróficas microbianas (Ej.: Vidussi *et al.*, 2011; Fouilland *et al.*, 2013; Medina-Sánchez *et al.*, 2013), son escasos los estudios que han considerado la interacción múltiple entre UVR, nutrientes y temperatura (Doyle *et al.*, 2005), o entre UVR, nutrientes y régimen de mezcla (Helbling *et al.*, 2013), tanto sobre poblaciones individuales como sobre comunidades o sus interacciones tróficas (Bouvy *et al.*, 2011; Fouilland *et al.*, 2007). Hasta donde conocemos, solo Bouvy *et al.* (2011) han analizado el efecto de la acción conjunta de UVR, nutrientes y temperatura sobre interacciones bióticas (predación y parasitismo) en redes tróficas microbianas en un lago costero de la región mediterránea.

Los ecosistemas acuáticos oligotróficos con bajo contenido en DOC, donde la UVR penetra hasta capas profundas de la columna de agua, son particularmente vulnerables a los efectos de UVR (Häder *et al.*, 2007; Rose *et al.*, 2009). En estos ecosistemas, el efecto de UVR sobre las comunidades planctónicas puede estar acentuado por la baja disponibilidad de nutrientes y está muy influido por el régimen de mezcla vertical de la columna de agua, que determinará tanto la exposición de los organismos a UVR como la distribución de los nutrientes. Un caso particular de ecosistemas vulnerables a los efectos del cambio global son los lagos de alta montaña (Psenner, 2003; Parker *et al.*, 2008), particularmente los de regiones templadas (Aguilera *et al.*, 2013). Estos ecosistemas están naturalmente expuestos a condiciones extremas tales como elevado flujo de UVR, bajas temperaturas o baja concentración de nutrientes y DOC (Psenner, 2003; Catalan *et al.*, 2006, 2009). A pesar de estas características y debido a la gran adaptación de los organismos que habitan estos ecosistemas, pequeños cambios en la estructura física del sistema pueden causar respuestas detectables a muy corto plazo (Psenner, 2003; Catalan *et al.*, 2006; Parker *et al.*, 2008), lo que ha favorecido que los lagos de alta montaña hayan sido propuestos como centinelas del cambio global (Catalan *et al.*, 2006, 2009). Además, estos lagos, poseen redes tróficas sencillas aunque con interacciones bióticas complejas (Carrillo *et al.*, 2006), por lo que constituyen un excelente laboratorio natural donde cuantificar y testar cambios predichos en futuros escenarios de cambio global (Psenner, 2003). Muchos de estos lagos han sido propuestos como ecosistemas de referencia para ser incluidos en estudios comparativos

intersistémicos centrados en la búsqueda de patrones de sensibilidad frente a perturbaciones ambientales .

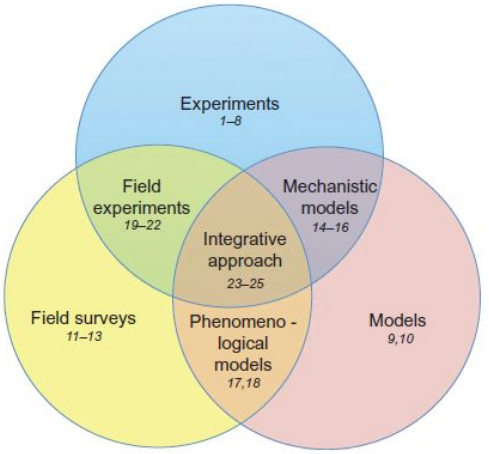

Figura 3: Clases y combinaciones de aproximaciones usadas para investigar las consecuencias ecológicas del cambio climático a través de experimentos, modelos y estudios de campo (Stewart et al. 2013, Fig. 2).

Entre las herramientas empleadas para la cuantificación de los efectos del cambio global (Fig. 3), es fundamental la monitorización a largo plazo, generando valiosas series temporales que permiten modelar las tendencias en las últimas cuatro décadas (Bullejos *et al.*, 2010; Tiberti *et al.*, 2010; Villar-Argaiz *et al.*, 2012; Birck *et al.*, 2013). Sin embargo, para la predicción de las consecuencias ecológicas del cambio global son necesarias aproximaciones experimentales (Stewart *et al.*, 2013), que además permiten discriminar entre potenciales procesos generadores de los cambios en las dinámicas de las poblaciones sobre escalas temporales largas (Ej.: Delgado-Molina *et al.*, 2009; Medina-Sánchez *et al.*, 2013). Las aproximaciones experimentales, a diferencia de los estudios observacionales y las aproximaciones matemáticas, proporcionan una información realista de los efectos del

cambio global sobre los ecosistemas acuáticos, ya que permiten controlar y manipular aquellos factores bióticos o abióticos de interés manteniendo el resto de los factores dentro de su variabilidad natural. El diseño experimental apropiado dependerá del nivel de organización biológica que queramos integrar en nuestro estudio y de los mecanismos de respuesta que se requiere dilucidar (Stewart *et al.*, 2013, ; Fig. 4). Así, en el caso de experimentos diseñados para el estudio de niveles de organización superiores como el de comunidad o ecosistema es apropiado el uso de meso- y microcosmos, que permiten la consideración de una mayor complejidad biológica y más largas escalas temporales de estudio (Stewart *et al.*, 2013), y pueden proporcionar una mayor comprensión de los mecanismos subyacentes a las respuestas observadas (Benton *et al.*, 2007), siempre que la elección del volumen del encerramiento y la escala temporal de estudio se adecúen a nuestro objetivo de estudio. De hecho, los resultados obtenidos con su utilización han demostrado su realismo y son extrapolables para comprender el resultado neto y el modo de interacción de los factores sobre los ecosistemas naturales (Benton *et al.*, 2007; Spivak *et al.*, 2010).

0.2. Cuantificación y caracterización de efectos interactivos: Consideraciones y problemática.

El cambio global incluye alteraciones en numerosos factores que se van a convertir en "conductores" de los cambios producidos en los ecosistemas. Existe una tendencia a denominar "estresores" a aquellos factores cuyos valores se hayan significativamente desviados de los valores habituales (Boyd y Hutchins, 2012), término con connotaciones negativas y que implica la expectativa de un efecto depresor sobre todos los organismos del ecosistema. Sin embargo, cualquier cambio en las condiciones abióticas va a influir de forma directa o indirecta negativamente sobre unos organismos pero también positivamente sobre otros, siendo estos últimos los que mejor se adapten al ecosistema alterado (Nogales *et al.*, 2011). Por ello, es más adecuado hablar de "conductores", o simplemente factores, cuando se alude a aquellas alteraciones abióticas que producen una respuesta biológica

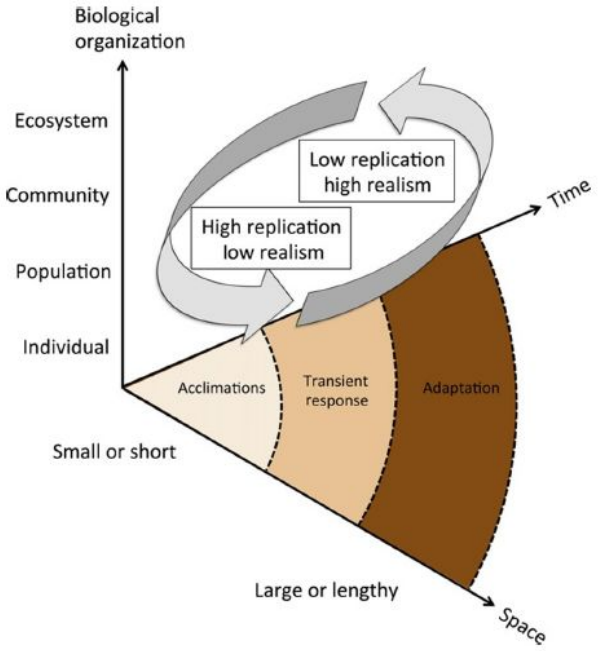

Figura 4: Diagrama conceptual que representa un diseño experimental ideal (Stewart et al. 2013, Fig. 3).

cuantificable, que puede ser desde depresora hasta estimuladora (Boyd y Hutchins, 2012). El término "estresor" por tanto definiría a aquellos cambios ambientales que resultarán en un efecto negativo (Vinebrooke *et al.*, 2004; Boyd y Hutchins, 2012) y con referencia a un determinado organismo o grupo de organismos.

Tales factores pueden actuar con diferente intensidad, frecuencia y distribución espacial (Paine *et al.*, 1998), pero no actúan de forma aislada en la naturaleza, sino que generalmente interaccionan para generar impactos combinados difíciles de predecir a priori en base a los efectos de los factores aislados (Folt *et al.*, 1999; Vinebrooke *et al.*, 2004). De hecho, a partir de estudios multifactoriales se ha comprobado que las interacciones son más comunes que los efectos aditivos (Darling y Côté, 2008). En base a estas evidencias, en los últimos años, se ha manifestado un creciente interés por el estudio de los efectos interactivos con objeto de obtener resultados más realistas y comprender cómo la interacción de factores mitigará o intensificará la respuesta de los ecosistemas ante el cambio global (Darling y Côté, 2008, y referencias).

Sin embargo, todavía no se ha establecido un criterio común para cuantificar la magnitud de los efectos interactivos y para determinar su naturaleza; asimismo, no existe un consenso sobre una clasificación general o tipología de efectos interactivos. Así, tras una primera diferenciación en efectos aditivos e interactivos (no aditivos), encontramos una mayor controversia cuando se trata de sub-clasificar estos últimos. Conceptualmente podríamos clasificar los efectos interactivos en sinérgicos y antagónicos (**?**Christensen *et al.*, 2006; Crain *et al.*, 2008). Así, la definición más sistemática de sinergismo sería un efecto interactivo mayor al esperado a partir de la suma de los efectos de los factores individuales (>aditivo); y por el contrario de antagonismo cuando el efecto interactivo es menor al esperado a partir de la suma de los efectos de los factores individuales (<aditivo) (**?**Christensen *et al.*, 2006; Crain *et al.*, 2008). Sin embargo, al trasladar a la práctica las anteriores definiciones, se evidencia la ambigüedad de las mismas por lo que es esencial definir si estos términos están siendo empleados en un sentido mecanicista (efectos de los factores) o basado en el resultado neto sobre los organismos (respuestas de los organismos) (Boyd y Hutchins,

2012). Por ello se han desarrollado aproximaciones matemáticas, por medio del empleo de ecuaciones e índices (Christensen *et al.*, 2006; Crain *et al.*, 2008; Elser *et al.*, 2009; Allgeier *et al.*, 2010, 2011; Brown *et al.*, 2012), frente a las tradicionales aproximaciones más conceptuales (Ej.: Folt *et al.*, 1999; Medina-Sánchez *et al.*, 2006). Esto justifica que resultados similares obtenidos a partir de distintos experimentos sean categorizados de diferentes maneras. Dependiendo del tipo de organismos y de los factores con los que se está trabajando pueden emplearse en ocasiones los términos de co-tolerancia, co-limitación o co-contaminación acompañando los términos de sinergismo y antagonismo o bien sustituyéndolos. Hay que ser por tanto cautos en la interpretación de la nomenclatura empleada en los estudios de interacción de factores, puesto que diferentes términos pueden referirse al mismo significado (Ej.: "potenciador" en lugar de "sinergismo") del mismo modo que descripciones imprecisas (ej.: "incrementó", "mejoró", "potenció") podrían ser realmente ejemplos de efectos interactivos, aditivos o por el contrario no ser significativos (Vanhoudt *et al.*, 2012).

Como complemento de la clasificación de la naturaleza de los efectos interactivos, igualmente interesante resulta la cuantificación de la fuerza o magnitud de tales efectos. Sin embargo, por su mayor complejidad, son muchos menos los trabajos que cuantifican dicha magnitud. Así, (Allgeier *et al.*, 2010), describieron el Índice de Efectos Interactivos (Interaction Effect Index, IEI), que proporciona una medida relativa del grado de no aditividad en respuestas a múltiples factores, más como recursos que como "estresores".

$$IEI = ln[respuestaAB/(respuestaA + respuestaB)] \tag{1}$$

donde "*respuesta A*" es la repuesta al factor A; "*respuesta B*" es la repuesta al factor B; y "*respuesta AB*" es la respuesta a la acción conjunta del factor A y B.

Un valor de IEI próximo a cero indicaría no aditividad, mientras que la fuerza relativa del efecto interactivo incrementaría a medida que el valor de IEI se aleja de cero, tanto en sentido positivo como negativo (Allgeier

et al., 2010, 2011). El IEI compara respuestas netas al efecto de uno o varios factores, ya que sus valores no son corregidos con el tratamiento control (Allgeier *et al.*, 2010). Sin embargo, este índice no permite la distinción entre diferentes clases de respuestas no aditivas, y la incorporación de una transformación logarítmica en su obtención puede hacer que respuestas multiplicativas aparezcan como aditivas, dificultando el estudio de efectos interactivos (Harpole *et al.*, 2011).

Diferentemente, un índice normalizado por el tratamiento control da una idea del tamaño de los efectos de los factores en cuestión. Uno de estos índices es el propuesto por (Brown *et al.*, 2012), en donde se analiza cómo el efecto interactivo observado difiere del efecto interactivo esperado (establecido a partir de los efectos individuales de los factores) y en base a esta diferencia se establece la magnitud de la interacción, incorporando además la consideración del modelo aditivo y multiplicativo (Folt *et al.*, 1999).

$$I = Observado_{AB} - Esperado_{AB} \tag{2}$$

donde: $ObservadoAB$= efecto AB-control; $EsperadoAB$= efecto A + efecto B. El tamaño del efecto de un solo factor se obtiene a partir de la diferencia del valor medio del tratamiento con el factor A o B y el control.

(Luo *et al.*, 2008) desarrollaron un índice (I), normalizando por el control, para interacciones de hasta tres factores (I^3) y que, como el IEI, también evalúa la importancia relativa de los efectos interactivos frente a los efectos individuales. Su índice considera en su cálculo tanto los efectos individuales de los factores así como los de las interacciones de menor rango. Permite, por tanto, discriminar la importancia relativa de las interacciones de dos (I^2) y tres (I^3) factores respecto a los efectos de los factores individuales. Debido a las consideraciones realizadas en el cálculo de I, la aplicación de éste índice es muy adecuada para realizar estudios comparativos entre ecosistemas afectados por similares factores de estrés, y permite determinar cuáles de ellos serían más vulnerables a cambios simultáneos en

determinados factores y cuáles lo serían a perturbaciones de un factor en particular.

En el estudio de interacción de factores es importante reconocer la escala temporal de actuación de los factores considerados. Así, cambios en determinados factores ambientales que operan simultáneamente en un ecosistema pueden actuar como conductores o factores de estrés dando lugar a efectos interactivos, aunque individualmente tales factores presenten diferentes escalas temporales de actuación. Encontramos en la naturaleza factores que pueden ejercer de estresores de una forma crónica, como es el caso de UVR (Helbling *et al.*, 2003b; Tedetti y Sempéré, 2006), la cual, aunque con distinta intensidad dependiendo de la latitud (Meador *et al.*, 2009) y altitud del ecosistema (Blumthaler *et al.*, 1997), actúa de forma permanente en los ecosistemas. Por otra parte, existen factores que actúan en forma de pulsos sobre los ecosistemas, como puede ser la disponibilidad de recursos (Yang *et al.*, 2008). Se considera que un factor opera de forma pulsada cuando se producen cambios en sus valores medios al combinarse una baja frecuencia, una gran magnitud y una corta duración (Yang *et al.*, 2008). Estos pulsos pueden originarse por diversas causas como pueden ser causas climáticas (Ej. Lluvias torrenciales, tormentas de polvo) o por su acumulación espacial o temporal y posterior liberación (Yang *et al.*, 2008). Aunque los pulsos son eventos de corta duración, por su carácter acumulativo, sus efectos ecológicos pueden tener una mayor persistencia en el tiempo (Svensen *et al.*, 2002; Falkner y Falkner, 2003; Yang *et al.*, 2008). Por el contrario, otros factores pueden ejercer su acción estresante de forma abrupta, como es el caso de la temperatura. Así, por ejemplo los eventos tormentosos(Adams *et al.*, 2010) u olas de frío o calor (Thompson *et al.*, 2013) pueden drásticamente modificar la temperatura aérea y acuática. Sin embargo, a pesar de la gran relevancia ecológica de los eventos climáticos extremos, la mayor parte de estudios de cambio global se han centrado exclusivamente en el estudio y simulación experimental de la alteración de los valores medios de los factores climáticos (Benedetti-Cecchi *et al.*, 2006; Pincebourde *et al.*, 2012; Kreyling y Beier, 2013). La temporalidad de diferentes factores puede ser interdependiente. Por ejemplo, una alteración climática abrupta, como un evento tormentoso, puede resultar en un cambio de temperatura y en un

pulso de recursos si lleva asociada agua de escorrentía. Puede darse, por tanto, una coincidencia temporal de los niveles estresantes de diferentes factores ambientales (Pincebourde *et al.*, 2012), Ej.: coincidencia de olas de calor y tormentas de polvo en lagos de la región mediterránea durante el verano.

Además de las características de los factores, es importante considerar el tipo de organismos objeto de estudio, su metabolismo y tiempos de generación (Crain *et al.*, 2008), así como las interacción de especies, diversidad y redundancia de especies, relaciones tróficas, historia ecológica y tipo de ecosistema (Vinebrooke *et al.*, 2004). A pesar de los numerosos estudios demostrando los efectos de los factores de cambio global sobre la abundancia de las poblaciones, composición de la comunidad o fisiología de los organismos (Sala *et al.*, 2000), existen alteraciones menos obvias sobre las redes de interacción entre organismos (Tylianakis *et al.*, 2008). Sin embargo, debido a las dificultades para cuantificar cambios en las interacciones tróficas, existe escasez de trabajos que aborden el estudio de la interacción de factores abióticos sobre las interacciones bióticas (Tylianakis et al., 2008). Las relaciones tróficas son especialmente sensibles a las alteraciones ambientales, ya que están condicionadas por los cambios en la fisiología, abundancia o diversidad (Tylianakis *et al.*, 2008), y constituyen la base del funcionamiento de los ecosistemas. Por tanto, son necesarios más estudios que aborden los efectos de cambio global sobre las relaciones de los organismos.

0.3. La interacción alga-bacteria en un contexto de cambio global

El estrés ambiental altera la fisiología de los organismos, influyendo a su vez en las interacciones bióticas y por tanto iniciando efectos que se pueden transmitir en forma de cascada a lo largo de toda la red trófica (Pincebourde and Woods, 2012). El microplancton representa la mayor parte de la biomasa acuática planctónica y es el responsable de la productividad y del reciclado de nutrientes en los ecosistemas acuáticos (Paerl *et al.*, 2003; Fouilland y Mostajir, 2010). Así, el fitoplancton, a pesar de representar sólo

el 1 % de la biomasa fotosintética del planeta, es el responsable del 45 % de la producción primaria (Falkowski *et al.*, 2004). Por su parte, el bacterioplancton inmoviliza y mineraliza la práctica totalidad de los elementos con importancia biogeoquímica en los ecosistemas acuáticos (Newton *et al.*, 2011) haciéndolos disponibles para niveles superiores de las redes tróficas pelágicas a través del bucle microbiano. Debido a su papel clave y su posición basal en las redes tróficas, pequeñas perturbaciones ambientales que afecten a estos dos eslabones puede ser amplificada a niveles tróficos superiores (Sarmento *et al.*, 2010).

En numerosos ecosistemas acuáticos, principalmente en ecosistemas con baja concentración de DOC de origen alóctono, ha sido descrito un acople alga-bacteria a través de una relación comensalista, de forma que la producción bacteriana heterotrófica está sustentada por el carbono orgánico excretado por el fitoplancton (EOC Baines y Pace, 1991; Aota y Nakajima, 2001; Carrillo *et al.*, 2002; Lasternas y Agustí, 2013). El estado trófico del ecosistema pueden influir en la fuerza de la interacción alga-bacteria (Morán *et al.*, 2002; Rochelle-Newall *et al.*, 2008), definida como la proporción de las demandas bacterianas de carbono (BCD) que son satisfechas por el carbono excretado por el fitoplancton (EOC) (Morán *et al.*, 2002).

La disponibilidad de nutrientes, la UVR, temperatura u otras variables abióticas, también pueden modular el tipo de relación existente entre fitoplancton y bacterioplancton. Cambios en la concentración de carbono orgánico y de nutrientes inorgánicos pueden modificar la interacción alga-bacteria (Danger *et al.*, 2007; Liu *et al.*, 2012). Así, en condiciones ambientales caracterizadas por una baja disponibilidad de fósforo (P), fitoplancton y bacterioplancton pueden mantener una relación de competencia por este recurso limitante (Aota y Nakajima, 2001; Liu *et al.*, 2012; Carrillo *et al.*, 2008b). Sin embargo, a pesar de la mayor capacidad competitiva del bacterioplancton por el P cuando éste se encuentra a bajas concentraciones (Grover, 2000), el bacterioplancton puede ser incapaz de desplazar al fitoplancton si es dependiente del C excretado por este último (EOC Danger *et al.*, 2007). Del mismo modo una entrada de carbono orgánico disuelto alóctono (DOC) puede favorecer una mayor actividad y crecimiento bacteriano y por tanto una mayor demanda de nutrientes inorgánicos,

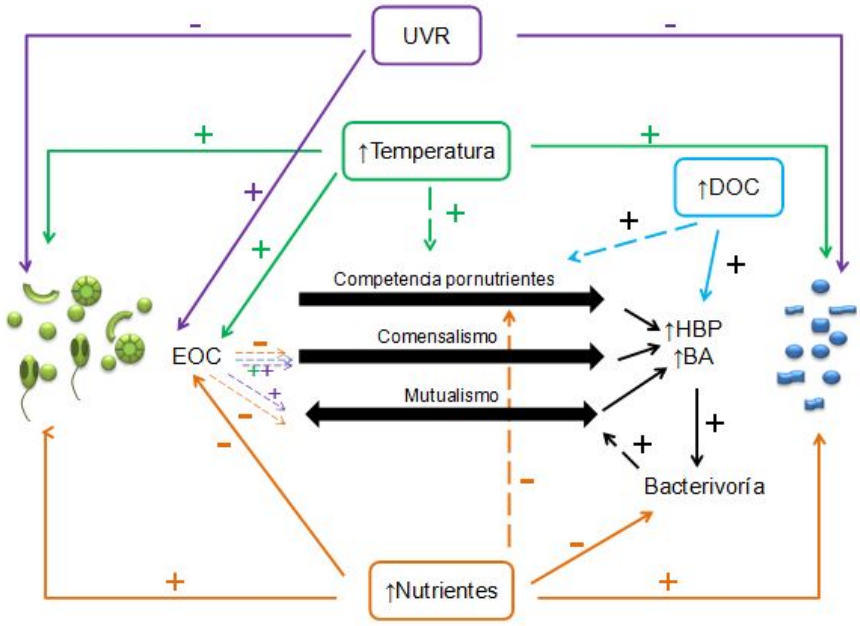

Figura 5: Efecto de factores de cambio global sobre la relación alga-bacteria.

promoviendo una relación de competencia con el fitoplancton (Liu *et al.*, 2012).

Puede también establecerse un mutualismo entre fitoplancton y bacterioplancton en ambientes con baja concentración de DOC y nutrientes, a través de un metabolismo mixotrófico, si el fitoplancton cubre las demandas de carbono del bacterioplancton a través de la liberación de EOC y además encuentra en la ingestión del bacterioplancton una fuente de nutrientes minerales (Medina-Sánchez *et al.*, 2004; Mitra *et al.*, 2014).

Otros factores como la UVR o la temperatura también pueden influir en el sentido de la relación alga-bacteria. Así, una mayor liberación de C producida por un incremento de temperatura (Morán *et al.*, 2006) o por algas estresadas por UVR (Carrillo *et al.*, 2002) puede estimular el crecimiento bacteriano (si estas bacterias están relativamente adaptadas a esta radiación en el caso de UVR), reforzando el acople de la relación comensalista alga-bacteria. También un incremento de la temperatura puede promover un comportamiento heterótrofo en organismos mixótrofos (Wilken *et al.*, 2013) que puede potenciar la ingestión de bacterias, conduciendo a una relación alga-bacteria de depredador-presa y reduciendo la relación de competencia por nutrientes en ecosistemas oligotróficos. Por tanto, las condiciones ambientales y la alteración de las mismas van a determinar el funcionamiento de los ecosistemas acuáticos, la preponderancia de la cadena de pastoreo o del bucle microbiano y el carácter autotrófico o heterotrófico de los mismos.

0.4. Objetivos e hipótesis

Los objetivos genéricos de esta tesis son:

1. Analizar los efectos interactivos de múltiples factores relacionados con cambio global (UVR, nutrientes, temperatura, mezcla vertical, incremento en la irradiación media), sobre la estructura, metabolismo e interacciones tróficas de comunidades microplanctónicas de ecosistemas oligotróficos de la región Mediterránea.

2. Cuantificar la magnitud y fuerza de interacción entre dichos factores de estrés para predecir la vulnerabilidad de ecosistemas acuáticos marinos y epicontinentales en un futuro escenario de cambio global.

Estos objetivos generales han sido abordados a través de la consecución de los siguientes objetivos específicos:

Objetivo 1: Determinar la magnitud del efecto interactivo entre UVR e incremento en la intensidad de la irradiancia sobre comunidades algales y bacterianas y su interacción comensalista en lagos de contrastante transparencia a UVR.

Hipótesis 1: El incremento de la intensidad de la irradiancia acentuará los efectos dañinos de UVR sobre la producción primaria y bacteriana heterotrófica, incrementando la fuerza de la relación comensalista alga-bacteria debido a un incremento en excreción de C algal. Estos efectos serán más intensos en lagos opacos a UVR debido a la menor aclimatación a UVR de los microorganismos en ecosistemas con alto contenido en DOC.

Objetivo 2: Establecer la naturaleza de la interacción entre UVR, mezcla vertical y nutrientes minerales sobre el metabolismo algal y bacteriano en lagos de alta montaña con distinta transparencia a UVR.

Hipótesis 2.1: La radiación fluctuante actuará sinérgicamente con UVR y nutrientes disminuyendo la producción bacteriana y algal en lagos opacos a UVR.

Hipótesis 2.2: Este efecto sinergista sobre el bacterioplancton será el resultado de efectos negativos directos e indirectos, a través de la disminución en la disponibilidad de carbono de origen algal, principalmente en lagos menos transparente a UVR.

Objetivo 3: Evaluar cómo la entrada de nutrientes minerales y el incremento de temperatura modulan la fuerza de interacción comensalista alga-bacteria en lagos de alta montaña con bajo contenido en DOC.

Hipótesis 3.1: La disponibilidad de carbono autóctono es el principal recurso que limita la producción bacteriana heterotrófica en lagos de alta

montaña del Sur de la Península Ibérica.

Hipótesis 3.2: La entrada de nutrientes minerales y un incremento de temperatura atenuarán el efecto negativo de UVR sobre la producción primaria y bacteriana, mejorarán el acople entre fotosíntesis y crecimiento algal, produciéndose menor excreción de carbono algal, lo que debilitará la fuerza de interacción comensalista alga-bacteria.

Objetivo 4: Cuantificar la magnitud del efecto interactivo de UVR y la entrada de nutrientes inorgánicos y temperatura sobre la estructura y metabolismo del microplancton en ecosistemas alta montaña y marinos. Nuestro objetivo fue determinar la respuestas de los organismos en función del tiempo de acción de los distintos factores de estrés: (i) permanente (UVR); (ii) esporádico (pulsos de nutrientes; pulsos: combinación de baja frecuencia, gran magnitud, y corta duración); (iii) abrupto, (temperatura: eventos de olas de calor).

Hipótesis 4.1: Un pulso de fósforo promoverá el desarrollo algal y bacteriano y atenuará el efecto dañino de UVR a medio plazo.

Hipótesis 4.2: Fluctuaciones de la temperatura actuarán antagónicamente disminuyendo los valores de producción primaria y bacteriana. Este efecto tendrá mayor magnitud en ecosistemas con menor rango de tolerancia a variaciones en la temperatura.

Objetivo 5: Evaluación a largo plazo de la interacción UVR y pulsos consecutivos de nutrientes minerales sobre la estructura y funcionamiento de la red trófica microbiana en un ecosistema modelo a media altitud en la Región Mediterránea.

Hipótesis 5: Los pulsos acumulativos de nutrientes y UVR determinaran un cambio en la composición de la comunidad microplankton a largo plazo, con un mayor desarrollo de compartimiento bacteriano y de lo bacterívoros (algas mixotróficas y ciliados) y un disminución del compartimento de autótrofos estrictos.

Con objeto de abordar estos objetivos,

1. Se llevaron a cabo estudios experimentales multifactoriales comparativos en ecosistemas oligotróficos de la Península Ibérica que difieren en sus características intrínsecas:
   - Distinta transparencia a UVR por su contenido en DOC (capítulo I, II)
   - Distintas exposición a UVR por su altitud ecosistemas marinos, media y alta montaña diferencias 3000m (capítulo I, II, IV, V)
   - Ecosistemas con marcadas diferencias en temperatura media de la columna de agua de entre 5 y 10 grados (capítulo, III, IV)
   - Contrastante estructura física de la columna de agua: Estatificado vs mezclados (capítulo I, II)
2. Se han seguido distintas escalas temporales de análisis:
   - Experimentos a corto plazo con acción simultánea de los factores de estrés ensayados.
   - Análisis a medio plazo con diseño Split-plot.
   - Análisis a largo plazo aplicando perturbaciones consecutivas por nutrientes
3. Finalmente, en las distintas aproximaciones experimentales se utilizaron como variables respuesta: Variables metabólicas:
   - Producción primaria, liberación de productos extracelulares.
   - Producción bacteriana.
   - Respiración de bacteriana y total de la comunidad microplanctónica.
   - Procesos de depredación de bacterias.

   Variables estructurales:
   - Concentración de Clorofila a,
   - Abundancia y biomasa de nano- y picoplancton autótrofo, picoplancton heterótrofo, nanoflagelados, ciliados

Capítulo 1

Modulation of algal-bacterial interaction by solar radiation in two optically contrasting oligotrophic lakes

1.1. Abstract

Global warming leads to shallower epilimnion, causing organisms to be exposed to higher levels of ultraviolet (UVR, 280-400 nm) and photosynthetically active radiation (PAR, 400-700 nm), which could affect primary and bacterial production as well as the commensalistic algal-bacterial relationship. The combined effects of UVR and reduction in the depth of the upper mixed layer (UML) were assessed on variables related to the metabolism of algae and bacteria, during in situ experiments performed with natural microplanktonic communities from two oligotrophic lakes with contrasting UVR-transparency (clear vs. opaque) of southern Spain. The negative UVR effects on epilimnetic primary production (PP) and on heterotrophic bacterial production (HBP) were higher in the UVR-opaque than in the UVR-clear lake, and stronger on the algae than on the heterotrophic bacterial communities. The harmful UV-B (280-315 nm) effect was intensified at high mean irradiance. Under UVR and high mean irradiance, the algal-bacterial relationship was strengthened in the UVR-clear lake, where excreted organic carbon (EOC) rates exceeded the bacterial carbon demand (BCD). This did not occur in the UVR-opaque lake. The greater UVR damage to algae and bacteria and the weakening of their commensalistic interaction found in the UVR-opaque lake indicates that these ecosystems would be especially vulnerable to stressors related to global change. Thus, our findings may have important implications for the carbon cycle in oligotrophic lakes of the Mediterranean region.

1.2. Introduction

Rising levels of greenhouse gases (mainly CO_2), attributed to human activities, have led to an increase of 0.56°C in the Earth's surface temperature over the past 150 years (Solomon *et al.*, 2007b). Model predictions indicate greater temperature increases, ranging from 1.5°C (under the CO_2 scenario B1) to 6.4°C (under the scenario A1FI high CO_2 emissions) by the end of the century. Major changes in precipitation have accompanied these temperature variations and are expected to become more pronounced (Solomon *et al.*, 2007b). These climate changes affect aquatic ecosystems by increasing water temperature, altering mixing regimes, shortening the thaw time and the duration of ice cover, and/or strengthening water-column stratification (De Senerpont Domis *et al.*, 2013). These alterations in physical conditions have different effects on primary and bacterial production, plankton growth, nutrient supply, and trophic interactions, among other ecological processes (De Senerpont Domis *et al.*, 2013). In addition, variations in stratification patterns are known to strongly affect biogeochemical cycles (Van de Waal *et al.*, 2009). Higher temperatures in the upper layers of freshwater bodies increase density differences between the upper mixed layer (UML), or epilimnion, and deeper waters, augmenting the vertical temperature gradient, and thus the stratification. This process has contrasting effects on nutrient and light availability for organisms' growth. On the one hand, stratification reduces the flow of nutrients from deep and nutrient-rich areas into the UML, limiting their availability for growth (Huisman *et al.*, 2006). On the other hand, stratification traps phytoplankton populations in surface layers, increasing the light availability for growth but also exposing them to higher levels of ultraviolet radiation (UVR, 280-400 nm). In this regard, it has been widely reported that greater exposure to UVR exerts an inhibitory effect on autotrophic and heterotrophic organisms (Häder, 2011), and that UV-B (280-315 nm) in particular, harms primary and bacterial production (Carrillo *et al.*, 2002), enzymatic activity (Korbee *et al.*, 2012), and cell viability (Helbling *et al.*, 1995), among other effects. However, it has been also reported (Aas *et al.*, 1996; Medina-Sanchez *et al.*, 2002; Gao *et al.*, 2007a) that UVR does not produce negative effects and it can even

stimulate bacterial production and photosynthetic activity. These opposite effects may be attributable to the differential acclimation capacity of organisms in severely UVR-stressed ecosystems (Medina-Sanchez *et al.*, 2002; Ruiz-González *et al.*, 2013) or to differences in physical-chemical factors (e.g. temperature or nutrient content) among ecosystems (Harrison *et al.*, 2009).

With respect to physical factors, it has been experimentally demonstrated (Helbling *et al.*, 1994) that vertical mixing can alter UVR-induced effects on planktonic organisms by generating a regime of fluctuating irradiance, with high values near the surface and low at the bottom of the UML. The depth of the UML also influences the mean UVR and PAR irradiance received by organisms and the duration of their residence in the photoactive zone (Neale *et al.*, 2003). Studies on the interactive effects of UVR and vertical mixing on algae (Helbling *et al.*, 1994; Neale *et al.*, 2003) and bacteria (Bertoni *et al.*, 2011) have shown that these organisms can recover from UVR-induced damage when UVR exposure is subsequently reduced or avoided. The outcome of damage vs. repair depends not only on the amount of damaging UVR received, but also on photo-repair wavelengths (UV-A, PAR) to which organisms are subsequently exposed during the fluctuating radiation regime. Moreover, the effects of different mixing depths, and thus of different mean irradiances (MIR), can act synergistically or antagonistically with UVR, depending on the composition, structure, and size of the species as well as on the environmental conditions (Villafañe *et al.*, 2007). For instance, Barbieri y Villafan (2002) found that the impact of UVR in Patagonian coastal waters was negative or positive depending on the fraction of the euphotic zone (Z_{eu}) that was mixed; thus, UVR was used for photosynthesis when vertical mixing reached ∼90 % of the Zeu, but carbon fixation was reduced by UVR when the UML was shallow (∼60 % of the Z_{eu}). Besides increased stratification of the water column, more extreme rainfall events and storms are predicted in many parts of the World in the global-change scenario (Stocker *et al.*, 2013). This would increase the amount of allochthonous dissolved organic matter (DOM) reaching inland and coastal aquatic ecosystems, reducing the penetration of incident UVR (Rose *et al.*, 2009). The UVR filtering characteristics of coloured DOM

(CDOM) result in a more effective attenuation of shorter (UV-B) than longer (UV-A, 315-400 nm) wavelengths, as also observed for stratospheric ozone. These changes would modulate the exposure of aquatic organisms to UVR (Williamson *et al.*, 2010), making it more complex to predict the interactive effects of UVR and stratification on the planktonic community.

Recent experiments carried out by our group have demonstrated that fluctuating irradiance increases the harmful UVR effects on primary producers in oligotrophic mountain lakes with high DOM, whereas the opposite effects were detected in those with low DOM content (Helbling *et al.*, 2013). Several authors have highlighted the importance of the quality of the radiation, which can interact with DOM and either increase or decrease the availability of organic carbon for bacteria (Pérez y Sommaruga, 2007). However, despite the key role of phytoplankton and heterotrophic bacteria production as a link between the microbial and grazing food webs, no comparative studies on algae-bacteria commensalistic relationships have been done in ecosystems with high- and low-DOM contents. Thus, at present, the information available concerning the interactive effects of radiation quality and increased MIR as a consequence of stratification on algae-bacteria interactions so far do not exist.

A growing body of literature supports the strong dependence of planktonic heterotrophic prokaryotes on organic matter released in situ by phytoplankton in the upper layers of aquatic ecosystems (Baines y Pace, 1991). It has also been demonstrated that UVR exposure in the upper layers of the water column can increases the proportion of photosynthate released as exudates (Carrillo *et al.*, 2008b; Korbee *et al.*, 2012), which would stimulate the growth of UVR-resistant bacteria (Xenopoulos y Frost, 2003) and give rise to a coupled algae-bacteria relationship in clear oligotrophic lakes (Carrillo *et al.*, 2002). Coupling between phytoplankton and bacterioplankton has been defined as the capacity of the carbon (C) released by algae to support the bacterial carbon requirement (Morán *et al.*, 2002) and will therefore differ depending on: (i) the availability of alternative (allochthonous or autochthonous) carbon sources (Gasol *et al.*, 2009), and (ii) the limitation of inorganic nutrient (Medina-Sánchez *et al.*, 2010, 2013; López-Sandoval *et al.*, 2011). Although the bacterial dependence on C released

by phytoplankton is a well established aquatic microbiological paradigm, it is currently under renewed debate because the application of different conversion factors to raw data and modelled rates could substantially alter this paradigm (Morán *et al.*, 2011). Furthermore, few data are available on the possible effects of global warming on this relationship or on C flux into aquatic food webs. With this background, the aim of the present study was to improve our understanding about the interactive effects of UVR exposure and increased MIR (as a consequence of increased stratification) on (i) phytoplanktonic and heterotrophic bacterial production and (ii) the commensalistic relationship between them in lakes with different transparency to UVR. We hypothesised that the interactive effects of UVR and increased MIR will accentuate the harmful UVR effects on primary production (PP) and heterotrophic bacterial production (HBP), thus resulting in a greater C release by algae, which will strengthen the commensalistic algae-bacteria relationship. These effects will be more acute in UVR-opaque than in UVR-clear lakes, due to lesser acclimation of organisms to UVR in the former case. To test our hypothesis, we carried out in situ experiments to assess the combined impact of solar radiation (i.e., quality) and increased MIR on metabolism of algae and bacteria, and their commensalistic relationship in two oligotrophic lakes with contrasting transparency to UVR in the Mediterranean Region.

1.3. Methods

1.3.1. Model ecosystems

The study was performed during September 2011 in two Spanish oligotrophic lakes: La Caldera Lake in Sierra Nevada National Park (37° 03'N; 3° 19'W, 3050 m a.s.l.) (Granada) and La Conceja Lake in Ruidera Natural Park (38° 55' N; 2° 47' W, 850 m a.s.l.) (Ciudad Real). La Caldera (hereafter called the "UVR-clear" lake) is a mixed oligotrophic (TP <0.3 μM, Chl *a* <5 μg L^{-1}) high-mountain lake above the treeline on a siliceous bedrock in a glacial cirque (Carrillo *et al.*, 2006). The lake has a mean depth of 4.3 m, with a maximum depth inter-annually variable from 2 to

14 m. The lake is highly transparent and receives high UVR irradiance levels (Carrillo *et al.*, 2002; Helbling *et al.*, 2013). Largely autochthonous in origin, the DOC reaches only low concentrations, <0.08 μM. The pelagic community is relatively simple, and characterized by the absence of autotrophic picoplankton and size overlap between algae and heterotrophic bacteria (Medina-Sanchez *et al.*, 2002). La Conceja (hereafter called the "UVR-opaque" lake) is a stratified oligotrophic lake (total phosphorus [TP] <0.03 μM and Chl *a* <5 μg L^{-1}), although it has an elevated nitrate concentration which can exceed 800 μM due to agricultural use of the land. This lake has a surface area of 29 ha and maximum depth of 14 m. The DOC content ranges from 0.15 to 0.25 μM. The autotrophic community is composed by pico- and nanoplankton autotrophs (Rojo *et al.*, 2012).

1.3.2. Experimental setup

To assess the interactive effects of solar radiation quality ("UVR" factor) and increased mean irradiance ("MIR" factor) on PP, HBP, TPR (<45 μm fraction) and BR (<1 μm fraction in the UVR-clear lake alone), samples were collected from the surface (0-0.5 m) epilimnetic water. An acid-cleaned 6-L horizontal Van Dorn sampler was used to collect the water that was pre-screened through a 45-μm mesh to remove large zooplankton prior to the experiments. Samples for PP were placed in 50-mL quartz flasks and those for HBP, TPR, and BR in 25-mL quartz flasks. In the UVR-opaque lake, samples for PP, HBP and TPR analyses were also gathered from the hypolimnetic water below the thermocline at 6 m depth, where UV-B did not reach the cells. The idea behind sampling two communities in the UVR-opaque lake was to compare the natural algal and bacterial communities that had different light histories and acclimation to solar radiation when exposed to similar light quality treatments and irradiance conditions. Since this sharp contrast did not occur in the clear lake, only samples from the 0-0.5 m were used in these experiments.

The experimental design consisted of three (for TPR and BR), four (for PP, HBP) or two (for TPR in the UVR-opaque lake) "UVR" treatments combined with the two MIR conditions: 1) The UVR treatments (triplica-

tes for each condition) were: (i) PAB: full solar radiation, uncovered quartz flasks; (ii) PA: exclusion of UV-B (280-320 nm), wrapping the flasks with Folex 320 film (Folex, Germany); (iii) P, control: exclusion of UVR (280-400 nm), wrapping the flasks with Ultraphan UV Opak395 film (Digrefa, Germany); and (iv) Dark: wrapping the flasks with opaque material (aluminium foil). The optical properties of the filters used for the radiation treatments have been published elsewhere [34]; the filters were replaced before each experiment and tested using a double-beam spectrophotometer (Perkin-Elmer Lambda 40). 2) The MIR treatments were: (i) high MIR, samples incubated at 0.5 m depth; and (ii) low MIR, samples subjected to vertical mixing from 0 to 5 m depth. To simulate these reductions in the depth of the UML (i.e. from 5 m to near the surface) two round trays containing the samples were exposed in situ to solar radiation. One tray was placed at 0.5 m depth (high MIR) subjected to irradiance oscillations associated to waves at the surface. Transient thermoclines trapping phytoplankton very close to the surface have previously been detected in aquatic environments (Neale *et al.*, 2003); in the present study, this high irradiance condition simulates a worst-case stratification scenario. The second tray was vertically moved between the surface and 5 m depth to simulate the irradiance changes in the upper 5 m of the water column (low MIR). The speed of movement was 1 m every 2 min, achieved by a custom-made mixing simulator, using a frequency-controlled DC motor (Maxon motor, Switzerland) to impose a linear transport rate on the vessels from the surface to the mixing depth and back. The tray was placed on a boat anchored in a deep area of each lake in such a manner as to avoid shadows or any type of interference from the shoreline or boat. All incubations lasted for 3.5 h centered on local noon, and a total of 10 cycles (from the surface to 5 m depth to the surface again) were completed for the low MIR condition. Unfortunately, space restrictions within the trays prevented the performance of all experimental treatments in the UVR-opaque lake for TPR, which was measured only in samples exposed to PAB and P in the high and low MIR treatments. The overlapping between autotrophic and heterotrophic picoplankton precluded the measurements of BR in the UVR-opaque lake.

1.3.3. Physical measurements

Incident solar radiation was continuously monitored by means of a BIC radiometer (deck unit, Biospherical Instruments Inc., CA, USA) that has three channels in the UVR region of the spectra (305, 320, and 380 nm) and one broad-band channel for PAR (400-700 nm). Vertical profiles of solar radiation in the water column were performed at noon using a BIC radiometer (underwater unit) with temperature and depth sensors, in addition to the aforementioned channels. Vertical profiles of temperature and pH in the water column were measured using a multiparameter probe (Turo Water Quality Analysis T-611 Sandy Bay, Tasmania, Australia). These profiles were done daily at noon, and the temperature data were used to estimate the strength and depth of the epilimnion in the water column.

1.3.4. Chemical and biological analyses

Chemical and biological variables were sampled with a 6-liter Van Dorn sampler at the deepest central station at four depths in the UVR-clear lake (surface, 5, 8, and 10 m) and six in the UVR-opaque lake (surface, 2, 4, 6, 8, and 10 m). Water samples were taken to determine the bacterial abundance (BA, 20 mL), phytoplankton species composition and abundance (250 mL), and chlorophyll a concentration (Chl *a*, 1L). Samples were also collected for the chemical determination of total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), nitrate (NO_3^-), and soluble reactive phosphorus (SRP). The samples for TDN, TDP, nitrate, and SRP analyses were filtered through GF/F Whatman filters (47 mm in diameter) before the determinations. Samples for TP and TDP were persulfate-digested at 120°C for 30 min and determined (as for SRP) using 10-cm quartz cuvettes (following the acid molybdate technique, APHA, 1992). TN and TDN samples were also persulfate-digested and measured as NO_3^- by means of the ultraviolet spectrophotometric screening method (APHA, 1992). Blanks and standards were run in all procedures.

1.3.5. Chl *a* fluorescence

Chl *a* fluorescence parameters of the photosystem II were measured at different depths in the water column by using a pulse-amplitude-modulated fluorometer (Water PAM, Walz, Germany). Samples were gently pumped from each depth (using an aquarium pump) into a custom-made darkened flow-through measuring quartz cuvette (5 mL) connected to the pump via a dark silicon tube (5 mm diameter). The flow rate was ca. 250 mL per min, i.e. sufficient to minimize the time spent by cells (<1 min) in the silicon tube before the measurement. The intrinsic photochemical quantum yield (Y) was calculated with the equations of **?**:

$$Y = \Delta F : F'_m = F'_m - F'_t : F'_m \tag{1.1}$$

where F'$_m$ is the instantaneous maximum intensity of Chl *a* fluorescence in an irradiated cell induced by a saturating white-light pulse (∼5300 μmol photons m^{-2} s^{-1} in 0.8 s) in the presence of a weak actinic light, and Ft the steady-state fluorescence induced by a weak actinic light in light-adapted cells. These fluorescence measurements were made every 10 sec, with at least 6 measurements per depth. Comparisons with samples from the Van Dorn bottle showed that the measurements were not affected by pumping the phytoplankton into the cuvette.

1.3.6. Chl *a* concentration

For measurements of the Chl a concentration, water samples from different depths in the water column were filtered onto Whatman GF/F filters (25 mm in diameter), which were frozen at -20°C until their analyses. For Chl *a* analysis, samples were thawed and placed in centrifuge tubes (15 mL) with 5 mL of acetone (90 %) for 24 h in the dark at 4°C. Next, the samples were centrifuged, and the fluorescence of the supernatant was measured with a fluorometer (APHA, 1992) (LS 55 Perkin Elmer, USA).

1.3.7. Identification and cell counting

Samples for the identification and counting of phytoplankton were placed in 250-mL brown glass bottles and fixed with Lugol's reagent (approx. 1 % vol/vol). Sub-samples (100 mL) were settled for 48 h in Utermöhl chambers (Hydro-Bios GmbH), and species were then identified and counted using an inverted microscope (Leitz Fluovert FS, Leica, Wetzlar, Germany). BA was determined by the 4', 6-diamidino-2-phenylindole (DAPI) direct-count method described by Porter y Feig (1980). Water samples were fixed with neutralized formalin (2 %), stained with DAPI to a final concentration of 2.5 μg mL^{-1}, and then filtered through a 0.2-μm pore-size polycarbonate black Nucleopore filter. At least 400 cells per sample were counted by epifluorescence microscopy (Karl Zeiss AX10).

1.3.8. Primary production and excreted organic carbon

For PP measurements, samples of phytoplankton communities were placed in 50-mL round quartz flasks (three clear and one dark per radiation treatment), inoculated with 0.37 MBq of $NaH^{14}CO_3$ (specific activity: 310.8 MBq mmol^{-1}, DHI Water and Environment, Germany), and exposed to solar radiation in situ, as described above. The samples for PP were filtered through 0.2-μm Nuclepore filters (25 mm diameter), under low vacuum (<100 mm Hg) to minimize cell breakage. Excreted organic carbon (EOC) was measured on 4-mLaliquots from the filtrates (<0.2 μm). Both filters and filtrates were placed in 20-mL scintillation vials and acidified with 100 μL of 1 N HCl for 24 h (no bubbling) to remove inorganic radiocarbon before the addition of a liquid scintillation cocktail (Ecoscint A) to the vials. The amount of organic carbon was obtained by counting of disintegration per minute (dpm), using an autocalibrated scintillation counter (Beckman LS 6000 TA). The total CO_2 in the lake water was calculated from alkalinity and pH measurements (APHA, 1992). In all calculations, dark values were subtracted from the corresponding light values (more details in Carrillo *et al.*, 2002).

1.3.9. Heterotrophic Bacterial production

Samples for HBP measurements were placed in 25-mL quartz flasks and exposed in situ for 3.5 h under the radiation and MIR conditions as described above. Then, the HBP was determined in the dark by incorporating ^{3}H-thymidine (S.A= 52 Ci mmol^{-1}, Amersham Pharmacia) into the bacterial DNA. Briefly, ^{3}H-thymidine was added to independent sets of five (three replicates + two blanks per treatment) sterile microcentrifuge tubes filled with 1.5 mL of the pre-exposed samples to a final (saturating) concentration of 15.2 nM. The vials were then incubated at in situ temperature in the dark for 1 h. After incubation, the incorporation of ^{3}H-thymidine was stopped by adding (6 % final concentration) of trichloroacetic acid (TCA). Likewise, blanks were TCA-killed before the radiotracer was added. After the cold TCA extraction, the precipitate was collected by centrifugation at 14000 rpm for 10 min. The conversion factor 1.5×10^{18} cell mol^{-1} was used to estimate the number of bacteria produced per mol of incorporated ^{3}H-thymidine. The factor 20 fg C cell^{-1} was applied to convert bacterial production into C (Lee y Fuhrman, 1987).

1.3.10. Respiration rates

Samples for TPR and BR measurements were placed in 25-mL quartz flasks and exposed in situ for 3.5 h under the radiation and MIR conditions described above. TPR and BR rates were measured using optode sensorspots (SP-PSt3-NAU-D5-YOP; PreSens GmbH, Germany) and an optic-fibre oxygen transmitter (Fibox 3; PreSens GmbH, Germany) connected to a computer. Data were recorded using the OxyView 3.51 software (PreSens GmbH). The system was calibrated by a two-point calibration, together with data of atmospheric pressure and temperature before each experiment, following the manufacturer's recommendations. Measurements were made at the initial time (t0) and then every hour during 8 h. Every oxygen measurement was done during 30 sec with a frequency of 1 datum per sec; only the last 10 data points of each measurement were used in our analysis to ensure the stability of the data. Oxygen data were then adjusted to a

linear model via least-squares regression. Slope of the regressions provided the oxygen consumption rates (μM O_2 h^{-1}) (Warkentin *et al.*, 2007).

Oxygen was converted into carbon units using a respiratory quotient of 1 (del Giorgio y Cole, 1998). The bacterial carbon demand (BCD) is the HBP plus BR. The bacterial growth efficiency (BGE) is the proportion of C entering the bacterial pool that is incorporated into the biomass and was calculated as BGE=HBP:BCD.The absence of size-overlapping between algae and bacteria in the UVR-clear lake (Medina-Sanchez *et al.*, 2002) allowed for a direct measurement of BR. This, however, was not possible in the UVR-opaque lake, where picoplankton autotroph and bacteria coexisted in the < 3 μm fraction. Therefore, BCD in this lake was estimated by assuming that BR values lies within two limits: (i) a conservative value of 75 % of TPR, which is an average value based on data reported for oligotrophic waters (Lemée *et al.*, 2002); and (ii) a potential minimum value of 50 % of TPR (Pakulski *et al.*, 2007), comparable with direct measurements made in this study on the TPR vs. BR in La Caldera lake (Herrera et al., unpubl. data).

1.3.11. Data calculation and statistical analysis

The effect size of the UV-B and UV-A were quantified as:

$$\text{UV-B}(\,\%) = 100\times[(PP_P - PP_{PAB}) : PP_P) - ((PP_P - PP_{PA}) : PP_P)] \quad (1.2)$$

$$\text{UV-A}(\,\%) = 100 \times [(PP_P - PP_{PA}) : PP_P] \quad (1.3)$$

where PP_P, PP_{PA}, and PP_{PAB} represent the primary production in samples under the P, PA and PAB treatments, respectively. We used propagation errors to calculate the variance of the effect-size (as percentage) due to UV-B and .UV-A. The change (Δ) in the effect size of UV-B and UV-A, between the high and MIR treatments, was calculated as the difference of the effect size for each radiation band.

The effects of solar radiation quality ("UVR" factor) and increased mean irradiance ("MIR" factor) on the response variables were tested using

two-way ANOVA. When the interactive effects were significant, a post hoc Bonferroni test was used to determine significant differences among treatments. The normality (by Shapiro-Wilks' W test or Kolgomorov-Smirnov) and homoscedasticity (using Cochran, Hartley and Bartlett or Levene's tests) were checked for each data group before ANOVA application. HBP data from the hypolimnetic community in the UVR-opaque lake were log-transformed to meet ANOVA assumptions. Significance of the effect size of UV-B and UV-A on PP and HBP between high- and low MIR were evaluated using t-tests. Regression analyses were made to assess the dependence of EOC in controlling BGE for the experimental data in each lake. Statistica 7.1 software for Windows was used for the statistical analyses

1.4. Results

1.4.1. Physical, chemical, and biological variables in the water column

Figures 1.1a and b depict the penetration of solar radiation into the water column in both lakes. The lakes greatly differed in their transparency to UVR, but not to PAR. Thus, in the UVR-clear lake, the 1 % of the surface energy at 305 nm reached the bottom of the lake, whereas in the UVR-opaque lake most of the UVR energy was attenuated in the upper layers of the lake (1 % of the surface energy at 305 nm reached only ca. 1 m depth). This differential penetration of solar UVR resulted in two contrasting environments, with organisms being exposed to UV-B along the water column in the UVR-clear lake (Fig. 1.1a) but only in the upper 1-2 m of the water column in the UVR-opaque lake (Fig. 1.1b). This was related to the differential DOC concentrations between the lakes that reached values of 0.07 and 0.18 mM in the clear- and UVR-opaque lakes, respectively (Fig. 1.1c, d). Vertical temperature profiles also differed between the lakes: the temperature was 14°C, ranging only 0.4°C between the surface and bottom in the UVR-clear lake (Fig. 1.1c), whereas a weak thermal stratification between 2-3 m was detected in the UVR-opaque lake, where the temperature ranged from 22 to 19.5°C between the surface and bottom layers

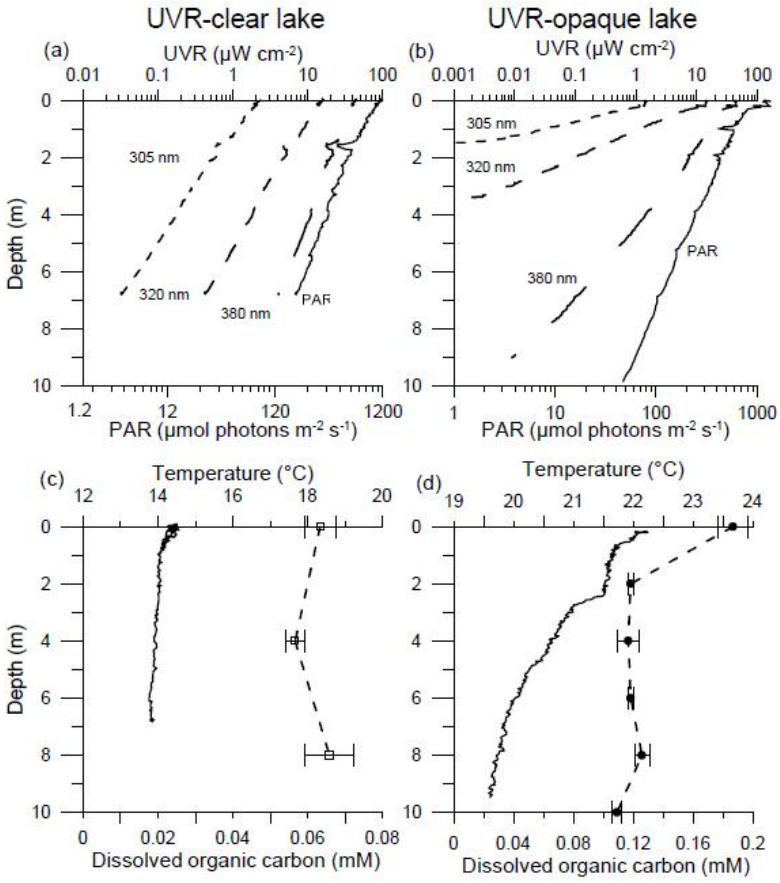

Figura 1.1: Depth profiles of the irradiance at 305, 320, and 380 nm, and PAR (400–700 nm) for (a) UVR-clear lake (Lake La Caldera) and (b) UVR-opaque lake (Lake La Conceja); DOC concentrations (μM) and vertical profiles of temperature (°C) as a function of depth in (c) UVR-clear lake and (d) UVR-opaque lake. Values of vertical attenuation coefficients (m^{-1}) were:$k_{d\ 305}$ = 4.84, and 0.61;$k_{d\ 320}$=2.53, and 0.52;$k_{d\ 380}$=0.93, and 0.34, $k_{d\ PAR}$=0.28, and 0.25, in the UVR-opaque lake and the UVR-clear lake, respectively.

Variable	UVR-clear lake	UVR-opaque lake
TN (μM)	21.50±1.54	787.1±10.7
TDN (μM)	20.71±1.46	786.4 ±12.9
NO_3^- (μM)	14.28±1.02	702.1±6.7
TP (μM)	0.10±0.003	0.06±0.012
TDP (μM)	0.051±0.002	0.038±0.012
SRP (μM)	0.02±0.001	0.018±0.012
Chl *a* (μg L^{-1})	2.02±0.42	2.66±0.46
AA (cell mL^{-1})$\times 10^3$	7.03±1.65	4.03±0.72
BA (cell mL^{-1})$\times 10^6$	1.94±0.17	1.28±0.21

Cuadro 1.1: Mean values of the main chemical and biological variables measured in the water column in Lake La Caldera (UVR-clear lake) and in Lake La Conceja (UVR-opaque lake). Values are mean (±SD) of concentrations for four (Lake La Caldera) or six (Lake La Conceja) depths of, inorganic, total and dissolved nitrogen (N) and phosphorus (P), Chlorophyll a, and algae and bacterial abundances. TN: Total Nitrogen; TDN: Total Dissolved Nitrogen; NO_3^-: Nitrate; TP: Total Phosphorus; TDP: Total Dissolved Phosphorus; SRP: Soluble Reactive Phosphorus; Chl *a*: Chlorophyll a concentration; AA: Algal Abundance; BA: Bacterial Abundance.

(Fig. 1.1d). The concentrations of total dissolved and inorganic forms of N and P were homogeneous in the water column in both lakes, therefore only mean values are reported in Table 1.1. TN values were higher in the UVR-opaque than in the UVR-clear lake, by up to one order of magnitude, and NO_3^- constituted most of the TN (90 % in the UVR-opaque and 68 % in the UVR-clear lake). By contrast, TP values were <0.16 μM and mostly in organic form in both lakes. The NO_3^-:TP ratio was >100 in the UVR-clear lake and >10.000 in the UVR-opaque lake, indicating a strong P limitation (Table 1.1).

Figures 1.2a and b show the vertical distribution of Chl *a* and Y in the two lakes. In the UVR-clear lake (Fig. 1.2a), Chl *a* concentrations had small variations with depth. However, Y had a significantly lower value at the sur-

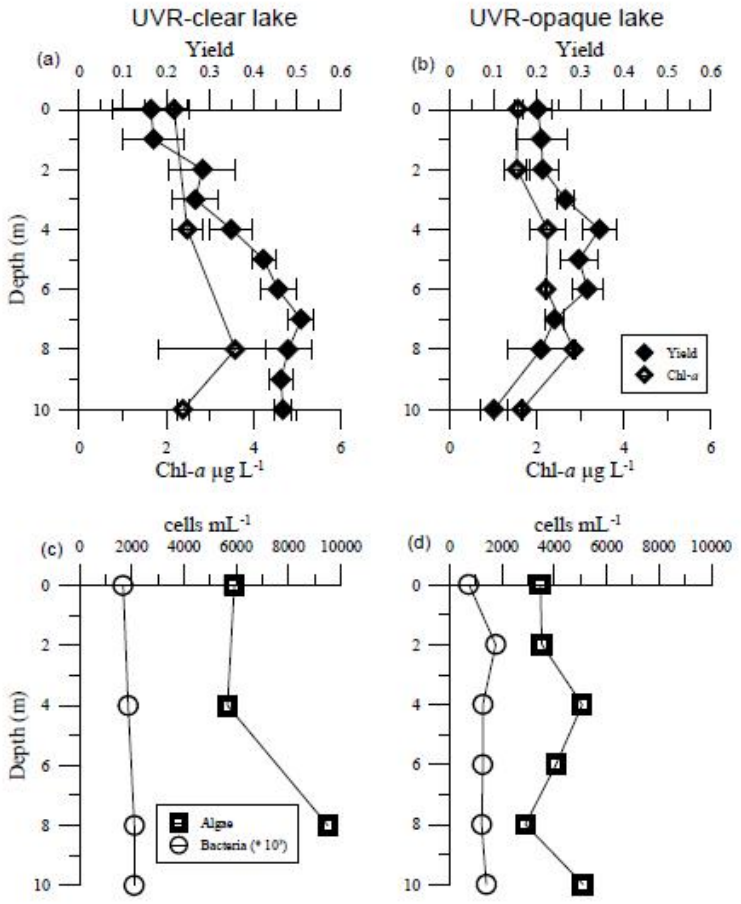

Figura 1.2: In situ photochemical effective quantum yield and Chl *a* (μg L^{-1}) in the UVR-clear lake (a) and the UVR-opaque lake (b); algae and bacterial abundance (cell mL^{-1}) in the UVR-clear lake (c) and the UVR-opaque lake (d).

	UVR-clear lake		UVR-opaque lake	
Wavelength	high MIR	low MIR	high MIR	low MIR
305 nm	3.90	1.40	1.44	0.16
320 nm	23.40	9.50	12.90	1.80
380 nm	60.10	31.50	47.90	12.80
PAR	1480	900	1428	824
$UV\text{-}A_{380}$:$UV\text{-}B_{305}$	15.41	22.50	33.26	80.00

Cuadro 1.2: Mean irradiances (MIR) during the incubations for 305 nm, 320 nm and 380 nm within the UVR wavelengths (μW cm^{-2} nm^{-1}) and for PAR (μmol photons m^{-2} s^{-1}). The ratio of the mean irradiances of 380 and 305 nm is also presented.

face (0-1 m) that steadily increased with depth. The change in Y from the surface down to 7 m was ca 0.4. In contrast, in the UVR-opaque lake (Fig. 1.2b), both Chl *a* and Y had slightly greater values at mid-water depths (4-6 m), reaching a difference between the surface and 4 m of <0.2. The vertical distribution of phytoplankton and bacteria also differed between the lakes: in the UVR-clear lake (Fig. 1.2c) bacterial abundance was rather homogeneous, but phytoplankton abundance increased with depth; however, in the UVR-opaque lake (Fig. 1.2d) the abundances of bacteria and phytoplankton were rather uniform with depth. Mean algal and bacterial abundance values were greater in the UVR-clear than in the UVR-opaque lake (Table 1.1). In terms of taxonomic composition, the Chlorophyceae *Monoraphidium* sp. represented >90 % of the total abundance of cells in the UVR-clear lake whereas the Bacillariophyceae *Cyclotella ocellata* was the dominant species in the UVR-opaque lake (>75 %).

1.4.2. Variations in solar MIR during experiments

The MIR for three wavelengths within the UVR and PAR region received by the samples under the experimental conditions are shown in Table 1.2. The MIR at 305nm, 320 nm and 380 nm in the UVR-clear lake were

2.8-, 2.5-, and 1.9-folds higher, respectively, in the high MIR than in the low MIR. The respective ratios between high and low MIR in the UVR-opaque lake were 8.7-, 7.1-, and 3.7- for the 305 nm, 320 nm, and 380 nm wavelengths, respectively. The energy ratio at 380 and 305 nm (i.e., UVA_380:UVB_305 ratio) had higher values in the UVR-opaque lake as compared to the UVR-clear lake, reflecting the lower penetration of UV-B in the former.

1.4.3. Joint effects of UVR and MIR on algal and bacterial metabolism in the UVR-clear lake

The PP values did not show significant differences between high- and low MIR in the PAB treatment, while samples under the PA and P treatments had significant higher PP values in high MIR as compared to the respective treatments at low MIR (Fig. 1.3a). A significant UVR×MIR effect was found for PP (Table 1.3) and according to our hypothesis, the high MIR resulted in higher UV-B (11.5 %) and UV-A (18.3 %) inhibition as compared to the low MIR (Table 1.4). Solar UVR at high MIR also significantly increased the rates of EOC, with significantly higher values in samples under the PAB and PA treatments (Fig. 1.3b). Like PP, HBP did not differ between PAB-high MIR and PAB-low MIR treatments. However, HBP was significantly lower under PA-high MIR than under PA-low MIR treatments (Fig. 1.3c) resulting in a significant UVR×MIR effect (Table 1.3). By contrast, only the "UVR" factor significantly affected BR (Fig. 1.3d, Table 1.3), with the lowest BR value determined in the PAB treatment at high-MIR (Fig. 1.3d). BGE had higher values in the PAB treatment at high MIR as compared to the other radiation treatments at high MIR; other comparisons between paired treatments did not result in significant differences of BGE (Fig. 1.3e). There was, nevertheless, a significant UVR×MIR interaction on BGE (Table 1.3). No relationship was found between EOC and BGE ($R^2 = 0.149$ $p>0.05$). Finally, to quantify the capacity of EOC released by algae to support the bacterial C demand (BCD) in each treatment, the BCD:EOC ratio (as a percentage) was calculated (Fig. 1.3f). Carbon released by algae resulted in excess to meet BCD (i.e., BCD:EOC values

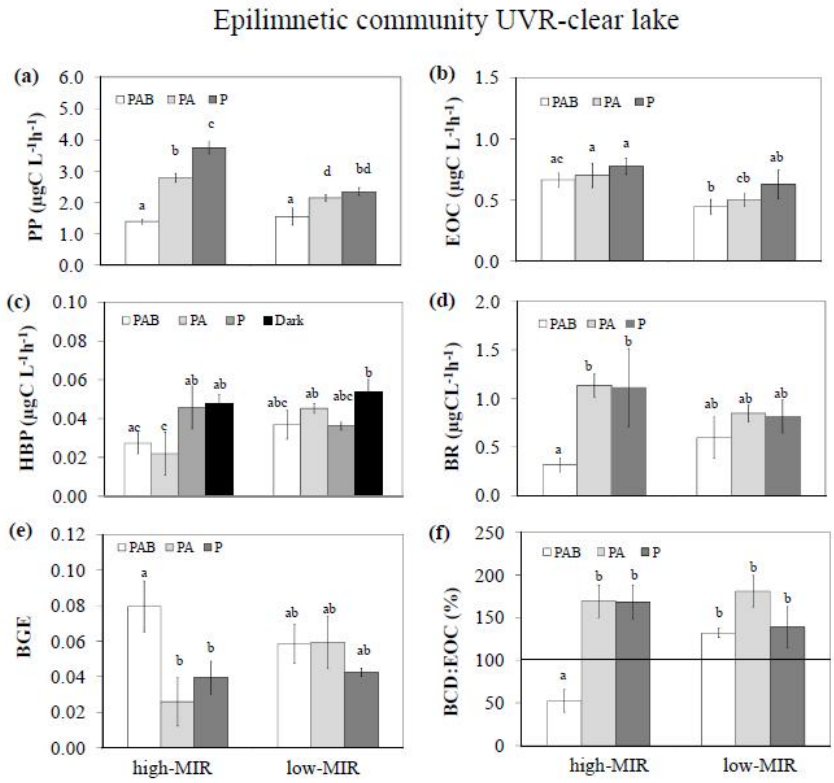

Figura 1.3: Metabolic variables of epilimnetic planktonic community under different stratification treatments in the UVR-clear lake. (a) Primary Production (PP,μg C L^{-1} h^{-1}); (b) Excreted organic carbon rates (EOC, μg C L^{-1} h^{-1}); (c) Heterotrophic Bacterial Production (HBP, μg C L^{-1} h^{-1}), (d) Bacterial Respiration (BR, μg C L^{-1} h^{-1}); (e) Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD):Excreted Organic Carbon (EOC; as a percentage). The lines on top of the bars are the standard deviation.

<100 %) only in the PAB treatment at high MIR (Fig. 1.3f), being this value significantly lower than the ones in all other treatments.

1.4.4. Joint effects of UVR and MIR on algal and bacterial metabolism in the UVR-opaque lake

UVR-fluxes exerted negative effects on both epilimnetic (Fig. 1.4) and hypolimnetic (Fig. 1.5) communities. For the epilimnetic community, PP was significantly lower in the PAB than in PA and P treatments at high MIR, while UVR did not affect PP at low MIR (Fig. 1.4a). A significant UVR×MIR effect on PP (Table 1.3) was determined, with the lowest PP at PAB-high MIR. The high MIR resulted in higher UV-B (40 %) and UV-A (27 %) inhibition (Table1.4). As for PP, EOC was significantly lower in the PAB than in PA and P treatments at high MIR, but not significant differences among radiation treatments at low MIR were determined (Fig. 1.4b). HBP showed no differences between high MIR and low MIR treatments except to Dark treatments where significant higher values was found at high- than at low MIR treatments, resulting a significant interactive effect of UVR×MIR on HBP (Table 1.3). Noticeably, a strong inhibition of UV-B and UV-A in high MIR and in low MIR conditions was found (Table 1.4). By contrast, the estimated BR was not significantly affected by any factor (Table 1.3; Fig. 1.4d shown $BR_{50\,\%}$), UVR was the only factor that significantly reduced BGE values in both low- and high MIR conditions (Fig. 1.4e). No relationship between EOC and BGE was found ($R^2 = 0.055$ $p>0.05$). The BCD:EOC (%) was <100 % for every experimental condition except for that under PAB in the high MIR, where the BCD:EOC (%) reached values from ∼100 % (assuming BR = 50 % of TPR) to 145 % (assuming BR = 75 % of TPR) (Fig. 1.4f). So, in this latter case (PAB-high MIR) EOC was not enough to meet BCD.

For the hypolimnetic community (Fig. 1.5), UVR was the only factor that significantly inhibited PP. Samples under the PAB and PA treatments had significantly lower PP values than those under the P in both high- and low MIR (Fig. 1.5a). The EOC rates (Fig. 1.5b) were significantly lower in the PAB and PA treatments as compared to those in the P treatment

				PP		EOC				HBP				BR		BGE		BCD:EOC (%)	
		df_1	df_2	$F_{df1,df2}$	p	$F_{df1,df2}$	p	df_3	df_4	$F_{df3,df4}$	p	df_1	df_2	$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p
UVR-clear lake																			
Epilimnetic	MIR	1	12	42.29	**0.000**	44.00	**0.000**	1	16	6.41	**0.022**	1	12	1.07	0.321	0.26	0.619	6.15	**0.029**
	UVR	2	12	124.12	**0.000**	6.33	**0.013**	3	16	8.65	**0.001**	2	12	12.38	**0.001**	7.22	**0.009**	35.47	**0.000**
	UVR× MIR	2	12	20.90	**0.000**	0.11	0.895	3	16	5.46	**0.009**	2	12	3.71	0.056	4.80	**0.029**	14.59	**0.001**
UVR-opaque lake																			
Epilimnetic	MIR	1	12	0.61	0.450	2.46	0.143	1	16	7.37	**0.015**	1	8	5.28	**0.05**	1.45	0.263	18.76	**0.002**
	UVR	2	12	6.78	**0.011**	9.78	**0.003**	3	16	27.96	**0.000**	1	8	0.14	0.72	46.13	**0.000**	14.42	**0.005**
	UVR× MIR	2	12	16.71	**0.000**	16.51	**0.000**	3	16	6.38	**0.005**	2	8	0.63	0.45	0.06	0.810	44.86	**0.000**
Hypolimnetic	MIR	2	12	0.33	0.574	4.33	0.060	1	16	32.98	**0.000**	1	8	0.29	0.604	6.01	**0.040**	4.65	0.063
	UVR	2	12	41.58	**0.000**	52.75	**0.000**	3	16	12.05	**0.000**	1	8	8.39	**0.020**	0.15	0.711	0.81	0.394
	UVR× MIR	2	12	0.39	0.688	3.21	0.076	3	16	7.98	**0.002**	2	8	0.90	0.372	5.24	0.061	1.99	0.196

Cuadro 1.3: Results of the two-way ANOVA of the interactive effect of "UVR" (PAB, PA, P, Dark) and "MIR" (low-and high mean irradiance) factors on carbon incorporation of algae (PP, μg C L^{-1} h^{-1}), and Excreted Organic Carbon (EOC, μg C L^{-1} h^{-1}), Heterotrophic Bacterial Production (HBP, μg C L^{-1} h^{-1}), Bacterial Respiration (BR, μg C L^{-1} h^{-1}) was directly measured in the UVR-clear lake or it was calculated as 50 % of Total Planktonic Respiration (TPR) in the UVR-opaque lake; Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD):Excreted Organic Carbon (EOC; as a percentage). Numbers in bold indicate, p <0.05. df1, df2, and df3, df4, are the degrees of freedom.

at high MIR. No significant differences among MIR treatments were determined when comparing each radiation treatment (Fig. 1.5b). HBP was significantly inhibited only by UV-B (Fig. 1.5c), whereas it was stimulated by PA and P in the high MIR (Fig. 1.5c). At low MIR, however, UVR did not affect HBP. Therefore, high MIR triggered the inhibition due to UV-B by 45.6 % (Table 1.4). Only UVR, as a single factor, significantly affected BR (Table 1.3), with the lowest values under the PAB-low MIR treatment (Fig. 1.5d), whereas only the MIR factor affected BGE, with the lowest BGE values in the PAB- high MIR treatment (Fig. 1.5e). The BCD:EOC was <100 % under all conditions (assuming both BR = 50 % or 75 % of TPR) indicating the EOC was capable of supporting BCD (Fig. 1.5f).

Summarizing, and taking into account the changes (Δ) in UVR inhibition (UV-B and UV-A) on PP and HBP with increased MIR (Table 1.4), our results reveal greater UV-B sensitivity of: (i) epilimnetic algae and heterotrophic bacteria communities in the UVR-opaque lake than in the UVR-clear lake; (ii) epilimnetic algae than heterotrophic bacteria in both lakes; and (iii) hypolimnetic heterotrophic bacterial than algae community in the UVR-opaque lake. In addition, significant interactive UVR×MIR effects were observed on the BCD:EOC (%) only in the epilimnetic communities (Table 1.3). Thus, partially supporting our hypothesis the BCD:EOC (%) significantly decreased under PAB-high MIR treatment in the UVR-clear lake but increased in the UVR-opaque lake.

1.5. Discussion

The main outcome of our work was that we demonstrated that the increased MIR associated to stratification of the water column altered the commensalistic algal-bacterial relationship in oligotrophic lakes. The present study is the first, so far, directly assessing the interactive effects of UVR and changes in MIR on algae and bacteria and their commensalistic relationship in freshwater ecosystems. Furthermore, in our complex experimental approach we simulated reductions in the depth of the UML due to the stratification of the water column (one of the potential consequences

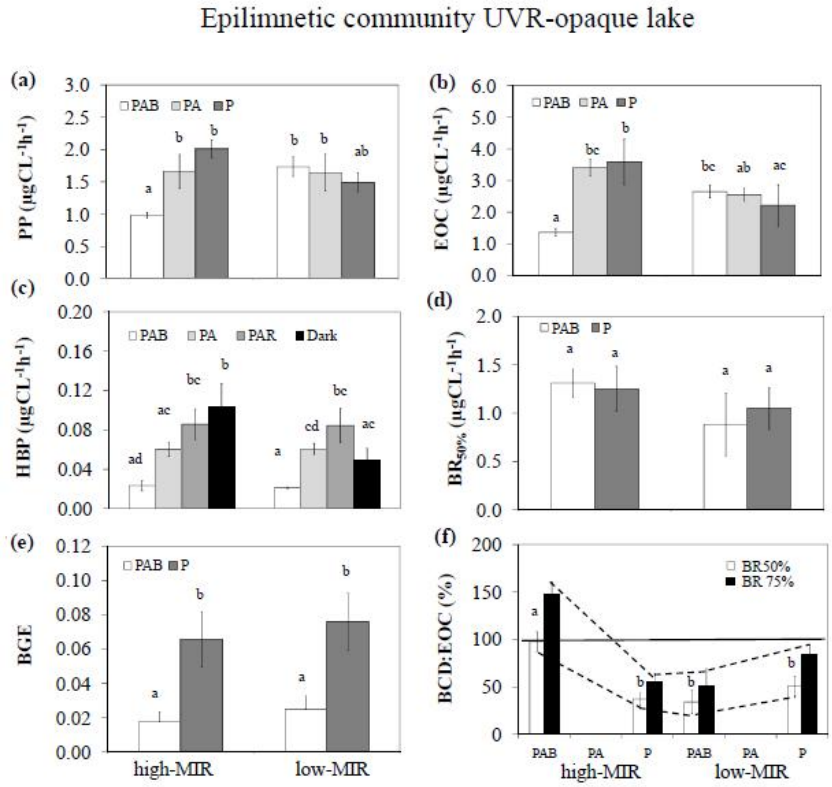

Figura 1.4: Metabolic variables of epilimnetic planktonic community under different stratification treatment in the UVR-opaque lake. (a) Primary Production (PP, μg C L^{-1} h^{-1}); (b) Excreted organic carbon rates (EOC, μg C L^{-1} h^{-1}); (c) Heterotrophic Bacterial Production (HBP, μg C L^{-1} h^{-1}), (d) Bacterial Respiration ($BR_{50\,\%}$,μg C L^{-1} h^{-1}) calculated as 50 % of Total Planktonic Respiration (TPR); (e) Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD): Excreted Organic Carbon (EOC; as a percentage) calculated assuming a BR as50 % and 75 % of Total Planktonic Respiration (TPR). The lines on top of the bars are the standard deviation.

		PP					HBP			
		%UVB	Δ%UVB	%UVA	Δ%UVA		%UVB	Δ%UVB	%UVA	Δ%UVA
UVR-clear lake										
Epilimnetic	high MIR	37.3±2.4	11.55	25.6 ±7.6	18.32		2.7±18.3	-20.3	51.9±26.7	110.2
	low MIR	25.7±5.0		7.3±7.1		23.0±1.5		-58.3	0.2	
UVR-opaque lake										
Epilimnetic	high MIR	33.7±4.2	40.00	17.4±13.9	27.41		42.9±6.2	-4.2	30.0±8.7	1.2
	low MIR	-6.3±10.9		-10.0±23.5		47.1±2.0		28.±6.7		
Hypolimnetic	high MIR	27.2±22.5	0.09	20.8±28.9	-5.98		52.1±5.8	45.6	12.0±24.4	-11.5
	low MIR	27.1±5.6		26.8±12.8		6.5±12.2		23.6±2.6		

Cuadro 1.4: Effect size of UV-B and UV-A on primary production (PP, μg C L^{-1} h^{-1}); and bacterial heterotrophic production (HBP, μg C L^{-1} h^{-1}) in the experimental conditions. The change (Δ) in effect size of UV-B and UV-A was calculated as difference effect size of UV-B and UV-A between high- and low MIR. Numbers in bold indicate $p<0.05$

of global warming); under these conditions, we measured the extracellular carbon release by algae, and directly determined the BR because these are the key variables implied in the bacterial carbon demand to C-supply ratio. Moreover, since a strong feedback between physical processes (e.g. mixing, stratification) and changes in DOC concentration in small lakes have previously been reported (Read y Rose, 2013), we further achieved an advance in our knowledge by investigating two oligotrophic ecosystems that differed in their UVR penetration in the water column due to their DOC content. This provides a framework for disentangling the complex processes that underlie biological interactions under changing physical (stratification, UVR) and chemical (DOC) conditions, which can then modify the C flux in aquatic ecosystems.

1.5.1. Sensitiveness of algae and bacteria to UVR with increased MIR due to stratification

Despite the physical and ecological differences between the two lakes, PP and HBP responses to the joint effect of UVR and MIR were quite similar in that the latter augmented the effect size of UVB, mainly on the epilimnetic communities in both ecosystems. This effect reached a higher magnitude in the UVR-opaque lake (Table 1.4), which coincided with a greater relative exposure to UV-B (9-fold) and an more accentuated decrease in the UV-A:UV-B ratio (58 %) at shallower layer in the opaque- than in the UVR-clear lake. This result agrees with the findings of higher UVR damage on primary producers in UVR-opaque lakes than in UVR-clear lakes as reported by (Baines y Pace, 1991), although in their study this response was found only under fluctuating irradiances. The results presented here indicate increased susceptibility to UVR of communities relatively less exposed to UV-B during their life cycles (Pakulski *et al.*, 2007). In addition, a higher sensitivity to UVR was found for epilimnetic algae than for bacteria mainly at high MIR, suggesting that photosynthetic processes are more sensitive under extreme conditions that mimic the global-warming scenario. This result contrasts to previous reports of greater UVR damage to bacterioplankton than to phytoplankton in oligotrophic waters of the

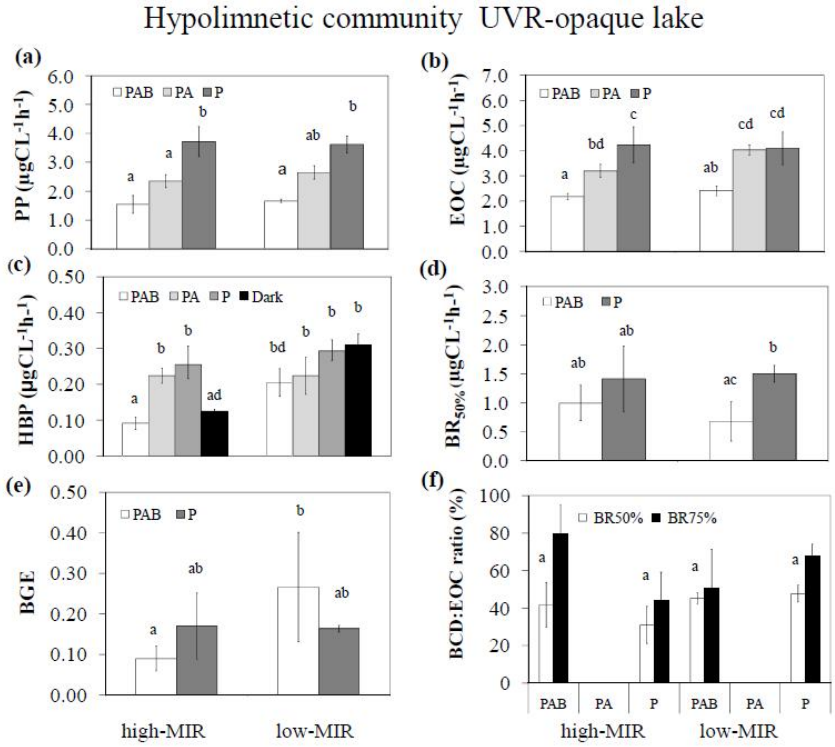

Figura 1.5: Metabolic variables of hypolimnetic community under different stratification conditions in the UVR-opaque lake. (a) Primary Production (PP,μg C L^{-1} h^{-1}); (b) Excreted organic carbon rates (EOC, μg C L^{-1} h^{-1}); (c) Heterotrophic Bacterial Production (HBP, μg C L^{-1} h^{-1}), (d) Bacterial Respiration ($BR_{50\,\%}$,μg C L^{-1} h^{-1}) calculated as 50 % of Total Planktonic Respiration (TPR); (e) Bacterial Growth Efficiency (BGE) and Bacterial Carbon Demand (BCD): Excreted Organic Carbon (EOC; as percentage) calculated assuming a BR as 50 % and 75 % of Total Planktonic Respiration (TPR). The lines on top of the bars are the standard deviation.

Mediterranean Sea (Bertoni *et al.*, 2011), the northern South China Sea (Yuan *et al.*, 2011), high-mountain lakes (Sommaruga *et al.*, 1999) and boreal lakes (Xenopoulos y Schindler, 2003).

Taken all together, these results show that stratification, by trapping the cells in a shallower epilimnion, with exacerbated UVR exposure, triggered the inhibitory effect of UVR on algal and bacterial metabolism. Because this negative effect was greater in opaque ecosystem to UVR because of their DOC content, we propose that the "ideal" photoprotective DOM may become harmful in a scenario of greater stratification and high UVR irradiance induced by global warming. Thus, UV-B may have additional harmful effects due to the free radicals (O_2^-, H_2O_2, OH^-) generated by photo-oxidation of the DOC (Sommaruga *et al.*, 1999; Pullin *et al.*, 2004), exacerbating the negative UVR effect in UVR-opaque lakes.

As expected, UVR was the main factor which affected the non-acclimated hypolimnetic community, and thus PP and HBP underwent negative UV-B and UV-A effects in both high- and low MIR. Nevertheless, HBP of the hypolimnetic community was stimulated by UV-A and PAR when exposed to shallower conditions. These results suggest that the hypolimnetic bacteria possessed photorepair mechanisms, via UV-A and PAR-promoted photolyase activity (DNA repair), which may be activated after 4 h of UVR and PAR exposure. This photorepair mechanism has a low energy cost and may be an important adaptive mechanism to attenuate the net negative effect of UVR when a non-UVR-acclimated bacterioplankton community is exposed to high PAR and UV-A intensity and harmful UV-B levels in ecosystems with low nutrient availability (Medina-Sanchez *et al.*, 2002). Notwithstanding, in agreement with our hypothesis, photorepair mechanisms were insufficient to completely counteract UVR-induced damage, this being concordant with a sharp decrease in the UVA:UVB ratio (58 %) in the upper layers (high MIR conditions). Moreover, the increased HBP found after exposure of samples to higher PAR intensity in the upper layers is consistent with the previously reported stimulatory effect of PAR on HBP (Morán *et al.*, 2001; Medina-Sanchez *et al.*, 2002; Pakulski *et al.*, 2007). Besides, a potential presence of aerobic anoxygenic phototrophic bacteria (Bertoni *et al.*, 2011; Masin *et al.*, 2012; Ferrera *et al.*, 2011) should not be ruled out to account for the

increased HBP under high PAR in UVR-opaque lake.

1.5.2. UVR and increased MIR effect on commensalistic algal-bacterial relationship

As noted above, UVR and high MIR exerted an interactive effect on both the algal and bacterial communities in the epilimnetic layer. These interactive effects were also reflected in algal C availability to support the bacterial C demand in both lakes. Quantification of the dependence of heterotrophic bacteria on organic substrate released by algae requires an accurate assessment of the BCD (Rojo *et al.*, 2012). Our study offers a quite precise estimate of the BCD, because both HBP and BR were directly measured in the UVR-clear lake, due to absence of size overlap between auto- and heterotrophic organisms. In the UVR-opaque lake, where segregation between both biological fractions was not feasible, BR was estimated from direct measurements of TPR and the reported percentages of the latter variable accounted for BR (i.e. 50 and 75 %, Lemée *et al.*, 2002; Robinson, 2008). This procedure brought about a min-max range where the actual BR should safely fall. In addition, its reliability is supported in that our estimated mean BGE and BR values fell within the range reported for oligotrophic ecosystems (Biddanda *et al.*, 2001; Amado *et al.*, 2013).

In the UVR-clear lake, BGE was increased under full-sunlight and high MIR conditions, reflecting greater changes in bacterial respiration than production. The reduction in BR and, as a consequence, the increase in bacterial growth efficiency could be interpreted as a tolerance-related mechanism under full-sunlight exposure in accordance with the non-inhibitory effect of UV-B on HBP found under shallower conditions. By contrast, in the UVR-opaque lake, BGE values were lower under full sunlight and high MIR (stratified conditions). The lack of the inhibitory effect of full sunlight (PAB vs. P) on TPR (and hence BR) concomitantly with a strong inhibitory effect of UV-B on HBP determined a reduction in bacterial growth efficiency according to the high sensitivity of the bacterial community. These results agree with previous laboratory findings of a negative UV-B effect on BGE or BR in some bacterial strains isolated from alpine lakes, but

a positive effect on others, suggesting a strain-specific response (Hörtnagl *et al.*, 2011). Nevertheless, changes in BGE are frequently observed when bacterial growth is limited by substrate availability (del Giorgio y Cole, 1998; López-Urrutia y Morán, 2007). Although our experiments were not specifically designed to test the role of organic substrates on BGE, we did not find a significant direct relationship between EOC and BGE in either lake. Thus, our data support the view that BGE can be altered by direct solar UVR impact.

Regarding the algal-bacterial relationship, it was noticeable that in the UVR-clear ecosystem, EOC rates increased with full sunlight and high MIR, reaching values that exceeded the C demand of a bacterial community which seemed to have undergone an inactivation or dormancy under PAB, reflected by lower respiration. This slowing of the bacterial metabolism, concomitant with an increase in the availability C released by algae, was the mechanism that determined the "coupling" algal-bacterial relationship. However, the fate of C released by algae could be transitory accumulation in lake water until its consumption by enhanced bacterial metabolic processes (growth and respiration) after an improvement in the light conditions, or could be definitively incorporated into the dissolved-C pool of the lake water. In the UVR-opaque ecosystem, particularly to the epilimnetic community, the strong inhibitory effect of UV-B under high MIR on PP (i.e. decreasing C incorporation) was also reflected in a lesser C release by algae under these conditions. These decreased EOC rates did imply a change in their capability to meet the BCD, which ranged from barely sufficiency (if a 50 % loss of TPR is assumed) to non-sufficiency (if a 75 % loss of TPR is assumed). Therefore, the estimated min-max interval for each experimental condition shows an unexpected trend to a weakening of the bacterial dependence on algal C under full-sunlight and high MIR in UVR-opaque lake, which may be induced by global warming. These results partially support our hypothesis because the interaction UVR×MIR strengthened the commensalistic algal-bacterial relationship in the UVR-clear lake, but weakened this relationship in the UVR-opaque lake (Fig. 1.3f and 1.4f). Moreover, they underline the capability of UVR in altering the efficiency of algal C excretion to support bacterial demands in optically contrasting

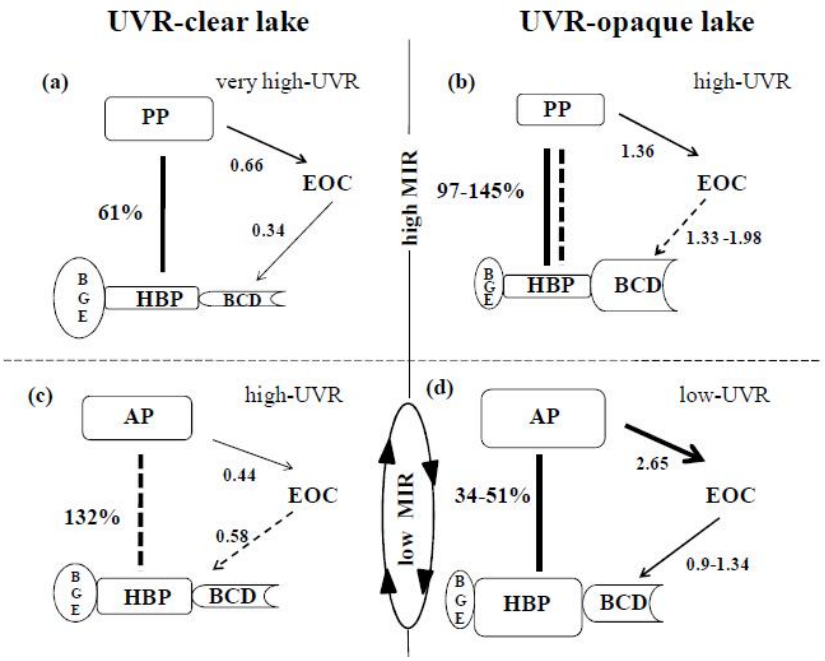

Figura 1.6: Changes in epilimnetic algal-bacterial relationship under PAB-high MIR and PAB-low MIR in UVR-clear lake (a, c) and UVR-opaque lake (b, d). The sizes of the boxes are proportional to the rates ((in μg C l^{-1} h^{-1}). The thicknesses of the arrows indicate the relative magnitude of a particular carbon flux. The broken-lines arrows indicate that EOC is not enough to satisfy the bacterial carbon demand (BCD). Thick black lines represent the BCD:EOC (%), indicating either coupled (solid lines) or uncoupled (broken lines) algal-bacterial relationship. PP: primary production, HBP: heterotrophic bacterial production, BGE: bacterial growth efficiency. Numbers are rates of (in μg C l^{-1} h^{-1}).

ecosystems. Since the interaction of UVR and stratification on this crucial biotic interaction in UVR-clear and UVR-opaque lakes has not been previously examined, more data is needed in order to generalize these responses by microbial organisms, not only on short- term (as considered in this study) but also on long term basis.

To summarize our findings, we propose a conceptual functioning model that fits both contrasting model ecosystems (Fig.1.6). According to the global-warming scenario: (i) the vertical stratification of aquatic ecosystems will intensify (van de Waal *et al.*, 2010); (ii) the depth of the mixed layer will be altered (micro-stratification in shallow lakes) (De Senerpont Domis *et al.*, 2013); and (iii) microbial communities and DOC will be confined within a highly irradiated layer. Based on our results, the interactive effect of UVR and stratification on the microbiota might strengthen the C flux through the microbial loop in the UVR-clear lake (or increasing the DOC pool in the lake) but weaken it in the UVR-opaque lake. Overall, our results reveal greater UVR damage in the UVR-opaque lake, despite of the lesser UVR flux and penetration, implying that these types of ecosystem might be especially vulnerable to factors related to global change.

Capítulo 2

Direct and indirect effects of vertical mixing, nutrients and ultraviolet radiation on the bacterioplankton metabolism in high-mountain lakes from southern Europe

2.1. Abstract

As a consequence of global change, modifications in the interaction among abiotic stressors on aquatic ecosystems have been predicted. Among other factors, UVR transparency, nutrient inputs and shallower epilimnetic layers could alter the trophic links in the microbial food web. Currently, there are some evidences of higher sensitiveness of aquatic microbial organisms to UVR in opaque lakes. Our aim was to assess the interactive direct and indirect effects of UVR (through the excretion of organic carbon -EOC- by algae), mixing regime and nutrient input on bacterial metabolism. We performed in situ short-term experiments under the following treatments: full sunlight (UVR+ PAR, >280 nm) vs. UVR exclusion (PAR only, >400 nm); ambient vs. nutrient addition [phosphorus (P; 30 μg P L^{-1}) and nitrogen (N; up to final N:P molar ratio of 31); and static vs. mixed regime. The experiments were conducted in three high-mountain lakes of Spain: Enol [LE], Las Yeguas [LY] and La Caldera [LC] which had contrasting UVR transparency characteristics [opaque (LE) vs. clear lakes (LY and LC)]. Under ambient nutrient conditions and static regimes, UVR exerted a stimulatory effect on heterotrophic bacterial production (HBP) in the opaque lake but not in the clear ones. Under UVR, vertical mixing and nutrient addition HBP values were lower than under the static and ambient nutrient conditions, and the stimulatory effect that UVR exerted on HBP in the opaque lake disappeared. By contrast, vertical mixing and nutrient addition increased HBP values in the clear lakes, highlighting for a photoinhibitory effect of UVR on HBP. Mixed regime and nutrient addition resulted in negative effects of UVR on HBP more in the opaque than in the clear lakes. Moreover, in the opaque lake, bacterial respiration (BR) increased and EOC did not support the bacterial carbon demand (BCD). In contrast, bacterial metabolic costs did not increase in the clear lakes and the increased nutrient availability even led to higher HBP. Consequently, EOC satisfied BCD in the clear lakes, particularly in the clearest one [LC]. Our results suggest that the higher vulnerability of bacteria to the damaging effects of UVR may be particularly accentuated in the opaque lakes and further recognizes the relevance of light exposure history and biotic

interactions on bacterioplankton metabolism when coping with fluctuating radiation and nutrient inputs.

2.2. Introduction

Among the organisms that constitute the pelagic community, bacterioplankton is an important compartment in the structure and function of ecosystems due to their key role in biogeochemical cycles (Cotner y Biddanda, 2002). Moreover, their biomass production and their trophic coupling to eukaryotes have a profound impact on elemental fluxes (Newton *et al.*, 2011). However, due to their particular characteristics (small cell-volume, general lack of pigmentation and short generation times), bacterioplankton are especially sensitive to environmental perturbations (Garcia-Pichel, 1994; Paerl *et al.*, 2003; Shade *et al.*, 2012). This sensitivity is particularly relevant in a scenario of global change, characterized by increased mean atmospheric temperatures, alteration in precipitation regimes (Giorgi y Lionello, 2008; Stocker *et al.*, 2013) and higher frequency of extreme weather events (Graham y Vinebrooke, 2009). These changes, together with increased atmospheric depositions (Goudie, 2009) and increased levels of ultraviolet radiation (UVR, 280-400 nm) (Manney *et al.*, 2011) are altering the natural characteristic of water masses (Stocker *et al.*, 2013; Häder, 2011). Natural levels of UVR can be stressors, with significant negative effects on most aquatic microorganisms (see review of Helbling *et al.*, 2003a). The direct negative effects of UVR on bacterioplankton include the damage to the DNA molecule (Jeffrey *et al.*, 1996; Hernández y Cormack, 2007), decrease in enzymatic activity (Herndl *et al.*, 1993) and reduction of membrane permeability (Chatila *et al.*, 2001; Buma *et al.*, 2003). All these direct effects can negatively affect heterotrophic bacterial production (HBP; Conan *et al.*, 2008; Bullock y Jeffrey, 2010). However, neutral or even stimulatory effects of UVR have also been reported (Aas *et al.*, 1996; Medina-Sanchez *et al.*, 2002, ;Durán *et al.* unpublised). These opposite results have been related either with the differential photoacclimation capacity of organisms (Pakulski *et al.*, 1998) or with the interaction of UVR with other environmental factors (Häder, 2011; Ruiz-González *et al.*, 2013). For example, the addition of nutrients can reduce UVR photoinhibition (Medina-Sánchez *et al.*, 2006) or, contrarily, unmask the damaging effect of UVR (Durán *et al.* unpublished). Also, dissolved organic matter (DOM) might contribute

to explain the differences in the observed effects on aquatic organisms by reducing UVR penetration in the water column (Rose *et al.*, 2009). Thus, high DOM contents can absorb UVR in upper water layers, therefore biota would receive less solar radiation (Williamson *et al.*, 2010) and so they would reduce damage caused by these wavelengths. However, recent studies (Harrison y Smith, 2011; Helbling *et al.*, 2013) showed that shade-adapted algae from ecosystems with high DOM content are more vulnerable to UVR under increased or fluctuating levels. Moreover, photochemical processes might influence organic C availability for bacteria (Ruiz-González *et al.*, 2013). Thus, recalcitrant DOM can be photolysed into more readily forms (Abboudi *et al.*, 2008), while labile DOM can result into more recalcitrant forms (Bastidas Navarro *et al.*, 2009).

Increases in temperature can modify the thermocline depth and accentuate the stratification of water column thus influencing UVR exposure of bacterioplankton within the upper mixed layer (UML) (Sahoo *et al.*, 2010; De Senerpont Domis *et al.*, 2013). Because the solar radiation spectrum have different penetration into the water column, with more rapid UV-B (280-315 nm) attenuation as compared to that of UV-A (315-400 nm) or photosynthetic active radiation (PAR, 400-700 nm; Hargreaves, 2003), organisms are not only affected to varying light intensities in the upper water layers but also to different light quality. As a consequence, the net effect of solar radiation on bacteria within an actively mixed water column will be a function of radiation exposure, with photoinhibition in near-surface waters, and physiological recovery when irradiance is attenuated in deeper waters (Ferrero *et al.*, 2006). Moreover, a persistent stratification would determine vertical nutrient gradients with higher accumulation below the UML, which can generate nutrient limitation in the epilimnion (Sarmento *et al.*, 2010; Song *et al.*, 2013). However, nutrient limitation in the epilimnion can be counteracted by an increase in wind speed and duration (Helbling *et al.*, 2013) if water mixing reaches the nutricline (Fouilland *et al.*, 2007). Also limitation by nutrients can be alleviated by the input of nutrients associated with atmospheric processes (Carrillo *et al.*, 2008a; Bullejos *et al.*, 2010; Lekunberri *et al.*, 2010) or water runoff (Moss *et al.*, 2012) which, may also contain DOM (Evans *et al.*, 2006). In turn, higher DOM concen-

tration contribute to higher temperature in the upper layers and therefore to shallower thermocline depths in small non-eutrophic lakes (Caplanne y Laurion, 2008; Read y Rose, 2013).

Eventually, changes in the physical structure of the water column would determine the primary production levels within the UML, and thereby the release of organic carbon associated with photosynthetic activity (Grubisic *et al.*, 2012; Helbling *et al.*, 2013). Because heterotrophic bacteria, unlike phytoplankton, have a metabolism that is not directly dependent on solar radiation, part of the UVR effects (i.e. indirect) on bacterioplankton might be mediated by phytoplankton excretion of organic carbon (EOC; Carrillo *et al.*, 2002; Medina-Sánchez *et al.*, 2004). In fact, there are numerous studies that recognize the importance of vertical mixing in affecting the responses of phytoplankton to UVR (Ej.: Helbling *et al.*, 1994; Neale *et al.*, 1998; Helbling *et al.*, 2008, 2013). However, due to the logistic complexity in experimentally mimicking vertical mixing (Ruiz-González *et al.*, 2013) and the difficulties to discriminate between direct and indirect effects of fluctuating radiation on bacterioplankton, less studies have considered the interactive effects of UVR and vertical mixing on bacteria (Jeffrey *et al.*, 1996; Huot *et al.*, 2000; Bertoni *et al.*, 2011; Galí *et al.*, 2013), and most of them indicated that fluctuating light tend to decrease the inhibition of HBP, altering the ratio of damaging to repair processes. Nevertheless it is known that constant UVR exposure, by favoring the uncoupling between photosynthesis and growth of phytoplankton, results in higher EOC (Carrillo *et al.*, 2008a; Korbee *et al.*, 2012), which modulates the bacterioplankton responses to UVR in ecosystems with low organic-carbon content (Medina-Sanchez *et al.*, 2002). Moreover, EOC also changed by fluctuant irradiance due to vertical mixing in high-mountain lakes (Helbling *et al.*, 2013).

The aim of this study was to test the direct and indirect interactive effects of UVR, nutrient-enrichment and mixing on the metabolism of bacterioplankton with different light histories. For this purpose we chose three oligotrophic high-mountain lakes from Spain with different biological and physical characteristics, with differences in UVR transparency as the most distinctive feature among them. We first analyzed how the joint action of mixing and nutrient-enrichment modified the effects of UVR on

bacterioplankton during short-term exposures. Previous studies (Helbling *et al.*, 2013) found a higher damage due to UVR exposure in autotrophic organisms of opaque lakes under mixing and after nutrient input (i.e., with higher inhibition of primary production and less carbon excretion), and a potential limitation of C for bacteria dependent on photosynthetic carbon. Therefore, with this background, we hypothesized that mixing, together with nutrient input would result in higher direct and indirect UVR damage on bacterioplankton in opaque as compared to clear lakes.

2.3. Methods

2.3.1. Study site

The study was carried out in three high-mountain lakes of Spain: Lake Enol (hereafter LE), located at Picos de Europa National Park at 1075 m.a.s.l., and Lake Las Yeguas (hereafter LY) and Lake La Caldera (hereafter LC), both located in Sierra Nevada National Park at 2800 and 3050 m.a.s.l., respectively. LE is an oligotrophic lake, with low light penetration in the water column and high influence of cattle activity (Velasco *et al.*, 1999; Helbling *et al.*, 2013) and has a mean chlorophyll *a* (Chl *a*) concentration at the surface of 2.1 μg Chl *a* L^{-1} (Helbling *et al.*, 2013). LY and LC are two small and shallow lakes, highly transparent and oligotrophic (Medina-Sanchez et al., 2010). LY has water inlets and outlets, but they are absent in LC (Medina-Sánchez *et al.*, 2010). Both Sierra Nevada's lakes have dissolved organic carbon (DOC) levels <1mg L^{-1} (Reche *et al.*, 2001) and are strongly P-limited, with a dissolved inorganic nitrogen: total phosphorus (DIN:TP) ratio 30-90 (by mass) (Carrillo *et al.*, 1996; Helbling *et al.*, 2013). LY and LC have a mean surface Chl *a* concentration of 1.1 and 0.7 μg Chl *a* L^{-1}, respectively (Helbling *et al.*, 2013). The three lakes receive pulses of nutrient from different origins: in LE they are mainly from cattle activity (López-Merino *et al.*, 2011), whereas in LY and LC they come from atmospheric Saharan dust containing high P levels (Morales-Baquero *et al.*, 2006).

2.3.2. Experimental design

Short-term experiments to assess the combined effects of vertical mixing, nutrient and UVR on HBP and bacterial carbon demand (BCD) were carried out in situ during summer of 2010: on 23 July in LE, on 10 September in LC, and on 13 September in LY. An acid-cleaned 6-L horizontal Van Dorn sampler was used to collect the water samples within the upper 3m of the water column. Before performing the experiments, water samples were filtered through a 45 μm-pore size mesh to remove large zooplankton. The experiments had a $2 \times 2 \times 2$ factorial design. Two radiation treatments (in triplicate) were implemented: (i) UVR+PAR (>280 nm; treatment UVR): using uncovered quartz flasks and (ii) exclusion of UVR (>400 nm; treatment PAR): covering the flasks with UV Opak 395 filter (Ultraphan, Difegra). The spectral characteristics of this filter are published elsewhere (Figueroa *et al.*, 1997). Samples were also exposed to two nutrient conditions: (i) ambient nutrient concentration (NP-ambient) and (ii) nutrient addition (NP-added). The nutrient-added treatments were obtained by adding Na_2HPO_4 to a final concentration of 30 μg P L^{-1} and NO_3NH_4 to a final N:P ratio of 31. In this way, we simulated and kept the proportion of nutrients input caused by pulses of Saharan dust as reported by Morales-Baquero *et al.* (2006).

The flasks were put in two trays, one at a fixed depth (STATIC) and one moving vertically (MIXING). The STATIC treatment was put at a fixed depth that varied between 1.3 and 1.4 m (according to their PAR attenuation coefficient, $k_{d\ PAR}$) to receive the mean irradiance of the upper 3 m of the water column (Helbling *et al.*, 1994). Vertical mixing in the MIXING treatment was simulated by transporting a tray up and down to 3 m depth at a speed of 1 m every 4 minutes. This speed was previously determined the day before experimentation by doing measurements of the effective photochemical quantum yield (Y) at different depths and at the surface and applying the model described in (Villafañe *et al.*, 2007). The incubations lasted 4 h, therefore a total of 10 cycles (surface-down to 3 m and back to the surface) were completed. The tray containing the samples was vertically moved in the water column by a custom-made mixing simula-

tor using a frequency-controlled DC motor (Maxon motor, Switzerland) to impose a linear and constant transport rate on the flasks from the surface to the bottom. The whole setup was placed on a boat that was anchored in a deep section of the lakes and it did not receive any shadows.

2.3.3. Physical and chemical analyses

Vertical profiles of solar radiation and temperature in the water column were obtained at noon with a submersible BIC compact 4-channel radiometer (Biospherical Instruments Inc., CA, USA), which has three channels in the UVR region of the spectra (305, 320 and 380 nm) and one broadband channel for PAR (400-700 nm) and a temperature sensor. Diffuse attenuation coefficients for downward irradiance (k_d) were determined from the slope of the linear regression of the natural logarithm of downwelling irradiance versus depth for each wavelength range considered (n>200 per profile). Temperature data were used to determine the strength of the thermocline and the depth of the epilimnion.

Water samples from the upper water layer were collected in triplicate in each lake to determine abiotic and biotic structural variables. Dissolved inorganic nitrogen (DIN) was considered as the sum of nitrate (NO^{3-}), nitrite (NO^{2-}), and ammonium (NH^{4+}), which were determined by UV-spectrophotometric screening, sulfanilamide and phenol-hypochlorite techniques, respectively (APHA, 1992). Total phosphorus (TP) was measured by analyzing 50 mL aliquots with the acid molybdate technique after persulfate digestion (APHA, 1992). Blanks and standards were done in all procedures. Dissolved organic carbon (DOC) values were determined by filtering the samples through pre-combusted (2 h at 500°C) glass-fiber filters (Whatman GF/F) and acidifying them with HCl. Samples were then measured in a total organic carbon analyzer (TOC-V CSH/CSN Shimadzu).

2.3.4. Analysis of biological structural variables

Phytoplankton abundance (PA) was obtained from samples that were put in 250-mL brown glass bottles and preserved with Lugol alkaline so-

lution (1 % vol vol^{-1}) and then stored until analysis. A volume of 50 mL was allowed to settle for 48 h in Utermöhl chambers (Hydro-Bios GmbH, Germany) and species were enumerated and identified using an inverted microscope (Leitz Fluovert FS, Leica, Wetzlar, Germany).

Bacterial abundance was determined by flow cytometry techniques (FACScanto II, Becton Dickinson Biosciences, Oxford, UK) from samples of water (three replicates and two controls for each experimental treatment) fixed with 1 % paraformaldehyde and stained with SYBR Green I DNA stain (Sigma-Aldrich) to a 1:5000 final dilution of initial stock (Zubkov *et al.*, 2007). Stained microbial cells were discriminated on bivariate plots of particle side scatter vs. green fluorescence. Yellow-green 1μm beads (Fluoresbrite Microparticles, Polysciences, Warrington, PA, USA) concentration standard was added at known dilution to determine absolute cell concentration and fluorescence (Zubkov y Burkill, 2006; Zubkov *et al.*, 2007).

2.3.5. Analysis of biotic functional variables

Heterotrophic bacterial production: Water samples for HBP measurements were placed in 10 mL-quartz flasks (three replicates and two blanks for each experimental treatment). Flaks were exposed in situ for 3 h under the different treatments previous to the radiotracer addition. HBP was determined by incorporating ^{3}H-thymidine (S.A= 46.5 Ci $mmol^{-1}$, Amershan Pharmacia) into the bacterial DNA (Fuhrman y Azam, 1982; Smith y Azam, 1992). ^{3}H-thymidine was added to each experimental flask to a final concentration of 16.6 nM (saturating concentration). Vials with the radiotracer were incubated during 1 h in situ under the different treatments. Therefore all flask sets were horizontally held during the total 4 h of incubations (3 h without radiotracer followed by 1 h with radiotracer) around noon.

After incubation, the incorporation of ^{3}H-thymidine was stopped with the addition of tricholoracetic acid (TCA, 5 % final concentration). Blanks were killed with 5 % TCA before addition of the radiotracer. Extraction was performed in cold 5 % TCA by keeping the vials in the ice for 20 min, after which the precipitate was collected by centrifugation (1600g for 10

min). Then, vials were rinsed twice with 1.5 mL of 5 % TCA to remove any residual unincorporated radioactivity. After that, scintillation liquid (Ecoscint A) was added for subsequent measurement in an autocalibrated scintillation counter (Beckman LS 6000TA). The conversion factor 1×10^{18} cell mol^{-1} (Bell, 1993) was used to estimate the number of bacteria produced per mol of incorporated thymidine. The factor 2×10^{-14} g C $cell^{-1}$ (Lee y Fuhrman, 1987) was applied to estimate the amount of carbon.

Respiration rates: Water samples for total planktonic respiration (TPR) (in the $<45\mu$m fraction) were placed in 10 mL-quartz flasks (three replicates for each experimental treatment) and exposed in situ for 4 h under the different treatments as explained above. TPR was assessed by using optode sensor-spots (SP-PSt3-NAU-D5-YOP; PreSens GmbH, Germany) and an optic-fiber oxygen transmitter (Fibox 3; PreSens GmbH, Germany) connected to a computer (Warkentin *et al.*, 2007). Data were recorded using the OxyView 3.51 software (PreSens GmbH) and measurements were made at the initial time and then every 8 h during 48 h. In order to estimate BR from TPR we used a potential maximum value of 75 % of TPR, which is based on data reported for oligotrophic waters (Lemée *et al.*, 2002) and a minimum value of 50 % of TPR, which is an average value based in previous studies in LC (Carrillo *et al.* submitted) and on data reported in other oligotrophic systems (Robinson *et al.*, 2002; Robinson, 2008). The consumption rates of oxygen (μM O_2 h^{-1}) by the bacterial community were converted into carbon units by using a respiratory quotient of 1 described for bacterioplankton (del Giorgio y Cole, 1998). Bacterial carbon demand (BCD) was calculated as HBP+BR.

Incorporated and excreted carbon: After the 4 h of in situ exposure to solar radiation, the samples were immediately filtered, under low pressure (<100 mm Hg) to minimize cell breakage, and fractionated in size fractions as follows: Samples from LE were filtered through 3 μm Whatman GF/D filters (25 mm diameter) and then through 0.7 μm Whatman GF/F filters (25 mm diameter)to determine the presence of picoautotrophs (3-0.7 μm) that might have incorporated some carbon. However, samples from LC and LY were filtered only through 1 μm Nuclepore filters (25 mm diameter) as previous studies (Carrillo *et al.*, 2002) already confirmed the absence of

picoautotrophs. The filters were put in 20-mL scintillation vials, and inorganic carbon was removed by adding 100 μL of 1 N HCl and allowing the vial to stand open in a hood for 24 h (no bubbling) (Lignell, 1992). Excreted organic carbon (EOC) was measured on 4 mL sub-samples, collected from the 0.7 or 1μm filtrate, that were put in 20-mL scintillation vials, together with 100 μL of 1N HCl to eliminate the excess inorganic radiocarbon.After acidification, scintillation cocktail (Ecoscint A)was added to both, samples for carbon incorporation and excretion, and counted usinga scintillation counter (Beckman LS 6000TA)equipped with an internal calibration source.The carbon incorporated and excreted was calculated based on the CPMs, and in all calculations the dark values were subtracted from the light values.

Bacterial carbon demand and their relationship with excreted organic carbon:

To assess the bacterial limitation by EOC (%BCD:EOC) we quantified if EOC rates were enough for sustaining the potential BCD (Morán *et al.*, 2002) as:

$$\%BCD : EOC = BCD \times EOC^{-1} \times 100 \tag{2.1}$$

2.3.6. Data treatment and statistics

The percentage of UVR-induced inhibition (UVR_{inh}) on HBP was calculated as:

$$UVR_{inh}(\%) = 100[(HBP_{PAR} - HBP_{UVR})]/HBP_{PAR} \tag{2.2}$$

where HBP_{PAR} and HBP_{UVR} represent the carbon produced in samples under the PAR and UVR treatments, respectively. The differences of $\%UVR_{inh}$ among treatments were evaluated by a t-test. We used propagation errors to calculate the variance of the UVR-induced inhibition.

To determine significant interactions among the radiation, nutrients and mixing regimes for the different variables, a three-way analysis of variance (ANOVA) was used. When significant effects were determined, a post

Variable	LE	LY	LC
$k_{d\ 305}$ (m^{-1})	3.15	0.62	0.30
$k_{d\ 320}$ (m^{-1})	2.28	0.58	0.26
$k_{d\ 380}$ (m^{-1})	1.22	0.26	0.17
$k_{d\ PAR}$ (m^{-1})	0.34	0.18	0.16
DOC (mg L^{-1})	2.23±0.45	1.01±0.11	1.07±0.08
TP (μM)	0.11±0.05	0.11±0.003	0.128±0.007
DIN (mM)	25.01± 0.44	6.67±0.2	11.78±0.53
DIN/TP	117.87±12.53	27.64±0.27	41.71±0.4
BA (cell mL^{-1})×10^5	25±19.2	4.14±0.24	1.22±0.22
PA (cell mL^{-1})	3599	2063	2000

Cuadro 2.1: Mean values of the main physical, chemical and biological variables of the studied lakes at the beginning of the experiment. Diffuse attenuation coefficients (k_d) of UVR at 305nm ($k_{d\ 305}$), at 320 nm ($k_{d\ 320}$) and at 380 nm ($k_{d\ 380}$) and PAR ($k_{d\ PAR}$); dissolved organic carbon (DOC); total phosphorus (TP); dissolved inorganic nitrogen (DIN); DIN/TP ratio expressed by weight; bacterial abundance (BA); phytoplanktonic abundance (PA)

hoc Fisher LSD test was used to determine significant differences among treatments. Data were checked for normal distribution with the Kolmorov-Smirnov test. Homocedasticity was verified with Cochran and Levene's test, and data were log-transformed when these conditions did not meet. Statistical analysis was performed using the program Statistica 7.0 for Windows (Statsoft 2001).

The points presented in Fig. 2.3 (UVR-induced inhibition of HBP as a function of the attenuation coefficient) were based on the results obtained in this study and, in order to extend the relationship obtained in our model lakes, we also used data from previous published studies and also unpublished data obtained by our research group. We used data by (Bertoni *et al.*, 2011) from their experiment 1 comparing static versus moving incubations.

Thus, we calculated the difference in the percentage of UVR_{inh} (calculated according to Eq. 2.2 from data reported in their Table 2) between values in mixing and static [i.e., 31.4 % (mixed) – 20.8 % (static)] treatments, and normalized it by a mean PAR irradiance of 259.2 W m^{-2}. We used the $k_{d\ 320}$ value of 0.425 reported in their Table 2. The second additional point is from experiments carried out by our research group in La Colgada Lake, in Ruidera Natural Park (Spain) (unpublished data). There, we obtained the difference between $\%UVR_{inh}$ (according to Eq. 2.2) in mixing and static treatments [i.e. 53.2 % (mixed) – -53.6 % (static)]. The differences were normalized by a mean PAR of 264.3 W m^{-2} and they were related with their $k_{d\ 320}$ (2.08) in the graph.

2.4. Results

2.4.1. Experimental conditions

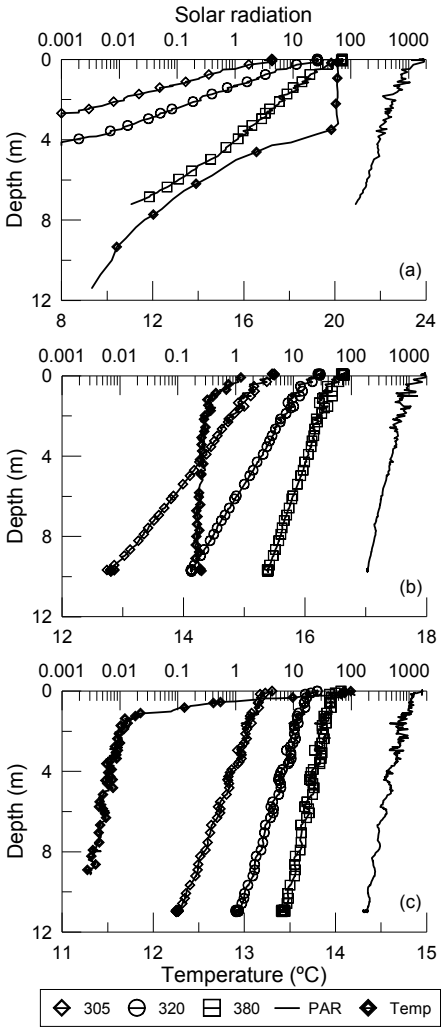

Figura 2.1: Solar irradiance and temperature as a function of depth in (a) Lake Enol (LE), (b) Las Yeguas (LY) and (c) La Caldera (LC).UVR data are expressed in μW cm^{-2}, and PAR in μmol photons m^{-2} s^{-1}

The main initial physical, chemical and biological characteristics measured in the epilimnion of the three studied lakes are shown in Table 2.1. The high DIN:TP ratios for the three lakes evidenced the strong likelihood of P-limitation in the upper layers (DIN:TP>12 by weight, sensu Morris y Lewis (1992). However, the DIN:TP ratio in LE (∼118) was ca. three-fold higher than in LY and LC due to the high concentration of DIN. Moreover, the DOC concentration (∼ 2.2 mg L^{-1}) was 2-fold higher in LE as compared to the other two lakes. In regard to the radiation conditions (Fig. 2.1, 2.1), LC was the most transparent, having the lowest k_d value i.e., $k_{d\ PAR}$ of 0.16 m^{-1}, followed by LY and LE (Table 2.1). Thus, and hereafter, we will refer LE as to the 'opaque' lake and LY and LC as to the 'clear' lakes. The highest mean surface irradiance during the experiments was received in LC, then followed by LY and LE (e.g. surface PAR irradiance: 1774, 1670 and 1558 μmol photons m^{-2} s^{-1} for LC, LY and LE, respectively). LE had the highest epilimnetic temperature value (Fig. 2.1a) with a marked epilimnion down to ca. 3.5 m depth, below which temperature steadily decreased to 9^{o}C at 12 m depth. In contrast, LY (Fig. 2.1b) was well mixed and temperature did not substantially vary in the water column; LC (Fig. 2.1c) showed a weak stratification with temperature decreasing from 14^{o}C at the surface to 12^{o}C at 1 m depth.

2.4.2. Joint effects of solar radiation, mixing and nutrient on bacterioplankton metabolism

In the clear lakes (LC and LY), interaction radiation×mixing×nutrient was found for HBP but not in the opaque lake (LE) (Table 2.2). LE had the

				HBP		BR		EOC		%BCD:EOC	
		df1	df2	$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p
LE											
	Radiation	1	16	27.092	**0.000**	5.007	**0.040**	13.618	**0.002**	5.500	**0.032**
	Nutrients	1	16	40.495	**0.000**	925.493	**0.000**	0.181	0.676	35.639	**0.000**
	Mixing	1	16	2.572	0.128	18.478	**0.001**	15.095	**0.001**	9.949	**0.006**
	Mixing×Radiation	1	16	11.179	**0.004**	1.944	0.182	0.816	0.380	0.929	0.350
	Mixing×Nutrients	1	16	1.623	0.221	14.331	**0.002**	0.360	0.557	11.854	**0.003**
	Radiation×Nutrients	1	16	2.274	0.151	3.157	0.095	20.792	**0.000**	5.983	**0.026**
	Radiation×Nutrients×Mixing	1	16	0.166	0.689	0.415	0.528	25.257	**0.000**	15.685	**0.001**
LY											
	Radiation	1	16	16.108	**0.001**	0.086	0.773	0.895	0.358	4.832	**0.043**
	Nutrients	1	16	16.920	**0.000**	21.558	**0.000**	22.912	**0.000**	0.840	0.373
	Mixing	1	16	0.094	0.764	3.538	0.078	72.986	**0.000**	4.832	**0.043**
	Mixing×Radiation	1	16	0.463	0.506	0.319	0.580	3.570	0.077	6.327	**0.023**
	Mixing×Nutrients	1	16	1.512	0.237	2.130	0.164	13.220	**0.002**	5.859	**0.028**
	Radiation×Nutrients	1	16	10.738	**0.005**	0.917	0.353	45.253	**0.000**	55.459	**0.000**
	Radiation×Nutrients×Mixing	1	16	25.373	**0.000**	0.016	0.901	24.965	**0.000**	50.450	**0.000**
LC											
	Radiation	1	16	17.155	**0.000**	3.664	0.073	0.088	0.771	11.024	**0.004**
	Nutrients	1	16	129.735	**0.000**	9.828	**0.006**	0.058	0.812	0.002	0.964
	Mixing	1	16	2.347	**0.003**	2.420	0.139	86.126	**0.000**	17.553	**0.001**
	Mixing×Radiation	1	16	49.149	**0.000**	0.258	0.618	83.738	**0.000**	25.783	**0.000**
	Mixing×Nutrients	1	16	7.562	**0.014**	14.766	**0.001**	177.981	**0.000**	5.799	**0.028**
	Radiation×Nutrients	1	16	0.139	0.714	9.865	**0.006**	2.624	0.125	20.387	**0.000**
	Radiation×Nutrients×Mixing	1	16	25.076	**0.000**	0.460	0.507	0.978	0.337	1.646	0.218

Cuadro 2.2: Result of the three-way analysis of variance (ANOVA) of the interactive effects of radiation, nutrient-addition, and mixing regime. Numbers in bold indicate significant effects. HBP: heterotrophic bacterial production; BR: bacterial respiration; EOC: excreted organic carbon; %BCD:EOC: Relationship between the bacterial carbon demand (BCD) and supply by algal excretion.

highest values of HBP, followed by LY and LC (Fig. 2.2). In LE, samples exposed to UVR in static conditions, had significantly higher HBP values than those exposed only to PAR (Fig.2.2a). Nutrient addition decreased HBP under UVR in both static and mixing treatments (Fig. 2.2a) as compared to values obtained without nutrient addition. While UVR stimulated HBP in static conditions regardless of the nutrient treatment, this effect decreased or almost vanished under mixing conditions (Fig. 2.2b). For LY, HBP was not affected by UVR exposure under static and ambient nutrient conditions, but HBP values increased in the PAR treatment in response to nutrient addition resulting in a high UVR inhibitory effect (UVR_{inh}=40 %; Fig. 2.2c, d). Under ambient nutrient conditions, mixing increased HBP under PAR as compared to static conditions, leading to an UVR inhibitory effect of 21 % (Fig. 2.2c, d). Under nutrient addition, mixing reduced HBP value in PAR treatment as compared to static conditions (Fig. 2.2c). In LC (Fig. 2.2e), UVR did not significantly affect HBP in static ambient nutrient conditions as compared to PAR-exposed samples. The addition of nutrients under UVR increased HBP in both static and mixing regimes, but nutrient-addition stimulated HBP under PAR only in the mixing regime. Hence, nutrient addition resulted in a UVR-stimulatory effect on HBP up to 58 % under static conditions. Despite HBP values increased after nutrient-addition under mixing regime, UVR was responsible for a strong inhibitory effect of HBP (47 %) under mixing, with no significant differences between nutrient treatments (Fig. 2.2f).

Figure 2.3 shows the relationship between the UVR-transparency, as

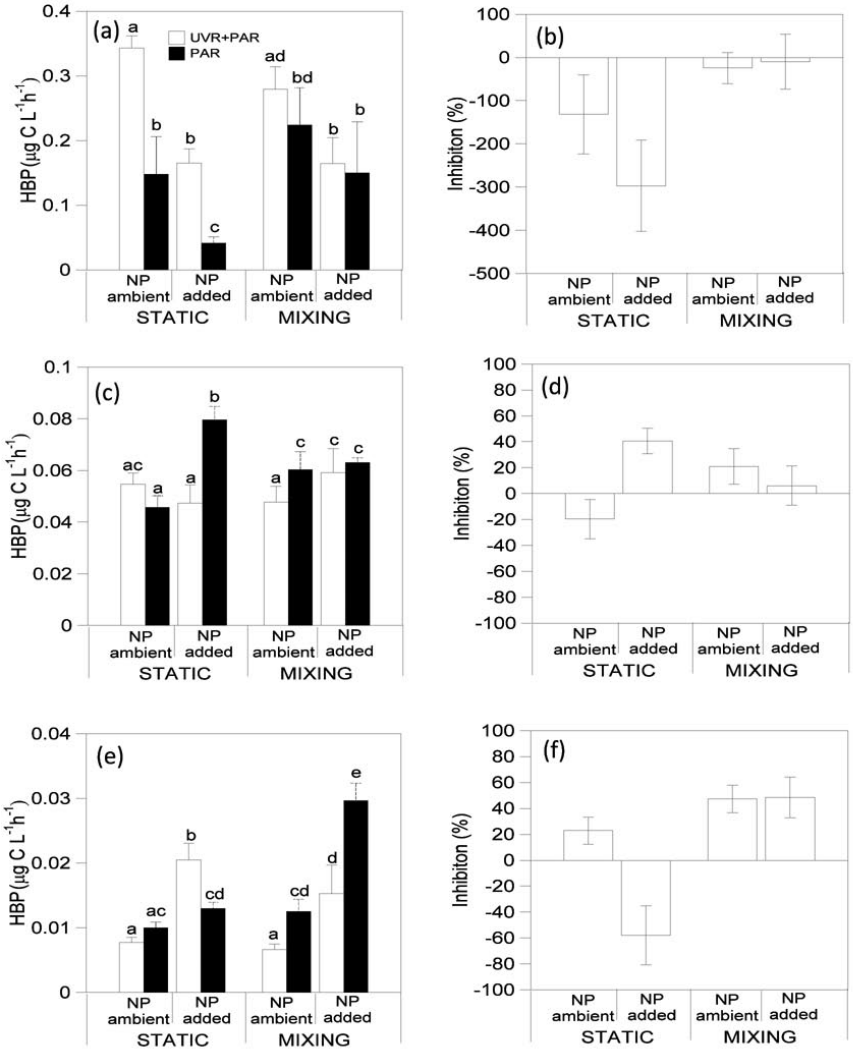
(a)
UVR+PAR
PAR
HBP(µg C L-1h-1)
0.4
0.3
0.2
0.1
0
a
b
b
c
ad
bd
b
b
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(b)
Inhibiton (%)
100
0
-100
-200
-300
-400
-500
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(c)
HBP(µg C L-1h-1)
0.1
0.08
0.06
0.04
0.02
0
ac
a
a
b
a
c
c
c
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(d)
Inhibiton (%)
100
80
60
40
20
0
-20
-40
-60
-80
-100
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(e)
HBP(µg C L-1h-1)
0.04
0.03
0.02
0.01
0
a
ac
b
cd
a
cd
d
e
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(f)
Inhibiton (%)
100
80
60
40
20
0
-20
-40
-60
-80
-100
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING

Figura 2.2: Heterotrophic bacterial production (HBP, in μg C L^{-1} h^{-1}) under different radiation (UVR+PAR vs. PAR), nutrient concentration (ambient vs. nutrient added) and mixing regime (static vs. mixing) in (a) Lake Enol (LE), (c) Las Yeguas (LY) and (e) La Caldera (LC). Percentage of inhibition of HBP (%) due to UVR under the different treatments in (b) LE, (d) LY and (f) LC. Negative values indicate stimulation by UVR and positive values indicate inhibition by UVR. Data are expressed as mean values $\pm$ SD (n=3). Letters indicate significant differences among treatments.

estimated by the attenuation coefficient at 320 nm ($k_{d\ 320}$), and the effects of mixing on the inhibitory effect of UVR on HBP. We found a decreasing inhibitory trend of UVR towards lower $k_{d\ 320}$ values (Fig. 2.3). Consistently with our observations, the data obtained by Bertoni *et al.* (2011) and from our own group in La Colgada Lake showed that the inhibitory effect due to mixing was higher in the more opaque lakes (high$k_{d\ 320}$) and decreased towards the more clear lakes (low $k_{d\ 320}$; r= 0.93, p-value<0.01). It is worth noting that the decrease in the inhibitory effect of UVR is much more pronounced when nutrients were added.

For BR, no significant interactive effects between the three studied factors were found for any ecosystem (Table 2.2). As in HBP, BR showed higher values in LE as compared to the other lakes (Fig. 2.4). In LE, BR increased in response to nutrient addition regardless the radiation and mixing regimes, but BR was neither affected by mixing nor by UVR (Fig. 2.4a). In LY, BR was stimulated by nutrient addition under UVR regardless the mixing regime and under PAR only under mixing. As determined in LE, mixing and UVR did not affect BR (Fig. 2.4b). Finally, for LC, while the addition of nutrients reduced BR in static conditions regardless the radiation conditions (Fig. 2.4c), mixing only reduced BR under UVR. Noticeably, nutrient addition unmasked the UVR stimulatory effect in mixing and static regimes.

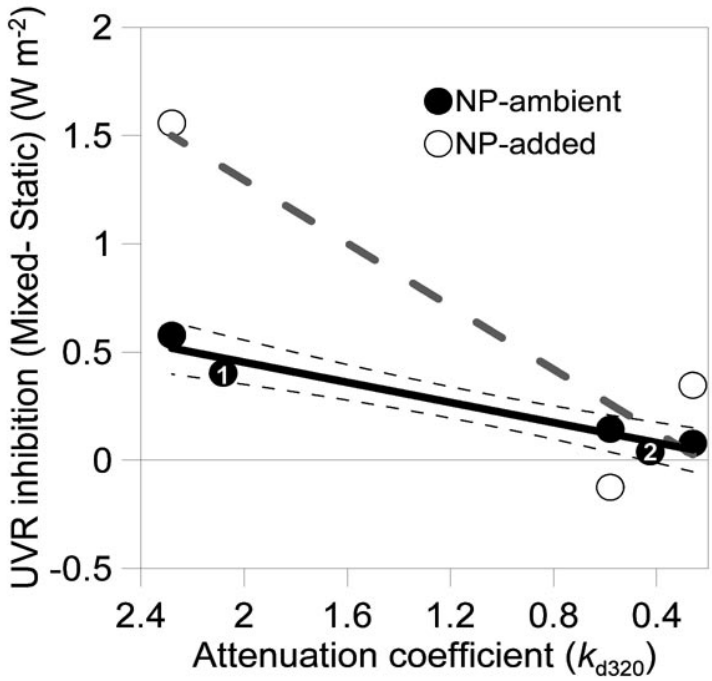

Figura 2.3: UVR inhibition of HBP in mixed minus static samples, normalized by the mean irradiance received, as a function of the attenuation coefficient ($k_{d\ 320}$) for samples without nutrient addition (black symbols), and with nutrient addition (white symbols). The black line represents the linear regression fit for the five lakes without nutrient addition (r^2=0.87, y= -0.0527+0.2418x), and the dashed grey line represents the fit for the three lakes with nutrient addition. The dashed thin lines are the 95 % confidence for the fit of the five lakes without nutrient addition. The additional data were calculated from (1) Bertoni et al. 2011 and (2) from unpublished data in lakes of Ruidera Natural Park (Spain).

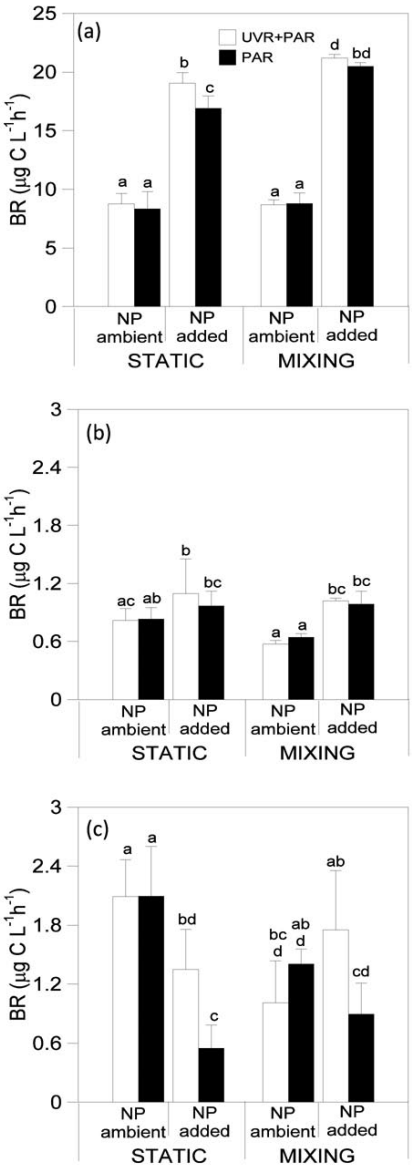
(a)
UVR+PAR
PAR
BR (µg C L-1h-1)
25
20
15
10
5
0
a a
b c
a a
d bd
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(b)
BR (µg C L-1h-1)
3
2.4
1.8
1.2
0.6
0
ac ab
b bc
a a
bc bc
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(c)
BR (µg C L-1h-1)
3
2.4
1.8
1.2
0.6
0
a a
bd c
bc d
ab d
ab
cd
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING

Figura 2.4: Bacterial respiration (BR; in μg C L^{-1} h^{-1}) under different radiation (UVR+PAR vs. PAR), nutrient concentration (ambient vs. nutrient added) and mixing regime (static vs. mixing) conditions in (a) Lake Enol (LE), (b) Las Yeguas (LY) and (c) La Caldera (LC). Only BR estimated as 50 % of total planktonic respiration (TPR) is represented in this figure, since significant differences among treatments are the same than for BR estimated as 75 % of TPR. Bars represent the mean BR values and the lines on top of them represent the standard deviation (SD; n=3). Letters indicate significant differences among treatments.

2.4.3. Joint effects of solar radiation, mixing and nutrient-addition on phytoplanktonic C fixation and excretion

The rate of phytoplankton carbon fixation was highly variable not only among lakes, but it also depended on the experimental conditions imposed to the samples (Fig. 2.5). In LE there were no significant differences between radiation treatments or nutrient addition in the >3μm fraction under the mixing condition, but in the 3-0.7 μm fraction samples receiving UVR had significant lower carbon fixation than those exposed only to PAR (Fig. 2.5a). The carbon fixed in the >3 μm fraction was significantlyhigher ($p < 0.05$) than in the 3-0.7 μm fraction, with the exception of both UVR treatments in static conditions. In LY (Fig. 2.5b) significant interactive effects between the three studied factors were determined (Table S1). All samples exposed to UVR had significantly lower carbon fixation than those exposed only to PAR. In addition, samples in the mixing regime had in general, significant higher carbon fixation as compared to the static ones. Also, and under mixing conditions, samples with addition of nutrients had higher carbon fixation than those without it for the same radiation treatment. In static samples, nutrient addition increased carbon fixation under UVR but not in samples receiving only PAR. Finally, in LC (Fig. 2.5c) there were interactive effects between mixing regime, radiation treatments

and nutrient addition (Table S1). Carbon fixation in the samples with addition of nutrients was significantly higher than those without it, in both static and mixing regimes. Also, nutrient addition "highlighted" the UVR effects under mixing conditions – with significantly higher carbon fixation in the PAR+P as compared to UVR+P, but there were no significant effects of UVR when nutrients were not added. In static samples, UVR had significant effects regardless the nutrient treatment.

The UVR-induced inhibition of carbon fixation in LE (Fig. 2.6a) was ca. 20 % under mixing in the 3-0.7 μm fraction, whereas under static conditions negative inhibition values were obtained (i.e., photosynthesis was enhanced by UVR). The effects of UVR and nutrients were especially evident in the > 3 μm fraction under the static conditions, with negative inhibition (-20 %) in samples without nutrient addition whereas it reached 48 % in those that did receive them. In LY (Fig. 2.6b) significant inhibition was determined in all treatments (60-80 %) but samples with nutrient addition and under static conditions were those having the lowest values of 34 %. Finally, phytoplankton from LC had UVR-induced inhibition ranging from 30 to 90 % in samples without nutrient addition under mixing and static conditions, respectively (Fig. 2.6c).

EOC was significantly higher in LE as compared to the other lakes (Fig. 2.7). There were significant interactive effects among radiation, mixing, and nutrient (Table 2.2) on EOC in the three lakes. In LE and under static and ambient nutrient conditions, EOC was higher in samples exposed to UVR (Fig. 2.7a), although nutrient addition reduced EOC in samples under UVR as compared to samples with no nutrient addition (Fig. 2.7a). There was no significant difference under mixing regime between PAR and UVR treatments (Fig. 2.7a). EOC was generally higher in static than in mixing regime in LY (Fig. 5b), whereas the opposite was observed in LC (Fig. 2.7c). In LY and LC, nutrient addition in the presence of UVR resulted in lowered EOC under static conditions, and higher EOC under mixing regime (Fig. 2.7b, c).

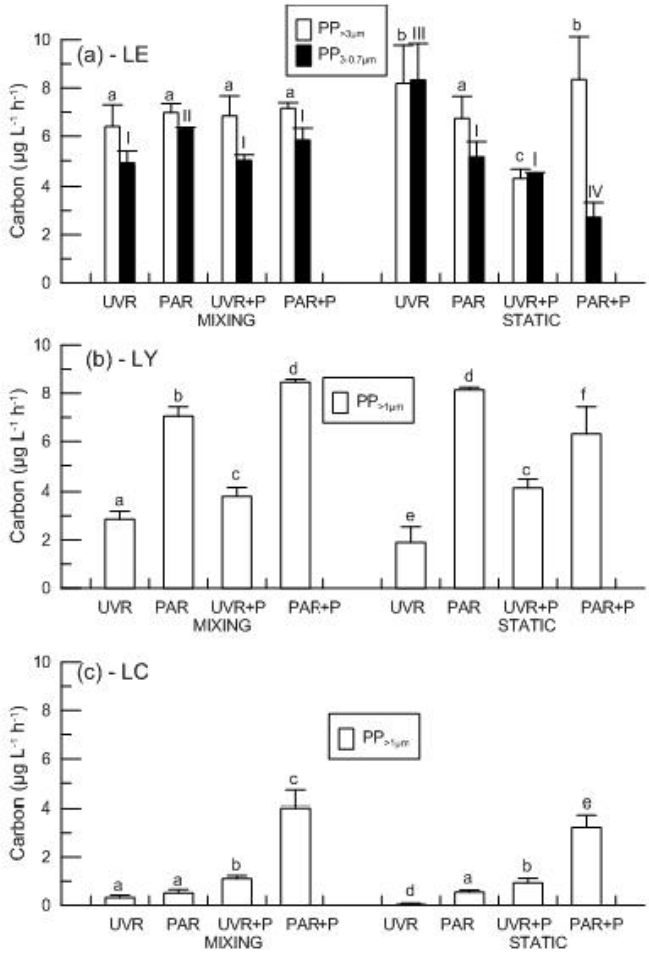

Figura 2.5: Carbon incorporation (in μg C L^{-1} h^{-1}) under different radiation conditions (UVR versus PAR alone), phosphorus concentration (ambient versus 30 μg P L^{-1}) and mixing regime (mixing versus static) in: a) Lake Enol (LE); b) Lake Las Yeguas (LY) and, c) Lake La Caldera (LC). The bars on top of the bars are the standard deviation whereas the letters and numbers indicate differences among treatments.

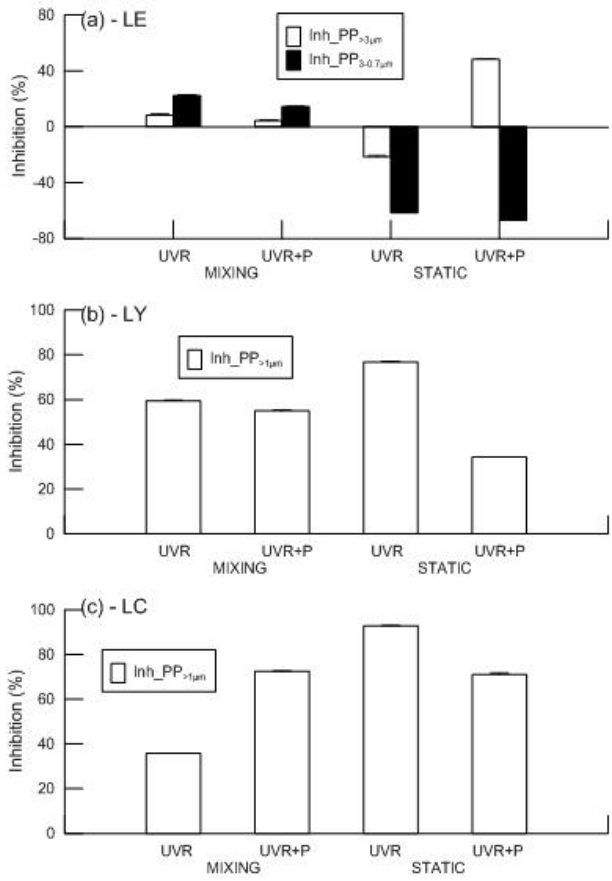

Figura 2.6: Percentage inhibition of carbon fixation (%) due to UVR under different phosphorus concentration (ambient versus 30 μg P L^{-1}) and mixing regime (mixing versus static) in: a) Lake Enol (LE); b) Lake Las Yeguas (LY) and, c) Lake La Caldera (LC). The bars on top of the bar are the standard deviation.

2.4.4. Joint effects of solar radiation, mixing and nutrient-addition on bacterial C demand and supply of algal C

In order to discriminate whether the effect of UVR on bacteria was mediated by their commensalistic interaction with algae, we compared bacterial carbon demands with the dissolved organic carbon excreted by algae (EOC).

Figure 2.8 depicts the ratio between BDC and EOC, i.e. an estimation of whether EOC meets the potential bacterial carbon demands for growth and respiration. In LE, under UVR and ambient nutrient conditions, EOC exceeded BCD (static) or was enough (under mixing) to meet BCD (i.e. %BCD:EOC$\leq$100; Fig. 2.8a). In LY and LC, EOC exceeded BCD under simultaneous UVR, mixed and nutrient-added conditions. However, EOC did not meet BCD only under UVR and ambient nutrient and mixing conditions in LY (Fig. 2.8b), and under UVR and nutrient added and static conditions in LC (Fig. 2.8c).

2.5. Discussion

This study fills a gap of knowledge about how vertical mixing modulates the UVR effects on bacterioplankton in optically different high-mountain lakes. We quantified how the interaction of UVR, nutrient inputs, and the epilimnetic mixing regime affected the bacterial metabolism both directly and indirectly, this latter through the excretion of organic carbon by algae. The three selected model ecosystems, with a gradient of UVR transparency, enabled us to understand how their optical characteristics modulated the interactive effects that the three studied factors exerted on the algae-bacteria relationship. Our experiments were made under realistic experimental exposure conditions resembling the epilimnetic vertical mixing during the middle ice-free period when both UVR flux and nutrient inputs reach a maximum in high-mountain Iberian lakes (Bullejos *et al.*, 2010; Villar-Argaiz *et al.*, 2012; Mladenov *et al.*, 2011).

Our study showed that, under static conditions, UVR alone stimulated

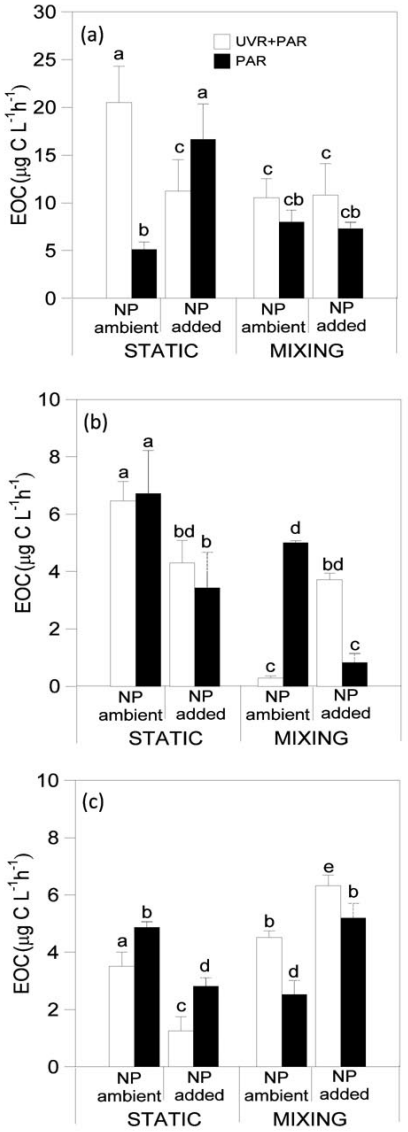
(a)
UVR+PAR
PAR
EOC(µg C L-1h-1)
NP ambient
NP added
STATIC
MIXING
(b)
(c)

Figura 2.7: Concentration of excreted organic carbon (EOC, in μg C L^{-1} h^{-1}) under the different radiation (UVR+PAR vs. PAR), nutrient concentration (ambient vs. nutrient added) and mixing regime (static vs. mixing) in (a) Lake Enol (LE), (b) Las Yeguas (LY) and (c) La Caldera (LC). Data are expressed as mean values ± SD (n=3). Letters indicate significant differences among treatments

HBP in the opaque, but not in the clear lakes. As a major finding, and in agreement with our initial hypothesis, mixing and nutrient addition resulted in higher negative UVR effect on the bacterioplankton metabolism as compared to static conditions in all systems and, this was more accentuated in the opaque system where mixing and nutrients annulled the strong UVR stimulation of HBP in opaque lake; by contrast, in the clear systems these factors unmasked an inhibitory effect of UVR on HBP. These results have important implications, as explained in the following paragraphs:

Firstly, our results challenge the long-standing assumption that vertical mixing (at ambient nutrient condition) counteracts the UVR-induced damage in aquatic ecosystems. This assumption is based on the modulating effect that mixing exerts on the ratio of UVR: PAR received by organisms which, consequently, reduced the damage to repair ratio from the upper water layers to the deeper ones (Neale *et al.*, 2003). Our results contrast with those reported for bacteria in marine clear-waters of Mediterranean Sea, where mixing slightly reduced photoinhibition on HBP (Galí *et al.*, 2013). Likewise, our findings are opposed to previous results showing the effect of vertical mixing decreasing photoinhibition established for autotrophic organisms in clear high-mountain lakes (Helbling *et al.*, 2013, ; Carrillo et al. unpublished).

Secondly, our results do not fully support the paradigmatic proposal of DOC as the "ozone of the underwater world" (Williamson *et al.*, 2010) protecting against harmful UVR. Thus, fluctuating radiation due to mixing in the opaque lake was responsible for the largest UVR negative effect in

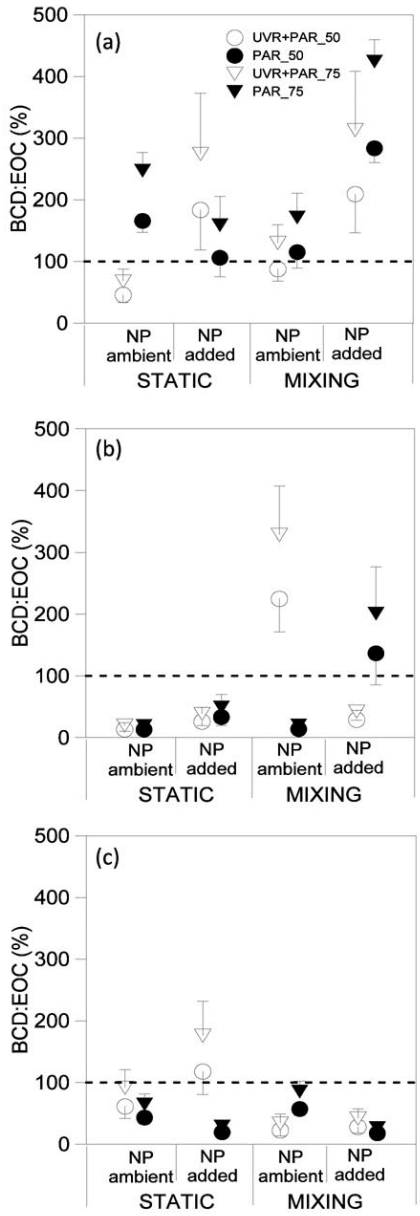
(a)
UVR+PAR_50
PAR_50
UVR+PAR_75
PAR_75
BCD:EOC (%)
500
400
300
200
100
0
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(b)
BCD:EOC (%)
500
400
300
200
100
0
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING
(c)
BCD:EOC (%)
500
400
300
200
100
0
NP ambient
NP added
NP ambient
NP added
STATIC
MIXING

Figura 2.8: Relationship between the bacterial carbon demand (BCD) and supply of carbon by algal excretion (EOC) as percentage, measured under the different radiation (UVR+PAR vs. PAR), nutrient concentration (ambient vs. nutrient added) and mixing regime (static vs. mixing) in Lake Enol (a), Las Yeguas (b) and La Caldera (c). A value of 100 % means a similar carbon demand and carbon supply. Triangles represent the mean value of %BCD:EOC (n=3) when considering bacterial respiration (BR) as 75 % of total planktonic respiration (TPR), whereas circles represent the mean value of %BCD:EOC (n=3) when considering BR as 50 % of TPR. The displayed vertical error bars embrace from the lowest error value for BR from 50 % to the highest error value for BR from 75 %. Error bars were calculated with error propagation.

the experiments (Fig. 2.3). This result is in agreement with previous studies that showed less acclimation of algae to cope with fluctuating UVR in high-DOC ecosystems (Villafañe *et al.*, 2004; Harrison y Smith, 2011; Helbling *et al.*, 2013). However, by assuming that mixed conditions represented the most realistic climate of light affecting HBP in each lake (Ruiz-González *et al.*, 2013), bacteria of the opaque lake were the most protected as no UVR inhibition was observed. In contrast, UVR under mixing inhibited HBP on the clear lakes. This finding in clear lakes was due to the increase in HBP under PAR rather than to a direct effect of UVR. This result suggests that fluctuating PAR could contribute to the enhancement of HBP through light-dependent mechanisms. Similar results were reported by Bertoni *et al.* (2011).

Thirdly, our findings reveal a non-generalized direct positive effect of P-pulse on the bacterial metabolism. Particularly for the opaque lake P-addition decreased HBP regardless the radiation quality or mixing regime. While literature indicates a widespread occurrence of P limitation of HBP in nutrient-limited waters (Bertoni *et al.*, 2011; Ogbebo y Ochs, 2008; Nelson y Carlson, 2011), we found that supplementation with P increased HBP

only in clear lakes. This result suggests that a resource as C released by autotrophs might be key in controlling bacteria growth under the joint action of UVR, mixing regime and nutrient-addition. In fact, a large number of studies have reported the bacterial dependence on EOC in oligotrophic ecosystems (Baines y Pace, 1991; Carrillo *et al.*, 2002; Medina-Sanchez *et al.*, 2002; Medina-Sánchez *et al.*, 2004; Morán *et al.*, 2002; Pugnetti *et al.*, 2010). The reduction of HBP after P addition, under mixing and UVR in the opaque lake is consistent with the higher metabolic cost due to respiration. The fact that EOC did not completely overcome C demands for growth strongly implicates that photosynthetic carbon acts as the main limiting resource for bacteria, despite the high DOC content in the lake, because of the reported preference of bacteria for the former (Medina-Sanchez *et al.*, 2002; Kritzberg *et al.*, 2005). Moreover, the absence of P stimulus in simultaneous primary production measurements carried out under identical experimental conditions (Helbling *et al.*, 2013) provides evidence to conclude that phytoplankton were not actively competing with bacteria for the available P. Thus, if P uptake by algae would have been higher than that of bacteria, supplementation with P in our experiments would have not decreased, or at the most not stimulated, HBP. A contrasting pattern was found for the clear lakes, where EOC satisfied BCD under the joint action of UVR, mixing regime and nutrient-addition. Thus, under UVR and fluctuating regime, P supplementation increased HBP, and these effects were concomitant with steady bacterial respiration.

Based on the results obtained in our model ecosystems, we propose a conceptual graphical model (Fig. 2.9) where we consider environmental drivers acting under ambient conditions and under global change predicted conditions (i.e. increasing nutrient inputs, DOM, wind forcing, temperature). The ecological impact of these drivers of global change is predicted to become more important affecting solar radiation penetration and mixing in aquatic systems (Caplanne y Laurion, 2008; Sarmento *et al.*, 2010). Our study indicates that this may cause alterations to the networks of biotic interactions by weakening the commensalistic relationship between algae and bacteria (i.e., %BCD:EOC) and accentuating bacterial limitation for the labile-C under predicted global change conditions (Fig. 2.9). Eventually

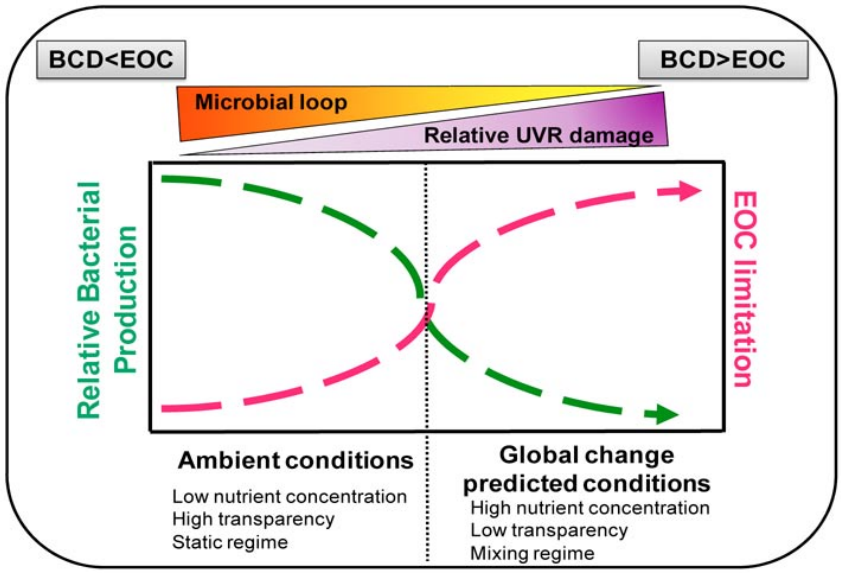

Figura 2.9: Conceptual graphical model of the interactive effects of increased inorganic nutrients, mixing regime and UVR transparency on the relative heterotrophic bacterial production (green line) and bacterial limitation by algal released C (red line). The green line represents the decreasing trend of HBP values under the interactive effects of the considered factors. BCD: bacterial carbon demand; EOC: excreted organic carbon.

bacteria vulnerability to UVR may affect the development of the microbial loop and particularly in the most opaque lakes.

Capítulo 3

Strength of the algal-bacterial relationship driven by UVR, nutrients, and temperature in high-mountain lakes of Sierra Nevada National Park: in situ experimental approach

3.1. Abstract

High-mountain lakes are increasingly considered ecosystems that are particularly sensitive to environmental perturbations and, hence, sentinels of global change. The aim of this study was to evaluate the interactive effect of ultraviolet radiation (UVR), nutrient inputs, and increased temperature (T) on phytoplankton and bacterioplankton metabolisms as well as on their trophic relationship. With this purpose, we performed short-term in situ experiments in two high-mountain lakes of Sierra Nevada, Spain, [La Caldera (LC) and Las Yeguas (LY)] with a 2×2×2 factorial design considering the following treatments: full sunlight (UVR+PAR) vs. UVR exclusion (PAR); ambient vs. nutrient addition; and ambient vs. increased T (T_+). Our results evidenced a higher sensitivity of bacterioplankton than phytoplankton since the joint action of increased T and nutrient-availability unmasked an inhibitory effect of UVR on heterotrophic bacterial production (HBP) without changes in its value; on the contrary, it reduced the inhibitory UVR effect on primary production (PP; in LC) or at least increased the PP value (in LY). Despite the similar response of phytoplankton and bacterioplankton from both lakes, the interaction of UVR, nutrient addition and T_+ had a different effect on algal-bacterial relationship, depending on the lake. Thus, in LC, the excretion of organic carbon by algae (EOC) was not adequate to meet the bacterial carbon demand (BCD), leading to an uncoupling in commensalistic algae-bacteria interaction. Contrarily, in LY, algal-bacterial coupling was accentuated by the interaction of UVR, nutrient-addition and T+, with EOC exceeding BCD. In a scenario of global change, these contrasting responses of algal-bacterial coupling might have important implications in microbial loop development, which might be reinforced in the warmer and less UVR-transparent lake (LY) but weakened in the colder and more UVR-transparent lake (LC). Climate-driven changes in water temperature and nutrient inputs may affect the strength of the algal-bacterial interaction with implications in the C-flux through food-web.

3.2. Introduction

High-mountain lakes are oligotrophic ecosystems naturally exposed to extreme conditions (e.g. high UVR fluxes, low temperature) but are concomitantly extremely sensitive to anthropogenic global change despite their relative isolation from human populations and their remoteness (Psenner, 2003; Nelson y Carlson, 2011). Hence, due to their particular geographic characteristics (e.g. high elevation, small catchment areas, etc.), high-mountain lakes constitute an excellent witness of global change and are increasingly being considered as excellent indicators of environmental change at the local and global scales (Catalan *et al.*, 2006; Parker *et al.*, 2008; Rose *et al.*, 2009, Chapter IV). However, high-mountain lakes may respond differently to environmental changes depending on catchment-area characteristics and because of their inherent physical and biological differences among lakes (Rose *et al.*, 2009).

Organisms in high-mountain lakes are per se subjected to a higher flux of ultraviolet radiation (UVR) than in other aquatic ecosystems because UVR increases with altitude (Blumthaler *et al.*, 1997). However, because of the decrease in the stratospheric ozone recorded since the1980s, a higher UVR flux is still reaching Earth's surface (Häder *et al.*, 2007; McKenzie *et al.*, 2011) and might accentuate the UVR effect on organisms in high-mountain lakes (Tucker y Williamson, 2011). Responses of planktonic organisms to the UVR range from inhibition to increased growth, and the intensity of the effects might also differ depending on organisms' metabolism (Jeffrey *et al.*, 1996; Perin y Lean, 2004; Sommaruga, 2003; Ogbebo y Ochs, 2008, Chapter IV). Thus, an inhibitory UVR effect has been reported in some studies on primary production (PP) and heterotrophic bacterial production (HBP) (Carrillo *et al.*, 2002; Conan *et al.*, 2008, Carrillo *et al.* submitted) in the upper water column, as well as damage to alkaline phosphatase activity (Korbee *et al.*, 2012; Tank *et al.*, 2005) and cell viability (Helbling *et al.*, 1995). However, lack or stimulatory effect of UVR on PP and HBP has been described by other authors (Aas *et al.*, 1996; Helbling *et al.*, 2001; Medina-Sanchez *et al.*, 2002; Helbling *et al.*, 2013, Chapter II, IV) These contrasting results might be the outcome of the UVR interaction with ot-

her environmental factors such as nutrient availability, temperature, and vertical mixing (Harrison *et al.*, 2009; Ruiz-González *et al.*, 2013).

Due to the evidence of current global warming (Stocker *et al.*, 2013), the influence of temperature (T) on planktonic response to UVR has not received attention until recently, with studies focused on indirect effects through an increase in the stability of thermal stratification or changes in water-mixing regime (Helbling *et al.*, 2013, Carrillo *et al.* submitted), as well as the direct effects of water heating on organisms facing UVR (Bullock y Jeffrey, 2010; Domaizon *et al.*, 2012; Fouilland *et al.*, 2013). The fact that temperature, contrary to UVR, decreases with elevation, makes high-mountain lakes likely to undergo some of the highest UVR:T ratios of any aquatic ecosystems (Williamson y Zagarese, 2003). The stimulatory effect of increased T on metabolic activity is well known (Sarmento *et al.*, 2010, and references therein), including molecular repair mechanisms for UVR damage (Hoffman *et al.*, 2003). However, although higher T has been found to alleviate photoinhibition of bacterial and phytoplanktonic community, e.g. in Antarctica and the Gulf of Mexico (Roos y Vincent, 1998; Doyle *et al.*, 2005; Bullock y Jeffrey, 2010), a rise in T in some oligotrophic ecosystems can prove negative for growth if basal metabolism costs rise whereas the energy or nutrient are not available to satisfy the demands (Degerman *et al.*, 2013, Chapter IV).

In the Mediterranean area, one of the most sensitive regions in the world to the effects of climate change (Giorgi y Lionello, 2008; Stocker *et al.*, 2013), the expected rise in T and higher frequency of extreme weather events, such as heat waves (Giorgi y Lionello, 2008), might have major consequences on UVR effects by changing the UVR:T ratio and thereby altering the ecosystem functioning. In addition, high-mountain lakes from the Mediterranean basin, due to their proximity to Saharan desert, are exposed to atmospheric inputs of mineral nutrient (Morales-Baquero *et al.*, 2006). This, together with land-use change and the predicted increase in droughts related to global change (Stocker *et al.*, 2013) might enhance the nutrient inputs in Mediterranean lakes. The inorganic nutrient influence altering the phyto- and bacterioplankton response to UVR has previously been reported in the literature (Medina-Sanchez *et al.*, 2002; Carrillo *et al.*,

2008b; Ogbebo y Ochs, 2008; Medina-Sánchez *et al.*, 2013).

However, beyond the effects of environmental changes on phyto- and bacterioplankton metabolisms, a more relevant issue is how those changes affect the relationship between these organisms, which is the basis of carbon flux in clear oligotrophic aquatic ecosystems (Morán y Alonso-Sáez, 2011; Medina-Sánchez *et al.*, 2004). In aquatic systems, a general bacterial dependence on algae through the excretion of photosynthetic carbon [EOC] has been described (Baines y Pace, 1991; Pugnetti *et al.*, 2010), this carbon source being preferred by bacteria even in lakes with considerable input of terrestrial carbon to subsidize their growth (Kritzberg *et al.*, 2005, 2006). Since UVR has been evidenced as an stimulator of EOC excretion as the result of the uncoupling between photosynthesis and algal growth (Carrillo *et al.*, 2008b; Korbee *et al.*, 2012), UVR might act by reinforcing a commensalistic algal-bacterial relationship (Carrillo *et al.*, 2002, Chapter I), increasing in the availability of the carbon released by phytoplankton to meet the bacterial carbon requirements (Morán *et al.*, 2002). The algal-bacterial commensalistic relationship and its strength is also related to other environmental factors, such as inorganic nutrient availability (Aota y Nakajima, 2001) or the vertical mixing regime (Chapter I, II). Thus, a higher nutrient availability might improve the coupling between photosynthesis and the algal growth, reducing algal C excretion (Berman-Frank y Dubinsky, 1999), which might constrain the C supply to satisfy the bacterial carbon demand.

With this background, the aim of the present study was threefold: first, to quantify the photoinhibition induced on phytoplanktonic and bacterial production by natural fluxes of solar UVR in different depths of water column. Second, we evaluate whether bacterial responses to UVR depend on the presence of algae. Third, our study assesses the interactive effect among UVR, nutrients, and T on phytoplanktonic and bacterial metabolism as well as on their commensalistic relationship. Our approach was to maintain organisms within a constrained depth subjected to high radiation to simulate that received by organisms trapped within a shallow upper mixed layer (UML) by strong stratification of water column. Therefore, we simulated an extreme condition of increased stratification due to global warming. We tested the hypothesis that, in the short-term, an increase in

T and nutrients availability will mitigate photoinhibition on phytoplankton and bacteria metabolism, and will uncouple algal-bacterial commensalistic relationship through the improvement of the coupling between photosynthesis and algal growth, resulting in shortages of organic C to satisfy BCD.

3.3. Methods

3.3.1. Study site

The experiments were performed in two model high-mountain lakes situated above the tree-line in Sierra Nevada National Park (Spain): La Caldera lake (hereafter LC) at an altitude of 3050 m.a.s.l. (37° 03' N; 3° 19' W) and Las Yeguas lake (hereafter LY) located at 2800 m.a.s.l. (37° 02' N; 3° 22' W). Both lakes have similar catchment areas (50 ha) (Morales-Baquero *et al.*, 1999), are oligotrophic lakes (total phosphorus [TP] <10 μg P L^{-1}, Chlorophyll a <5 μg L^{-1})(Reche *et al.*, 2001; Helbling *et al.*, 2013), and are highly transparent (Medina-Sánchez *et al.*, 2010) with a DOC concentration <50 μmol L^{-1} (Reche *et al.*, 2001). Both ecosystems undergo frequent inputs of atmospheric Saharan dust containing high P levels, with TN and TP ratio ranging from 10 to 50 in dust deposition (Morales-Baquero *et al.*, 2006).

3.3.2. Experimental design

UVR effect on algae and on bacteria in the presence vs. absence of algae at different depths

An experimental setup consisting in a 2×2 matrix (in LC) or 2×3 matrix (in LY) was designed in order to assess a potential shift in the UVR effect on primary production (PP) and heterotrophic bacterial production (HBP) with depth, i.e. the effect of the differential radiation reaching different layers of the lake. We performed a short-term experiment in LY and LC in August and September 2010, respectively. Two radiation treatments, full

sunlight (UVR+PAR, >280 nm; treatment UVR) and exclusion of UVR (>700 nm; treatment PAR), were considered at two lake depths (0.5 and 10 m) in LC and at three lake depths (0.5, 2, 7 m) in LY. For HBP, we also studied the bacterial response to UVR at different depths both in the presence and absence of algae with a factorial design 2×2×2 and 2×3×2 in LC and LY, respectively. Quartz flasks were used for the UVR treatments, whereas PAR treatments were applied using glass flasks covered with UV Opak 395 filter [Ultraphan, Difegra; the spectral characteristics of this filter are published elsewhere (Figueroa *et al.*, 1997). The treatments with the presence of algae were made by filtering water from the corresponding layer through 45-μm pore-size filter (for zooplankton removal) whereas the treatments with the absence of algae were made by filtering water through 1-μm pore-size filter. Water was collected from and incubated at the corresponding water-column depth.

Interactive effect of radiation, nutrients, and temperature on algae, bacteria, and their commensalistic relationship in the upper water layer

Short-term experiments to assess the combined effects of UVR, nutrient-addition, and increase in T on PP, HBP, and TPR were conducted in situ in each ecosystem in 2010 (August in LY and September in LC). The experiment in both ecosystems had a 2×2×2 factorial design (in triplicate for each treatment). Two nutrient conditions were implemented (i) ambient nutrient concentration (NP-ambient) and (ii) nutrient addition (NP-added). For each treatment, an integrated water sample was composed from equal volumes of water samples taken with an acid-cleaned 6-L horizontal Van Dorn sampler at three depths: upper and middle layers and at the bottom. The composite samples were pre-screened through a 45-μm mesh to remove zooplankton and then mixed in two acid-clean containers (6L). A container with no added nutrients served as control (NP-ambient) whereas the other one was nutrient-added with Phosphorus ([P] as Na_2HPO_4) to a final concentration of 30 μg P L^{-1}, and nitrogen ([N] as NO_3NH_4) to a final molar ratio of 30. In this way, we simulated the proportion of macronutrient input caused by pulses of Saharan dust, as previously shown by Morales-Baquero

et al. (2006).

Subsamples from each nutrient treatment were exposed to two radiation treatments (specified above UVR+PAR vs. PAR) and to two T treatments: (i) ambient temperature ($T_=$) and (ii) 5°C above ambient temperature (T_+). For this latter purpose, a set of flasks were incubated in situ in the lake as $T_=$ treatments (10°C in LC; 15°C in LY), and T_+ (15°C in LC; 20°C in LY) treatments were applied by using a thermostatically controlled bath on the lake shore. Water from the thermostatically controlled bath was constantly pumped to a container which had its interior painted black to prevent any light reflection and which was situated in a sunny location on the lake shore.

3.3.3. Physical analyses

Vertical profiles of solar radiation in the water column were determined at noon with a BIC compact 4-channel radiometer (Biospherical Instruments Inc., CA, USA), which had three channels in the UVR region of the spectra (305, 320, and 380 nm) and one broad-band channel for PAR (400-700 nm). Diffuse attenuation coefficients for downward irradiance (k_d) were calculated from the slope of the linear regression of natural logarithm of downwelling irradiance vs. depth for each wavelength range considered. A large sample size (pairs of irradiance and depth values, n>400) was used and a good fit (R^2 >0.95) was found for all regressions.

3.3.4. Sampling and analysis of biotic and abiotic structural variables

Water samples from different depths of the water column were collected in triplicate from both lakes to determine abiotic and biotic structural variables. Dissolved inorganic nitrogen (DIN) was considered the sum of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+), which were determined by UV-spectrophotometric techniques, sulfanilamide and phenol-hypochlorite techniques, respectively (APHA, 1992). Total phosphorus (TP) and reactive soluble phosphorus (SRP) were measured by analy-

sing 50 mL aliquots with the acid molybdate technique after persulfate digestion (APHA, 1992). In order to determine sestonic N and sestonic P, 500 mL or 1L, respectively, were filtered through pre-combusted (1 h at 550°C) 1.0-μm glass-fibre filters (Whatman GF/B) at low pressure (<100 mm Hg). Filters containing sestonic N were dried (24 h at 60°C) and kept desiccated until N analysis using a Perkin-Elmer model 2400 CHN elemental analyzer (Perkin-Elmer Corporation, Waltham, Massachusetts, USA).

Filters for sestonic P were analysed following the method described for TP. Blanks and standards were performed in all procedures. The sestonic N:P ratio was calculated on a molar basis. DOC values were determined by filtering the samples through pre-combusted (2 h at 500°C) glass-fibre filters (Whatman GF/F) and acidifying them with HCL. Samples were then measured in a total organic carbon analyser (TOC-V CSH/CSN Shimadzu).

For the assessment of the Chl *a* concentration, 0.5-1 L of water from each layer of the water column considered were filtered onto Whatman GF/F filters (25 mm in diameter) and frozen at -20°C until analysed. Subsequently, filters were thawed and placed in centrifuge tubes (15 mL) with 5 mL of acetone (90 %) for 24 h in darkness at 4°C. Then, the samples were centrifuged, and the fluorescence of the supernatant was measured with a fluorimeter (LS 55 Perkin Elmer, USA). A Chl *a* standard (Chl *a* from spinach, Sigma) was used to transform the fluorescence data into Chl *a* concentrations.

Samples for identification and enumeration of phytoplankton were preserved in 250-mL brown glass bottles containing Lugol alkaline solution (1 % vol vol^{-1}). A volume of 50 mL was allowed to settle for 48 h in Uthermol chambers (Hydro-Bios GmbH, Germany) and species were counted and identified using an inverted microscope (Leitz Fluovert FS, Leica, Wetzlar, Germany). Phytoplankton biovolumes were estimated from measurements of 10-20 cells of each species using an image analysis (Inverted microscope Axio Observer A1, Carl Zeiss – High-resolution microscopy camera Axiocam HRc, Carl Zeiss). Biovolumes were calculated according to Carrillo *et al.* (1995) and cell volume was converted to phytoplankton carbon using the conversion factors reported by Rocha y Duncan (1985).

Bacterial abundance (BA) was determined by a flow-cytometry techni-

que (FACScanto II, Becton Dickinson Biosciences, Oxford, UK) from samples of water (three replicates and two controls for each considered stratum of the water column) fixed with 1 % paraformaldehyde and stained with SYBER Green I DNA stain (Sigma-Aldrich) to a 1:5000 final dilution of initial stock (Zubkov *et al.*, 2007). Stained microbial cells were discriminated on bivariate plots of particle side scatter vs. green fluorescence. Yellow-green 1-μm beads (Fluoresbrite Microparticles, Polysciences, Warrington, PA, USA) were used as an internal standard of particle concentration and fluorescence (Zubkov y Burkill, 2006; Zubkov *et al.*, 2007).

3.3.5. Biotic functional variables

For primary production (PP) measurements, sets of 50-mL flasks (three clear and one dark for each experimental treatment) received 0.37 MBq of $NaH^{14}CO_3$ [SA: 310.8 MBq mmol^{-1}, DHI] and incubated in situ for 4 hours at midday (10:00 to 14:00 h), at the same depth where the water had been collected. All flask sets were horizontally held during the incubations. PP calculations were based on the ^{14}C method (Lignell, 1990). In brief, total organic carbon (TOC) was measured in 4-mL subsamples collected before filtration. To determine the ^{14}C retained in phytoplankton (PP), we filtered the samples through 1-μm Nucleopore filters (25 mm diameter). Low pressure (<100 mmHg) was applied in order to minimize cell breakage (more details on laboratory procedure in Carrillo *et al.* (2002). Excreted organic carbon (EOC) was measured in 4mL subsamples collected from the filtrates <1μm. The 4mL aliquots for TOC and EOC determination, as well as filters for PP determination, were put into scintillation vials, and inorganic carbon was removed by adding 100μL of 1N HCl and allowing the vial to stand open in a hood for 24h. After acidification, scintillation cocktail (Ecoscint A) was added to all the samples. The amount of carbon was determined from the desintegrations per min (dpm), counting with a scintillation counter equipped with autocalibration (Beckman LS 6000TA). In all calculations, dark values were subtracted from corresponding light values. The percentage of excreted organic carbon (%EOC) was calculated as:

$$\%\text{EOC} = EOC \times TOC^{-1} \times 100 \quad (3.1)$$

Samples for heterotrophic bacterial production (HBP) measurements were placed in 10-mL quartz flasks (three replicates and two blanks for each experimental treatment). The flasks for radiation× nutrients×T experiment were exposed in situ at 0.5 m for 3 h under the corresponding treatment prior to the radiotracer addition. HBP was determined by incorporating ^{3}H-thymidine (S.A= 46.5 Ci mmol^{-1}, Amershan Pharmacia) into the bacterial DNA [hereafter BPT] (Fuhrman y Azam, 1982; Smith y Azam, 1992). HBP was also quantified as the direct production of carbon by using ^{3}H-leucine (S.A= 60 Ci mmol^{-1},Amershan Pharmacia) to measure protein synthesis [hereafter BPL] (Simon y Azam, 1989), the main component of bacterial biomass. ^{3}H-thymidine or ^{3}H-leucine was added to each experimental flask to a final saturating concentration of 16.6 nM or 40.06 nM for ^{3}H-thymidine and ^{3}H-leucine, respectively. Vials with the radiotracer, incubated for one hour in situ at the same depth where the water was collected, were subjected to the different treatments. All flask sets were horizontally held during the entire incubation period (1h with radiotracer for the depth profile experiment; 3h without radiotracer followed by 1h with radiotracer for the radiation×nutrients×T experiment), symmetrically distributed around noon.

After incubation, the incorporation of ^{3}H-thymidine or ^{3}H-leucine was stopped with 5 % (final concentration, f.c.) tricholoracetic acid (TCA). Likewise, blanks were TCA-killed before the radiotracer was added. Extraction was performed by cold TCA (5 % f.c.) keeping the vials in ice for 20 min, after which the precipitate was collected by centrifugation (1600 g for 10 min). Then, vials were rinsed twice with 1.5 mL of TCA (5 % f.c.) to remove any residual unincorporated radioactivity. Finally, scintillation liquid (Ecoscint A) was added for subsequent measurement in an autocalibrated scintillation counter (Beckman LS 6000TA).

The conversion factor 1×10^{18} cell mol^{-1} (Bell, 1993) was used to estimate the number of bacteria produced per mol of incorporated thymidine. The factor 2×10^{-14} g C cell^{-1} (Lee y Fuhrman, 1987) was applied to estimate

the amount of carbon. In order to convert the incorporated ^{3}H-leucine into carbon, the conversion factor 1.55 Kg C mol^{-1} (Simon y Azam, 1989) was used. The different responses of BPT and BPL to the considered abiotic factors and their interaction were integrated as the BPL:BPT ratio.

Bacterial respiration (BR) was estimated from total planktonic respiration (TPR) and was used for calculating the bacterial carbon demand (BCD) as HBP plus BR. TPR was measured in 10-mL flasks by using optode sensor-spots (SP-PSt3-NAU-D5-YOP; PreSens GmbH, Germany) and an optic-fibre oxygen transmitter (Fibox 3; PreSens GmbH, Germany) connected to a computer. The consumption rates of oxygen (μM O_2 h^{-1}) were converted into carbon units by using a respiratory quotient of 1 (del Giorgio y Cole, 1998). To estimate BR from TPR, we used a conservative value of 75 % of TPR, (an average value based on data reported for oligotrophic waters (Lemee et al. 2002); and a potential minimum value of 50 % of TPR (Robinson, 2008). The last percentage is comparable with direct measurements made in a previous study on the TPR vs. BR in LC (Herrera *et al.* unpublished). Applying these two percentages, we established a potential minimum and maximum value of BR. In turn, we used these two BR values to calculate %BCD:EOC, getting two extreme values, which we considered to be the highest and lowest values of a potential range of values. The deviation standard was calculated for %BCD:EOC by using error propagation. The %BCD:EOC was calculated with BPT and BPL.

3.3.6. Statistical analyses

The inhibition by UVR as a percentage (UVR_{inh}) of the different functional variables was calculated as:

$$UVR_{inh}(\%) = ((PAR - UVR)/PAR) \times 100 \tag{3.2}$$

where PAR and UVR represent the mean values in the absence (PAR) or presence (UVR) of ultraviolet radiation for each nutrient or nutrient × temperature treatment. We used propagation errors to calculate the variance in inhibition due to UVR and PAR. The differences among treatments for %UVR_{inh} were evaluated by a t-test.

The UVR effect on PP with depth was tested using a two-way ANOVA. The effect of algae removal on HBP under both radiation treatments at different depths, as well as the interactive effect of radiation × nutrient × T on all functional variables, were tested by a three-way ANOVA. LSD post hoc test was used to determine significant differences between treatments. Data were checked for normal distribution with Kolmorov-Smirnov test and homoscedasticity was verified with Cochran's and Levene's tests. The data were log-transformed or exponentially transformed when these conditions were not met. Statistica 7.0 for Windows (Statsoft 2001) was used for the statistical analysis.

3.4. Results

3.4.1. Physical, chemical, and biological characterization of the water column

During the experiments, both lakes received a high flux of incident UVR (clear sky)., Despite that UVR reached the bottom in both lakes, $k_{d\ 320}$ values in LY doubled those of LC (Fig. 3.1). By contrast, the temperature had a homogeneous vertical profile (∼10°C in LC and ∼15°C in LY) in both lakes (Table 3.1). Also, sestonic N:P and DIN:TP ratios showed a uniform vertical profile in both lakes, yielding high values (DIN:TP>12 by weight, sensu Morris and Lewis 1988) although higher in LC than LY (Table 3.1). Similarly, BA and PA showed similar values all along the water column (Table 3.1). In both lakes, Chlorophyta was the dominant group (≈60-70 % of total PA in LC and >60 % in LY), followed by Chrysophyta in LC (≈30-40 % of total PA) whereas in LY the remaining groups (Chrysophyta, Cryptophyta, Bacillariophyta) showed similar abundance among them.

Despite the homogeneous vertical profile of physical-chemical and structural variables, UVR had different effects on PP, %EOC, and BPT depending on the lake and depth (Fig. 3.2). Thus, in LC, there was interactive radiation × depth effect on PP but not on %EOC, with a higher inhibitory UVR effect on PP only in the upper layer (Fig. 3.2a), whereas under UVR the %EOC increased regardless of the depth (Fig 3.2a). In LY, radiation ×

Variable	La Caldera (September)	Las Yeguas (August)
TN (μmol L^{-1})	14.7 (9.2-16.6)	10.3 (8.51-12.48)
TP (μmol L^{-1})	0.12 (0.08-0.20)	0.10 (0.07-0.15)
SRP (μmol L^{-1})	0.042 (0.031-0.048)	0.046 (0.039-0.057)
DIN (μmol L^{-1})	8.44 (8.08-8.64)	8.50 (8.08-9.15)
DIN:TP (μmol)	90 (42-162)	87 (56-129)
DOC (μmol L^{-1})	1.078 (0.998-1.158)	1.01 (0.9-1.12)
Chl *a* (μg L^{-1})	2.55 (2.09-2.98)	2.83 (1.97-4.46)
$N:P_{sestonic}$	44.1 (33-63)	54 (42-63)
PA (cell mL^{-1})	867 (1344-584)	738 (441-1307)
PB (μg C L^{-1})	3.6 (2.3-5.7)	5.10 (2.8-7.8)
BA (cell mL^{-1})$\times 10^5$	2.57 (0.34-4.13)	2.9 (2.37-3.44)
T (°C)	10.0 (10.2-9.8)	14.8 (15.2-14.3)

Cuadro 3.1: Mean values of the main physical, chemical and biological variables of the studied lakes during the periods of the experiments. Number in brackets shows the range values in the water column of each period in La Caldera and Las Yeguas. Total nitrogen (TN); total phosphorus (TP); soluble reactive phosphorus (SRP); dissolved inorganic nitrogen (DIN); DIN:TP ratio expressed by weight; dissolved organic carbon (DOC); phytoplanktonic abundance (PA); phytoplankton biomass (PB); bacterial abundance (BA); temperature (T).

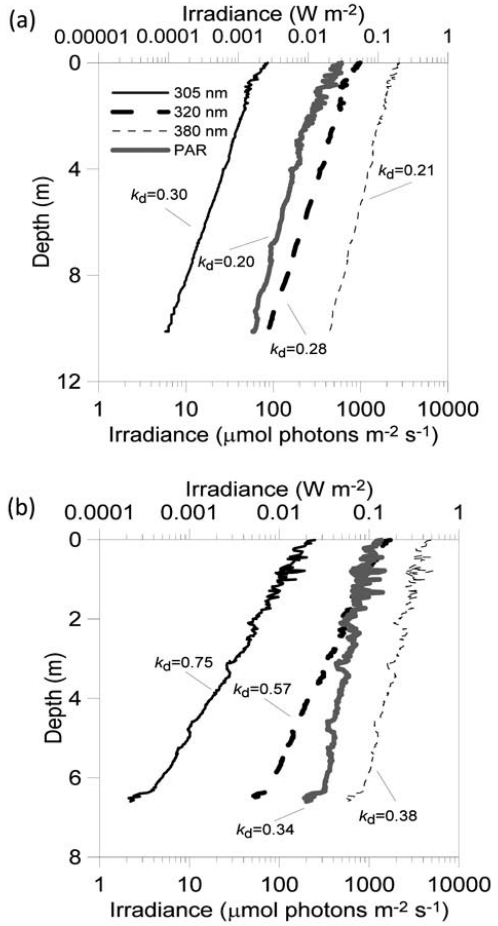

Figura 3.1: Solar irradiance as a function of depth in La Caldera (a) and in Las Yeguas (b). Irradiance data in the UVR range are expressed in W m^{-2} and PAR is in μmol photons m-2 s^{-1}. The diffuse attenuation coefficients (k_d) are also shown.

				La Caldera		Las Yeguas	
		df1	df2	$F_{df1,df2}$	p	$F_{df1,df2}$	p
PP							
	Depth	1	8	0.02	0.892	24.56	**0.000**
	Radiation	1	8	16.44	**0.004**	15.27	**0.001**
	Depth×Radiation	1	8	11.67	**0.009**	0.54	0.664
%EOC							
	Depth	1	8	0.97	0.354	42.06	**0.000**
	Radiation	1	8	46.76	**0.000**	436.00	**0.000**
	Depth×Radiation	1	8	4.84	0.059	25.76	**0.000**
BPT							
	Depth	1	16	22.68	**0.000**	285.27	**0.000**
	Algae	1	16	26.07	**0.000**	2078.97	**0.000**
	Depth×Algae	1	16	0.53	0.477	169.46	**0.000**
	Depth×Radiation	1	16	27.09	**0.000**	37.13	**0.000**
	Algae×Radiation	1	16	1.13	0.304	11.18	**0.003**
	Depth×Radiation×Algae	1	16	6.15	**0.025**	114.80	**0.000**

Cuadro 3.2: Results from the two-way analysis of variance (ANOVA) of the interactive effect of radiation and depth for primary production (PP) and percentage of EOC (%) and from the three-way ANOVA of the interactive effect of radiation, depth and presence of algae for bacterial production determined by incorporating ^{3}H-thymidine (BPT). Numbers in bold indicate significant effect on the considered variable.

depth exerted no effect on PP (Table 3.2), nor did UVR significantly affect PP at any depth (Fig. 3.2b). However, radiation and depth interacted significantly on %EOC while UVR diminished the %EOC at all the depths (Fig. 3.2b).

In both lakes, radiation × depth × algae exerted a significant effect on BPT (Table 3.2). In the presence of algae, UVR decreased BPT in the upper layer in LC and upper and middle layers in LY (Fig. 3.2c, d). In the absence of algae, BPT values decreased in both ecosystems regardless of the radiation treatments and depth, supporting a bacterial dependence on phytoplankton. The absence of algae only modified the UVR effect on BPT

in LY, (Fig. 3.2c, d), which disappeared in the upper and middle layers and was inhibitory at the bottom (Fig. 3.2c, d).

3.4.2. The interactive effect of solar radiation, nutrient input, and temperature increase on autotrophic C - incorporation and excreted organic carbon

In both lakes, radiation, nutrients, and temperature significantly interacted on PP, EOC and %EOC (Table 3.3). In LC, under $T_=$, samples exposed to UVR had significantly lower PP values than did those exposed only to PAR under both nutrient conditions (Fig. 3.3a), prompting an UVR inhibitory effect (Table 3.4). Under UVR, T_+ stimulated PP, regardless the nutrient treatment, as compared to $T_=$ treatments (Fig. 3.3a), resulting in a decrease in $\%UVR_{inh}$ (Table 3.4). In LY, UVR decreased PP values compared to PAR treatments (Fig. 3.3b) leading to an inhibitory UVR effect under all experimental conditions (Table 3.4). Under ambient nutrient conditions, T_+ increased PP in PAR treatment (Fig 3.3b), accentuating the inhibitory effect of UVR compared with $T_=$ conditions (Table 3.4). With T_+ and UVR, nutrient addition stimulated PP as compared to samples with ambient nutrient (Fig.3.3b).

For EOC, under $T_=$, samples exposed to UVR had significantly lower EOC values than did those exposed only to PAR in both lakes (Fig. 3.3c, d; Table 3.4). In LC, under $T_=$, nutrient addition reduced EOC values compared with ambient nutrient samples regardless the radiation treatment, whereas under T_+, nutrient addition decreased the EOC value under UVR but increased it under PAR (Fig. 3.3c). Thus, under T_+, nutrient addition resulted in an UVR inhibitory effect on EOC (Table 3.4). By contrast, in LY, under T_+, UVR raised EOC values compared with T= conditions, regardless nutrient treatment (Fig. 3.3d), but causing either a stimulatory UVR effect under ambient nutrient conditions or eliminating the UVR inhibitory effect generated by nutrient addition under $T_=$ (Table 3.4).

In relation to %EOC, higher values were found in LC than in LY, particularly under ambient nutrient conditions where samples exposed to UVR reached values of 62 % or 97 % under $T_=$ and T_+, respectively (Fig. 3.3e).

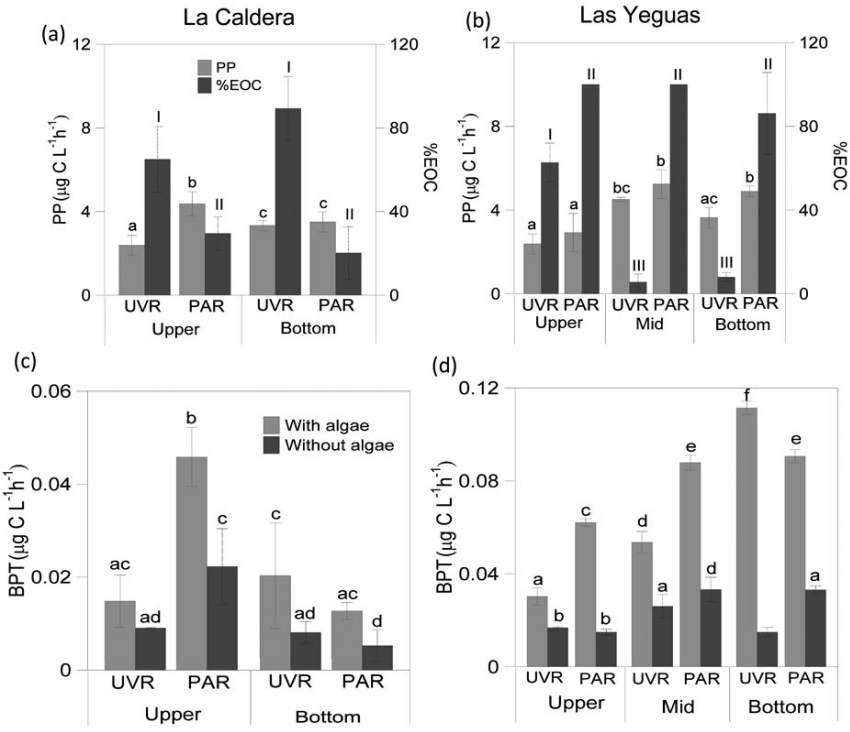

Figura 3.2: Rate of primary production (PP; in μg C L^{-1} h^{-1}) and percentage of excreted organic carbon (%EOC) in La Caldera (LC; a) and Las Yeguas (LY; b) under different radiation (UVR vs. PAR alone) at different water column depth. Rate of heterotrophic bacterial production determined by incorporating ^{3}H-thymidine (BPT; in μg C L^{-1} h^{-1}) in LC (c) and LY (d) under different radiation (see above) at different water depth and in present vs. absence of algae. Bars represent the mean PP and BPT values and error bars represent the standard deviation (SD; n=3). Letters indicate significant differences among treatments.

		df1	df2	PP		EOC		%EOC		BPT		BPL		BPL:BPT		BR	
				$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p	$F_{df1,df2}$	p
La Caldera																	
	Radiation	1	16	278.18	**0.000**	94.61	**0.000**	47.96	**0.000**	10.68	**0.004**	2.30	0.149	11.63	**0.003**	1.09	0.313
	Nutrients	1	16	7.58	**0.01**	19.23	**0.000**	93.08	**0.000**	0.17	0.683	9.12	**0.008**	11.07	**0.004**	0.01	0.929
	T	1	16	44.73	**0.000**	24.79	**0.000**	1.68	0.214	4.39	0.052	3.47	0.081	0.08	0.775	0.38	0.547
	T×Radiation	1	16	29.34	**0.000**	11.21	**0.004**	0.31	0.588	1.25	0.279	23.96	**0.000**	18.87	**0.001**	0.00	0.983
	T×Nutrients	1	16	2.35	0.145	4.38	0.053	17.40	**0.001**	14.51	**0.002**	17.90	**0.001**	46.28	**0.000**	0.16	0.699
	Radiation×Nutrients	1	16	4.03	0.062	34.70	**0.000**	89.43	**0.000**	3.66	0.074	0.07	0.797	1.63	0.219	2.63	0.124
	Radiation×Nutrients×T	1	16	5.91	**0.020**	31.20	**0.000**	33.01	**0.000**	0.12	0.736	26.85	**0.000**	19.73	**0.000**	0.47	0.502
Las Yeguas																	
	Radiation	1	16	92.83	**0.000**	3.59	0.076	0.03	0.875	15.59	**0.001**	3.33	0.087	9.34	**0.007**	0.00	0.970
	Nutrients	1	16	23.41	**0.000**	0.26	0.619	3.56	0.077	5.82	**0.028**	0.43	0.521	5.63	**0.030**	0.07	0.793
	T	1	16	35.17	**0.000**	78.74	**0.000**	2.23	0.155	17.60	**0.001**	33.03	**0.000**	0.97	0.339	5.65	**0.030**
	T×Nutrients	1	16	1.16	0.297	10.18	**0.006**	3.21	0.092	4.83	**0.043**	1.04	0.323	4.84	**0.043**	1.73	0.207
	Radiation×Nutrients	1	16	0.00	0.976	42.04	**0.000**	4.07	0.061	5.71	**0.029**	0.46	0.506	5.78	**0.029**	0.00	0.972
	Radiation×Nutrients×T	1	16	8.19	**0.011**	39.08	**0.000**	7.34	**0.015**	3.56	0.077	0.08	0.783	5.41	**0.033**	0.23	0.636

Cuadro 3.3: Result of the three-way analysis of variance (ANOVA) of the interactive effect of radiation, P-addition, and temperature (T). Numbers in bold indicate significant effect on the considered variable. PP: primary production; EOC: excreted organic carbon; %EOC: percentage of excreted organic carbon; BPT: heterotrophic bacterial production determined by incorporating ^{3}H-thymidine; BPL: heterotrophic bacterial production determined by incorporating ^{3}H-leucine; BPL:BPT: ratio between BPL and BPT; BR: bacterial respiration.

In LC, under $T_{=}$ and ambient nutrient conditions, samples exposed to UVR resulted in higher %EOC values than in samples exposed only to PAR (Fig. 3.3e). Under UVR, nutrient addition decreased %EOC in both temperature treatments as compared to values registered without nutrient addition (Fig. 3.3e), whereas T_{+} increased %EOC under ambient nutrient conditions as compared with $T_{=}$ (Fig. 3.3e). In LY, under T_{+} and nutrient-added conditions, UVR lowered %EOC in comparison with $T_{=}$ (Fig. 3.3f), resulting in an inhibitory UVR effect (Table 3.4).

3.4.3. Interactive effect of solar radiation, nutrient input and increase in temperature on HBP and on BR

No interactive effect of radiation, nutrients, and temperature on BPT were found in any ecosystem, but appeared only in BPL in LC (Table 3.3). Under $T_{=}$ and ambient nutrient conditions, UVR did not significantly affect BPT or BPL in any ecosystem compared with PAR treatments (Table 3.4). In LC, nutrient addition reduced BPT values under UVR and $T_{=}$ but increased them under PAR and T_{+} in comparison with ambient nutrient conditions (Fig. 3.4a). Hence, nutrient addition resulted in a UVR inhibitory effect on BPT under both T treatments (Table 3.4). In LY, under $T_{=}$, UVR slightly reduced BPT after nutrient addition whereas this effect was highly significant under T_{+}, in both cases as compared with the treatments without nutrient addition (Fig. 3.4b).This, as in LC, led to a

	La Caldera						
Treatment	PP	EOC	%EOC	BPT	BPL	BPL:BPT	BR
UVR	72^{a}	32^{a}	-79^{a}	ns	ns	ns	ns
UVR×NP-added	80^{a}	53^{a}	ns	45^{a}	-265^{b}	-574	ns
UVR×T_{+}	39^{b}	ns	-204^{b}	ns	ns	ns	ns
UVR×NP-added×T_{+}	35^{b}	86^{b}	60^{c}	42^{a}	73^{c}	ns	ns

	Las Yeguas						
Treatment	PP	EOC	%EOC	BPT	BPL	BPL:BPT	BR
UVR	36^{a}	47^{a}	ns	ns	ns	ns	ns
UVR×NP-added	52^{ab}	38^{a}	ns	52^{a}	ns	ns	ns
UVR×T_{+}	62^{b}	-229^{b}	ns	ns	ns	ns	ns
UVR×NP-added×T_{+}	33^{a}	ns	37^{a}	59^{a}	ns	-109	ns

Cuadro 3.4: Percentage of UVR inhibition (UVR_{inh}) on phytoplanktonic and bacterial variables under the indicated treatments. Different superscript letters indicate significant differences based on t-test among the different treatments. ns: not significant, indicates that differences between UVR and equivalent PAR treatment for each nutrient or nutrient×temperature treatment were not found (LSD-test>0.05). PP: primary production; EOC: excreted organic carbon; %EOC: percentage of excreted organic carbon; BPT: heterotrophic bacterial production determined by incorporating ^{3}H-thymidine; BPL: heterotrophic bacterial production determined by incorporating ^{3}H-leucine; BPL:BPT: ratio between BPL and BPT; BR: bacterial respiration.

inhibitory UVR effect under nutrient addition (Table 3.4).

Regarding BPL, in LC under $T_=$ conditions, nutrient addition increased BPL values under UVR compared with ambient nutrient conditions (Fig. 3.4c), leading to a stimulatory UVR effect (Table 3.4). By contrast, under T_+, nutrient addition reduced BPL under UVR (Fig. 3.4c), resulting in an UVR inhibitory effect (Table 3.4). In LY, T_+ had a stimulatory effect on BPL under ambient nutrient conditions regardless the radiation treatment as compared with $T_=$ treatments (Fig. 3.4d). Nutrients or radiation did not have any significant effect (Fig. 3.4d).

Because of the partially different responses of BPT and BPL to each experimental treatment, we calculated BPL:BPT ratio to integrate the bacterial response to each treatment. The BPL:BPT ratio under UVR, $T_=$ and ambient nutrient conditions had a mean value of 1 in LC and 11 in LY (Fig. 3.4e, f). In LC, the opposite effect of nutrient-addition on BPT and BPL under UVR and $T_=$ resulted in a significant increase of 11-fold comparing with ambient nutrient conditions (Fig. 3.4e), which generated a stimulatory UVR effect on this ratio (Table 3.4). Moreover, under UVR and ambient nutrient conditions, T_+ also increased the BPL:BPT ratio by 3-fold compared with the $T_=$ treatment (Fig. 3.4e). On the other hand, in LY, under UVR, the BPL:BPT ratio was modified only by the combined effect of nutrient-addition and T_+, which increased the ratio value in 1.6-fold in comparison to ambient nutrient and $T_=$ conditions (Fig. 3.4f) and triggered a stimulatory UVR effect on this ratio (Table 3.4).

With regard to BR, no significant interaction was found among radiation, nutrients, and temperature in any ecosystem (Table 3.3). In addition, no differences were found between treatments in the two ecosystem (Fig. 3.5) with the mean value ranging from 1.42 (50 % of TPR) to 2.14 $\times$ gC $L^{-1}h^{-1}$ (75 % of TPR) in LC (Fig. 3.5a) and 2.32 (50 % of TPR) to 3.45 $\times$ gC $L^{-1}h^{-1}$ (75 % of TPR) in LY (Fig. 3.5b).

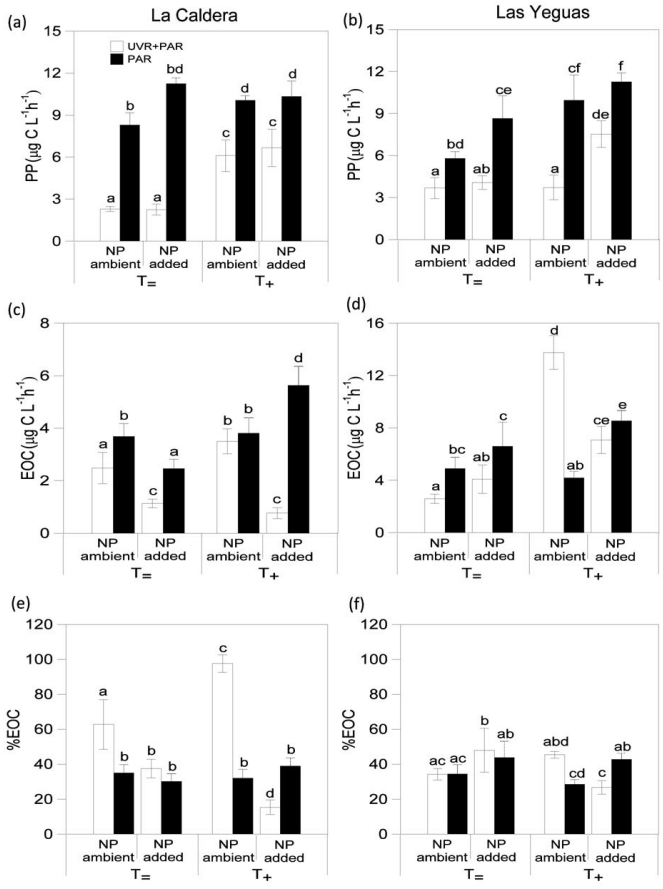

Figura 3.3: Rate of primary production (PP; in μg C L^{-1} h^{-1}), excreted organic carbon (EOC; μg C L^{-1} h^{-1}) and percentage of EOC (%EOC; as percentage) under different radiation (UVR+PAR vs. PAR), nutrient concentration (ambient vs. nutrient added) and temperature (ambient vs. increased) conditions in La Caldera (a, c, e) and Las Yeguas (b, d, f). Bars represent the mean PP, EOC and %EOC values and error bars represent the standard deviation (SD; n=3). Letters indicate significant differences among treatments.

3.4.4. Interactive effect of solar radiation, nutrient input, and increase in temperature on the strength of algal-bacterial relationship

Since HBP values decreased in absence of algae, we can assume a bacterial dependence on algae in both lakes (see above); therefore, we quantified the strength of algae-bacterioplankton coupling, i.e. the capacity of the carbon (C) released by algae to meet the bacterial carbon requirement demands (BCD). We calculated BCD from both BPT and BPL and compared it to EOC rates, through the BCD:EOC ratio (as a percentage). Significant differences were not found between %BCD:EOC calculated by using BPT or BPL under each experimental condition in either lake, and for this reason only data of %BCD:EOC calculated with BPT are shown in Fig. 3.6. Our findings showed that algae provided enough EOC to meet the bacterial demands, i.e. %BCD:EOC ratio<100 (Fig. 3.6a) in all treatments except those that were subjected to joint UVR and P-enrichment (in LC) or that represent the ambient control in LY (Fig. 3.6b).

3.5. Discussion

This study, examining the increasing demand for in situ experimentation to test the multiple-stressor effect on key processes on ecosystems, reports on the way in which biotic interactions such as the commensalistic algal-bacterial relationship respond to the joint impact of UVR, increased nutrient availability, and increased temperature in current and expected future scenarios of global change. High-mountain lakes have been described as witness ecosystems to environmental changes (Catalan *et al.*, 2006; Parker *et al.*, 2008). Two model ecosystems in Sierra Nevada National Park that differed mainly in the mean temperature of the water column and in their transparency to UVR were selected in order to provide a framework for evaluating the sensitivity of primary producers and decomposers in the water column. Also, we quantified the nature and magnitude of the interactive effect of multiple stressors on their metabolic activity, which had implications in determining the C flux through the microbial food web in

aquatic ecosystems.

3.5.1. Sensitiveness of algae and bacteria to UVR. Role of C released by algae

Under ambient conditions (depth profile experiments) phytoplankton was more susceptible to UVR effect in LC than in LY, particularly in the upper water layer, where %UVR_{inh} of PP duplicated that of LY. This higher sensitivity was reflected in higher %EOC values under UVR, supporting %EOC as a physiological stress indicator in ecosystems with high transparency to UVR (Carrillo *et al.*, 2008b; Korbee *et al.*, 2012). However, bacterioplankton did not differ between lakes in the response to UVR, and the removal of algae resulted in a noteworthy decrease in HBP, this being consistent with the findings of Aas *et al.* (1996) and Sommaruga *et al.* (1997), and supporting a bacterial dependence on algae (Carrillo *et al.*, 2002; Medina-Sanchez *et al.*, 2002). Notably, in LY in absence of algae, the bacterial sensitivity to UVR was masked in upper layers, probably as result of a stronger C limitation to growth than in LC, in line with the %BCD:EOC ratio (see below).

3.5.2. Modulation of sensitiveness of algae and bacteria to UVR by nutrient addition and warming. Propagation to algal-bacterial interaction.

Our results show a higher magnitude of response of algae and bacteria to UVR with increased nutrient availability and increased temperature in the colder and higher UVR-transparent lake (LC) than in the warmer and less UVR-transparent lake (LY). Thus, in LC, the joint action of nutrients and warming alleviated the strong negative UVR effect on PP, but, contrarily, triggered a negative UVR effect on HBP (with both tracers) which was partially modulated by indirect effects through competitive and commensalistic interactions between algae-bacteria. The boost in metabolic algal activity by the combined effects of nutrient and warming under UVR was evidenced by the increase in the net PP value and the reduction

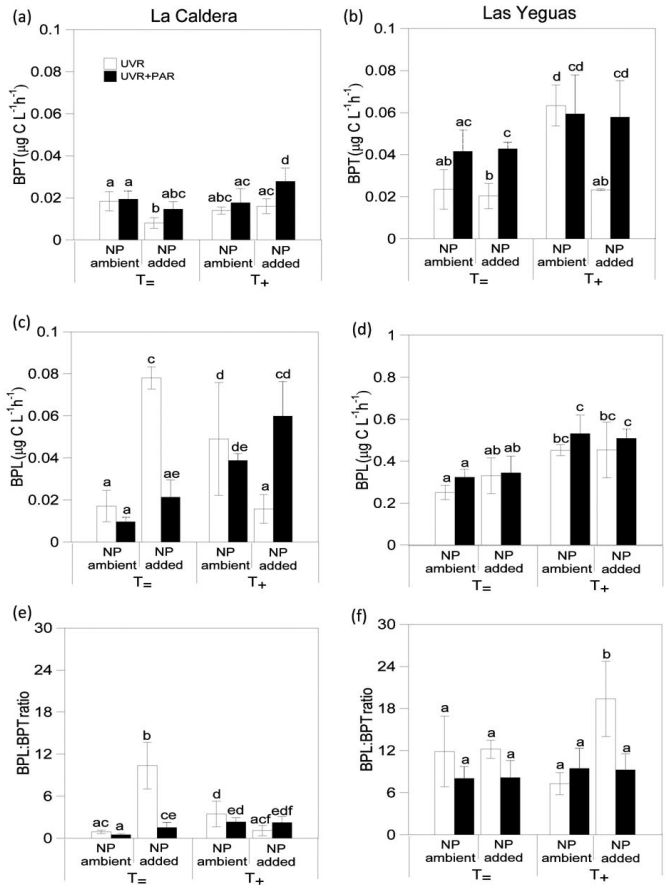

Figura 3.4: Rate of heterotrophic bacterial production determined by incorporating ^{3}H-thymidine (BPT; in μg C L^{-1} h^{-1}) and by incorporating ^{3}H-leucine (BPL; in μg C L^{-1} h^{-1}) and BPL:BPT ratio under different radiation (UVR+PAR vs. PAR), nutrient concentration (ambient vs. nutrient added) and temperature (ambient vs. increased) conditions in La Caldera (a, c, e) and Las Yeguas (b, d, f). Bars represent the mean BPT, BPL and BPT:BPL values and error bars represent the standard deviation (SD; n=3). Letters indicate significant differences among treatments.

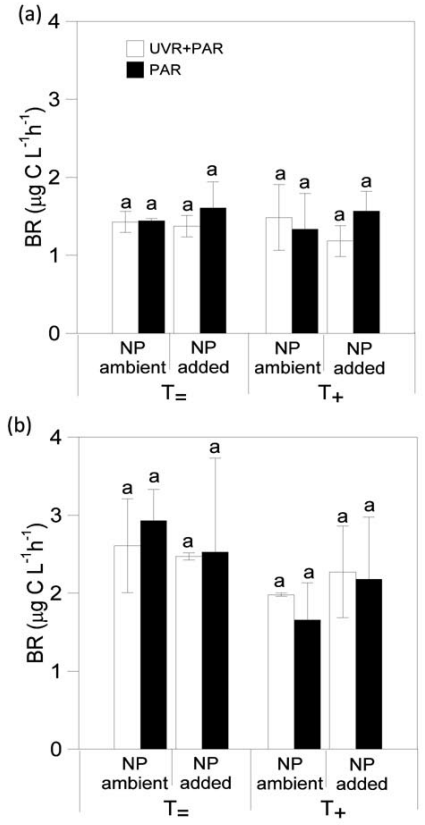

Figura 3.5: Bacterial respiration (BR; in μg C L^{-1} h^{-1}) under different radiation (UVR+PAR vs. PAR), nutrient concentration (ambient vs. nutrient added) and temperature (ambient vs. increased) conditions in La Caldera (a) and Las Yeguas (b). Only BR estimated as 50 % of total planktonic respiration (TPR) is represented in this figure, since significant differences among treatments are the same than for BR estimated as 75 % of TPR. Bars represent the mean BR values and the lines on top of them represent the standard deviation (SD; n=3). Letters indicate significant differences among treatments.

in absolute and percentage EOC values %EOC, reflecting the coupling between photosynthesis (^{14}C assimilation) and algal growth (Berman-Frank y Dubinsky, 1999; Carrillo *et al.*, 2002, 2008b) under higher temperature and nutrient availability. The increase in T (see UVR×T_+ treatment) was the main factor that reduced the damaging UVR effect in LC, through the disposing of the surplus fixed C (EOC and %EOC). This response could be interpreted as one possible pathway to maintain the Mehler reaction (**?**), leading to the reduction of inhibitory-UVR effect on PP. The nutrient addition played a crucial role in the reduction of the extracellular carbon release, reaching values lower than 20 % and reflecting balanced photosynthesis and algal growth (Berman-Frank y Dubinsky, 1999). However, we cannot dismiss the involvement in PP enhancement of potential repair processes, such as reparation of PSII through higher synthesis of D1 protein, dependent on temperature (Bouchard *et al.*, 2005; Sobrino y Neale, 2007). In the warmer lake (LY), increased PP occurred only with increased simultaneous nutrient and temperature, as reported by Degerman *et al.* (2013), suggesting colimitation. However, the increase in T alone (see UVR×T_+ treatment) accentuated the inhibitory UVR effect and augmented EOC. The contrasting responses to warming between the two ecosystems suggest that, in LY, the rise in temperature (from 15 to 20°C), reaching values far from those characterizing permanent high-mountain lakes of more than 5m depth (Bullejos *et al.*, 2014), even in warmer dry periods (Villar-Argaiz *et al.*, 2002), exceeded the optimum temperature for algal performance (Hoffman *et al.*, 2003; Sobrino y Neale, 2007) making the algae more susceptible to UVR.

Regarding bacterioplankton, under UVR, the generalized lack of positive response of BPT after nutrient addition and the increase in the negative UVR effect in both lakes, is consistent with the ability of algae to overcome bacteria in P-uptake (Xenopoulos *et al.*, 2002; Villar-Argaiz *et al.*, 2002; Carrillo *et al.*, 2008b). Nevertheless, this negative algae effect on bacteria could be reinforced in LC by the decrease in the extracellular matter released by P-enriched phytoplankton (Villar-Argaiz *et al.*, 2002) which was not sufficient to satisfy the BCD (%BCD:EOC >100 %). Another line of evidence supporting the bacterial competitive disadvantage for P and their C-limitation stem from the increase in the BPL:BPT ratio under $T_=$

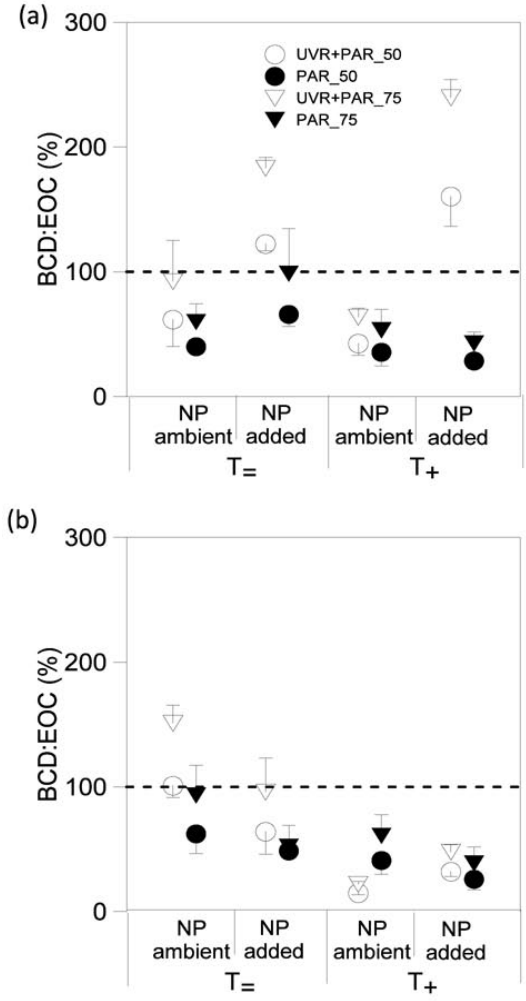
(a)
300
200
100
0
BCD:EOC (%)
UVR+PAR_50
PAR_50
UVR+PAR_75
PAR_75
NP ambient
NP added
NP ambient
NP added
T=
T+
(b)
300
200
100
0
BCD:EOC (%)
NP ambient
NP added
NP ambient
NP added
T=
T+

Figura 3.6: Relationship between the bacterial carbon demand (BCD) and supply of carbon by algal excretion (EOC) as percentage, measured under different radiation (UVR+PAR vs. PAR), nutrient concentration (ambient vs. nutrient added) and temperature (ambient vs. increased) conditions in La Caldera (a) and Las Yeguas (b). Due to differences between %BCD:EOC calculated by using BPT or BPL data were not found, only those obtained from BPT are represented. A value of 100 % means a similar carbon demand and carbon supply. Triangles represent the mean value of %BCD:EOC (n=3) when considering bacterial respiration (BR) as 75 % of total planktonic respiration (TPR), whereas circles represent the mean value of %BCD:EOC (n=3) when considering BR as 50 % of TPR. The displayed vertical error bars embrace from the lowest error value for BR from 50 % to the highest error value for BR from 75 %. Error bars were calculated with error propagation.

after nutrient-addition. Thus, values >1 might reflect an unbalanced physiological state of the bacterial community translated into an unbalanced growth(Shiah y Ducklow, 1997; del Giorgio *et al.*, 2011; Motegi *et al.*, 2013), which could be caused by worse growth conditions due to EOC limitation. The BPL:BPT ratio as a proxy of balanced or unbalanced growth is controversial (Hoppe *et al.*, 2006) due to the bacterial selectivity for leucine or thymidine uptake in freshwaters (Pérez *et al.*, 2010). Besides, BPL:BPT changes under UVR and nutrient-added conditions might be the result of the enhancement by nutrient addition of the activity of some groups with higher capability for leucine uptake than thymidine, as *β-Proteobacteria* (Pérez *et al.*, 2010; Sebastián y Gasol, 2013), one of the most abundant groups in LC (Reboleiro-Rivas *et al.*, 2013), rather than the outcome of changes in cell physiology. However, from a perspective of bacterioplankton compartment, under UVR, the joint action of the increase in T and the nutrient addition, resulted in no changes in BPL and BPT values that led to BPL:BPT ratio remained close to 1 and therefore bacterioplankton

presented balanced growth despite the damaging effect of UVR.

Therefore, in LC, consistent with our initial hypothesis, the lower %EOC under the joint action of UVR, nutrient addition, and T_+ would promote a better coupling between photosynthesis and algal growth with a fall in absolute EOC values, leading to a weakened algae-bacteria relationship. By contrast, in LY, there was enough EOC to meet BCD, suggesting that the increase in UVRinh on BPT after nutrient addition might be due to a direct effect of UVR on HBP (see competition for P) rather than indirect effects of UVR through the availability of carbon of algal origin. Along this line, the joint action of the increase in T and the nutrient addition resulted in a slight increase of BPL:BPT ratio, due mainly to the stimulatory effect of T_+, indicating the failure of the bacterial compartment to be stimulated in their growth through cell division despite the availability of EOC and nutrients. Therefore, contrary to our hypothesis and regardless of the intrinsic mechanisms, in both lakes, simultaneous action of an increase in T and nutrients triggered a negative UVR effect, and resulted in a non-generalized stimulus of bacterial growth.

3.5.3. Implications

Our results indicate that in the coldest and most transparent lake, where a dependency of bacterioplankton on EOC has been consistently evidenced (Carrillo *et al.*, 2002; Medina-Sanchez *et al.*, 2002; Medina-Sánchez *et al.*, 2004), in a global-change scenario (i.e. increases in temperature and nutrient-inputs) the commensalistic algal-bacterial relationship might become uncoupled. Consequently, the heterotrophic bacterial growth would be impaired, weakening the poorly developed microbial loop in this ecosystem (Medina-Sánchez *et al.*, 2004), against a reinforced grazing chain (Fig. 3.7). Contrarily, in the warmer and less transparent lake, where a weak algal-bacterial commensalistic relationship was evidenced, warming and nutrient-inputs would strengthen the algal-bacterial commensalistic relationship, leading to a higher C-flux through the microbial loop (Fig. 3.7), somewhat more developed in this ecosystem (Cruz-Pizarro *et al.*, 1994). Although caution should be exercised in extrapolating results from short-

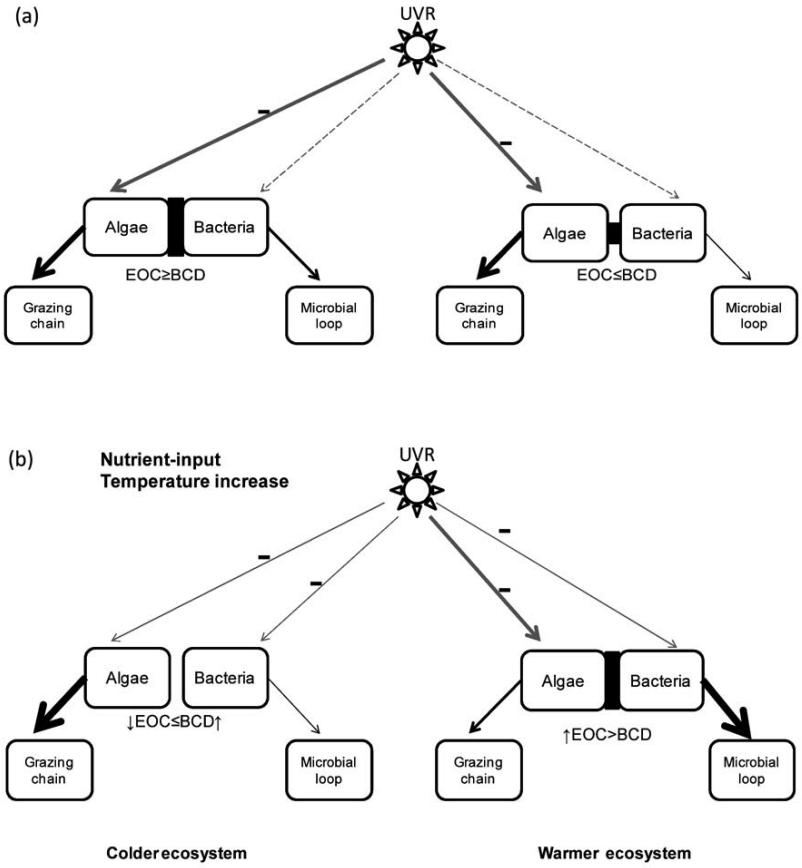

Figura 3.7: Algal-bacterial relationship and C-flux under ambient conditions (a) and under increased nutrient concentration and temperature (b). Grey arrows represent UVR effect on phyto- and bacterioplankton metabolisms, dashed arrows indicate non-significant UVR effect. Black arrows represent the sense of C-flux, the thickness of the arrows indicate the relative magnitude of the C-flux.

term experiments to long-term scale, our results can be considered to represent an initial trend of the algal-bacterial relationship under changing multiple environmental conditions. Despite that high-mountain lakes have been considered a rather homogeneous ecosystem class subjected to extreme conditions, their trophic webs may respond differently to environmental changes because of slightly different initial physical, chemical, and biological characteristics (Rose *et al.*, 2009). Thus, even in nearby high-mountain lakes exposed to a similar abiotic environment and with simple planktonic communities, the joint impact of global-change stressors can lead to contrasting planktonic structure and functioning, i.e. a relative dominance of the grazing chain against the microbial loop. This might have implications for C cycle, through the number of trophic levels involved in energy transfer from primary producers.

Capítulo 4

Quantifying interactive effects of UVR, phosphorus, and temperature on phyto- and bacterioplankton in two contrasted (marine vs. high-mountain) Mediterranean ecosystems.

4.1. Abstract

We performed in situ experiments in which three types of stressors (chronic: ultraviolet radiation [UVR]; pulsed: phosphorus [P]; and abrupt: temperature) were manipulated. A 2×2×3 split-plot design (UVR: ambient/exclusion; P: ambient/elevated; temperature: ambient/5°C higher/5°C lower) was carried out in a high-mountain lake (HML) and clear-water coastal marine ecosystem (CME). The aim was to quantify and compare the nature and magnitude of their interactive effect on phytoplanktonic and bacterial abundance and on primary [PP] and heterotrophic bacterial production [HBP]. Phytoplankton and bacteria showed a common pattern of metabolic response to UVR×P interaction, increasing their net values, but with UVR inhibiting PP and stimulating HBP after P-enrichment in both ecosystems. Temperature shift effect (T-shift) on the UVR×P interaction differed between the ecosystems, with a decrease in PP, HBP, and bacterial abundance in the HML but no change in these parameters in the CME. These findings are consistent with the higher relative magnitude of the three-way interactions found in the HML than in the CME. T-shift accentuated the strength of the antagonistic negative UVR×P effect on PP but attenuated the synergistic positive UVR×P effect on HBP in both ecosystems, which even became antagonistic negative in the HML with cooling. The effects of two- and three-way interactions were of a higher magnitude on HBP, whereas the single effects of UVR or P were stronger on PP. Based on these findings, phytoplankton is proposed as a sensitive damage biosensor and high-mountain lakes are proposed as sentinels of global change, due to their greater vulnerability.

4.2. Introduction

Global climate change is the result of multiple anthropic stressors that drive an accumulative impact on biodiversity and the functioning of ecosystems (Sala *et al.*, 2000; Steffen *et al.*, 2006). It is, therefore, important to assess interactions among multiple abiotic stressors acting at different rates and on regional (e.g. eutrophication, drought, increased ultraviolet radiation [UVR]) and global (e.g. ozone depletion, atmospheric dust, global warning) scales if we are interested in predict the effects of anthropic impacts on ecosystems. One of the most sensitive regions in the world to the effects of climate change is the Mediterranean basin where the interaction among stressors may be especially accentuated (Stocker *et al.*, 2013; Giorgi y Lionello, 2008). Among others, the increased UVR fluxes in the Northern Hemisphere (**?**) represent a worldwide stressor with far-reaching implications for organisms and ecological interactions (Häder *et al.*, 2007; Andrady *et al.*, 2012). Higher frequency of extreme weather events are expected to alter precipitation regimes and increase desertification in the Mediterranean region (Stocker *et al.*, 2013; Linares *et al.*, 2011; De Senerpont Domis *et al.*, 2013). As a consequence an increase in nutrient loads of phosphorus (P) and nitrogen (N), can occur shifting the functioning of oligotrophic lakes and coastal environments in the Mediterranean area (Assessment, 2005). The importance of these nutrient loads for aquatic ecosystems is supported by findings in numerous natural systems where resource pulses are often extreme events in a continuum rather than isolated occurrences (Yang *et al.*, 2008).

The Mediterranean area is also expected to experience an increase in maximum, minimum, and mean air temperatures (Zanis *et al.*, 2009) and in the frequency of heat waves during the 21^{st} century (Giorgi y Lionello, 2008; Tolika *et al.*, 2009). Despite the temperature effects have been largely interpreted in terms of changes in mean values, some studies have explicitly acknowledged that changes in the incidence of extreme events and in their temporal variability can have profound effects on ecosystems (Gaines y Denny, 1993; Easterling *et al.*, 2000). Moreover, abrupt changes in temperature would occur as a result of the microstratification near subsurface

in the aquatic ecosystems. These temperature extreme events that exceed physiological limits can cause widespread mortality of the organisms (Coma *et al.*, 2009). However, very little is known about the kinds of effects that temperature extremes have on natural systems.

Besides, account should also be taken of the duration of stressors, which can be permanent, e.g., UVR; sporadic, e.g., P-pulses (pulse = combination of low frequency, large magnitude, and short duration, sensu Yang *et al.* (2008); or abrupt, e.g., temperature. Changes in these multiple factors may potentially trigger complex interactive effects among them; therefore, it is important to determine their combined impact, which might be stronger (synergistic effect) or weaker (antagonistic effect) than the sum of their individual effects (Christensen *et al.*, 2006; Crain *et al.*, 2008). Likewise is relevant in a context of global change to quantify how the interaction among multiple factors will change the magnitude of their interactive effect.

The response of organisms to the interactive effect of stressors can also vary as a function of their metabolism, with reports of differences between autotrophic and heterotrophic organisms (Christensen *et al.*, 2006; Crain *et al.*, 2008). Hence, prediction of the net effect on organisms as well as the magnitude and strength of interaction among multiple factors is a complex task. Microplankton has been proposed as an ideal experimental model system for studying the consequences of global change, because these unicellular organisms are relatively easy to manipulate and have short generation times (Lürling y De Senerpont Domis, 2013). Phyto- and bacterioplankton, the two main components of the planktonic community, are usually exposed to high UVR levels (Garcia-Pichel, 1994; Häder, 2011). This exposure is accentuated in oligotrophic ecosystems, whose waters are more UVR-transparent due to their lower concentration of dissolved organic matter (DOM) (Häder *et al.*, 2011). Briefly, three main direct harmful effects of UVR (mainly UV-B) on phytoplankton and bacteria are: nucleic acid damage, e.g., cyclobutane pyrimidine dimmers (Hernández y Cormack, 2007; Helbling *et al.*, 2001); a reduction in enzymatic activity (Herndl *et al.*, 1993; Tank *et al.*, 2005); and a decrease in nutrient uptake due to impaired membrane permeability (Klamen y Tuveson, 1982; Hessen *et al.*, 1997). UVR also has an indirect negative effect through the photochemical production

of oxygen radicals that inhibit PP and HBP. However, UVR can exert some indirect positive effects by degrading recalcitrant DOM into smaller organic molecules readily assimilable by bacteria (Abboudi *et al.*, 2008); by releasing bound mineral nutrients for algal and bacterial uptake (Wängberg *et al.*, 1999); and by stimulating algal excretion of organic carbon (EOC), a substrate readily consumed by bacteria (Chatila *et al.*, 2001; Carrillo *et al.*, 2002). Accordingly, despite the well-documented direct harm caused to pelagic organisms by UV-B, some authors have found that the net effect of UVR is not negative and that it can even stimulate BP and photosynthetic activity (Aas *et al.*, 1996; Medina-Sanchez *et al.*, 2002; Gao *et al.*, 2007b; Helbling *et al.*, 2001). This apparent inconsistency may be due to the acclimation capacity of organisms in highly exposed UVR ecosystems through UVR-damage repair mechanisms, either photoenzymatic repair or dark repair, e.g., nucleotide excision repair (Matallana-Surget *et al.*, 2010; Sinha y Hader, 2002). In addition, net UVR effects depend on the intensity of the environmental stressors. Thus, an increase in nutrient availability can either reduce photoinhibition (Heraud *et al.*, 2005; Helbling *et al.*, 2013) or unmask UVR damage (Carrillo *et al.*, 2008a; Korbee *et al.*, 2012). Likewise, increases in temperature might have a positive influence by reducing photoinhibition of the bacterial and phytoplanktonic community (Roos y Vincent, 1998; Bullock y Jeffrey, 2010). However, the net response of algal and bacterial abundance to the interaction of these factors has received much less attention.

Although the interactions between UVR and P-enrichment (Medina-Sánchez *et al.*, 2006; Ogbebo y Ochs, 2008; Delgado-Molina *et al.*, 2009) and between UVR and temperature (Roos y Vincent, 1998; Rae y Vincent, 1998; Vidussi *et al.*, 2011; Lionard *et al.*, 2012) have been widely studied in relation to bacterioplankton and phytoplankton, few data are available on the interactive effect of these three factors on both trophic levels (Doyle *et al.*, 2005). Furthermore, no study has evaluated the interactive effects of UVR, nutrients and temperature on bacterial and phytoplanktonic functional variables, despite their key role in ecological interactions. With this background, the aim of the present study was to examine how the sensitivity of phytoplankton and bacteria to a chronic stressor (UVR)

may be modified by the combined action of pulsed (nutrient) and abrupt (temperature variation at short term) factors by quantifying the magnitude and nature of their interactive effect on PP, HBP, and their abundance. Two oligotrophic clear-water ecosystems in the Mediterranean region were selected for the study, a high-mountain lake (HML, La Caldera, Sierra Nevada, 3050 m.a.s.l.) and a coastal marine ecosystem (CME, Cabo de Gata, Mediterranean Sea).

These ecosystems are subjected to contrasting underwater irradiance due to their marked difference in altitude and therefore incident UVR-flux. They receive P-pulses from the atmosphere (Morales-Baquero *et al.*, 2006; Marañón, 2010) and also, especially in the case of the CME, from terrestrial sources. Both ecosystems undergo temperature fluctuations, although these are much less extreme in the CME. Based on reports in the literature on oligotrophic clear-water ecosystems and current environmental change trends (Stocker *et al.*, 2013), we hypothesized that pulsed nutrient input (P) would enhance net algal and bacterial development and counteract the harmful effect of UVR. This enhanced development of phytoplankton and bacteria may be impaired if they experience abrupt variations of temperature related to the cold or warming extreme events characteristic of this region (Baldi *et al.*, 2006).

This hypothesis was tested in two steps. In a first comparative analysis of the HML (La Caldera) and CME (Cabo de Gata), we determined the response of PP and HBP and abundance to P additions under UVR exposure in mid-term incubation (1 wk), using P concentrations that simulated current inorganic nutrient pulses from allochthonous inputs (Morales-Baquero *et al.*, 2006; Marañón, 2010). In a second comparative analysis, we evaluated the influence of temperature shift (T-shift) on these responses over the short term (12 h). The key variables were measured in an identical manner in both ecosystems, facilitating comparison of their biological responses and vulnerability.

4.3. Methods

4.3.1. Study site

The study was performed in two aquatic ecosystems: La Caldera, a high-mountain lake above the tree line at an altitude of 3050 m.a.s.l. in Sierra Nevada National Park (Spain, 37° 03' N; 3° 19' W), and the sea of Cabo de Gata in the Western Mediterranean (36° 33' N; 2° 16' W). La Caldera is oligotrophic (total phosphorus [TP] <0.3 μM, chlorophyll *a* <1 μg L^{-1}) and receives mineral-nutrient inputs from sporadic Saharan dust depositions (Villar-Argaiz *et al.*, 2001). The water temperatures range from 4°C to 17°C during the ice-free season and the lake does not stratify. The lake water is highly transparent (Secchi depth to at least 14 m), having a low dissolved organic carbon (DOC) concentration (<1 mg C L^{-1}) that allows >1 % UVR to penetrate to the bottom of the lake (Carrillo *et al.*, 2008a). Ciliates and heterotrophic nanoflagellates are scarce and were not detected during the present study period. In Cabo de Gata, the nutrient balance depends on the annual hydrological cycle. The nutrient concentration in the euphotic layer is reduced during much of the cycle, due to the thermal stratification of the water column, but is somewhat higher from March through May, when vertical mixing takes place (Ramírez *et al.*, 2005, and references therein). Nevertheless, nitrate (NO_3^-) and soluble reactive phosphorus (SRP) concentrations remain relatively low all year round, and the input of nutrients can be assumed to play a role in controlling the productivity of this system (Reul *et al.*, 2005). The average surface water temperature ranges from 15°C to 21°C (Zabaleta, 1976; REDIAM, 2011).

4.3.2. Experimental design

A mid-term experiment was conducted in situ in each ecosystem in 2009, from August 27 through September 3 in the HML and from September 21 through September 26 in the CME. The experiment had a 2×2×3 factorial design (in triplicate): two radiation treatments [UVR+PAR, 280-700 nm] and exclusion of UVR [Photosynthetic Active Radiation (PAR), 400-700 nm]; two nutrient treatments [ambient nutrient concentration (P-ambient)

and P addition (P-added: P_+)]; and three temperature treatments (5°C above ambient temperature [T_+], ambient temperature [$T_=$], and 5°C below ambient temperature [T_-]). The experiment had a split-plot design, with light and nutrient treatments at main-plot level and temperature at subplot level.

Each treatment used three microcosms, i.e., clear polythene 20-L cylinders. Polyethylene plastic that transmits ∼60 % [280 nm] and >80 % [400-700 nm] was used for the UVR and PAR treatments with the addition of a Plexiglas UF3 cover (a long-wave-pass plastic that transmits 90 % of PAR but blocks UVR<390nm) for the PAR treatments. Each microcosm was filled with water that had been filtered (45 μm pore size for zooplankton removal) from the photic layer receiving >1 % UVB. Microcosms were incubated at 0.5 m depth, in situ at HML or in two open tanks placed on the desk a boat (to avoid wave damage) at CME. In the latter case, the interior of the tanks was painted black to prevent any light reflection, and sea water was constantly pumped into them in order to replenish the water surrounding the microcosms and keep it at ambient temperature.

For the P-added treatments, KH_2PO_4 was added to a final concentration of 30 μg P L^{-1}, based on previous observations of a maximum response of the microbial food web at this concentration in a high-mountain lake [57] and within the range of experimental time-integrated P-additions in the Mediterranean sea (Agawin *et al.*, 2004; Duarte *et al.*, 2005). Microcosms with no added nutrient and no UVR exposure served as controls. Microcosms were gathered after one week and the contents of each were divided into three samples for incubation under one of three conditions: in situ at $T_=$ (17°C in HML; 20°C in CME) or in thermostatically controlled chambers at T_+ (22°C in HML; 25°C in CME) or T_- (12°C in HML; 15°C in CME). Incubations were performed overnight (12 h) from 2-3 September in the HML and from 25-26 September in the CME.

4.3.3. Physical analyses

Vertical profiles of solar radiation in the water column were determined at noon with a BIC compact four-channel underwater radiometer (Biosp-

herical Instruments Inc., CA, USA), which has three channels in the UVR region of the spectra (305, 320, and 380 nm) and one broad-band channel for PAR (400-700 nm), as well as temperature and depth sensors. Diffuse attenuation coefficients for downward irradiance (k_d) were determined from the linear regression slope of the natural logarithm of downwelling irradiance versus depth for each wavelength range considered. A large sample size (pairs of irradiance and depth values, n >160) was used, and a good fit (R^2 >0.95) was obtained for all regressions.

4.3.4. Abiotic and biotic structural variables

Samples for dissolved inorganic nitrogen (DIN) and total phosphorus (TP) were analyzed on the same day as their collection in the HML, whereas CME samples for nutrient variables were frozen at -30°°C until their analysis, which was performed in a Technicon AutoAnalyzer (TrAAcs 800, Bran-Leubbe) following Ramírez *et al.* (2005). DIN was considered the sum of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+), which were determined by UV-spectrophotometric screening, sulfanilamide, and phenol-hypochlorite techniques, respectively [60]. TP was determined by analyzing 50-mL aliquots with the acid molybdate technique after digestion with a mixture of potassium persulfate, boric acid, and sodium hydroxide at 120°C for 30 min (APHA, 1992) [60]. Up to 500 mL (for seston N) or 1L (for seston P) per replicate were filtered through pre-combusted (1 h at 550°C) 1.0-μm glass fiber filters (Whatman GF/B) at low pressure (<100 mm Hg). Filters containing seston N were dried (24 h at 60°C) and kept desiccated until N analysis using a Perkin-Elmer model 2400 CHN elemental analyzer (Perkin-Elmer Corporation, Waltham, Massachusetts, USA). Seston P was analyzed following the method described for TP. Blanks and standards were performed in all procedures. The sestonic N:P ratio was calculated on a molar basis. DOC values were determined by filtering the samples through pre-combusted (2 h at 500°C) glass-fiber filters (Whatman GF/F) and acidifying them with HCL. Samples were then measured in a total organic carbon analyser (TOC-V CSH/CSN Shimadzu).

Nanophytoplankton abundance (PA) was determined by the method of

(Uthermöl, 1958) in samples preserved in glass bottles with alkaline (freshwater system) or acidic (marine system) Lugol's solutions. Phytoplankton diversity was estimated by the Shannon-Wiener index (H'), calculated as:

$$\text{H'} = -\sum_{1}^{x} pi \log_2 pi \tag{4.1}$$

where x is the number of taxa and pi is the relative abundance of taxon i ($\sum pi$=1). We did not assess the PA after temperature treatments because the phytoplanktonic generation time exceeded the incubation time (>12 h) at the different temperatures. In CME, phytoplankton abundance was also determined by flow cytometry analysis (using a Becton Dickinson FACScan flow cytometer, Mercado *et al.*, 2006) in order to account both autotrophic picoplankton abundance (APPA) and PA. No autotrophic picoplankton was found in the HML, similar to previous reports on this ecosystem (Medina-Sánchez *et al.*, 2004). Bacterial abundance (BA) was determined by the 4', 6-diamidino-2-phenylindole (DAPI) direct-count method described by Porter y Feig (1980). Water samples were fixed with neutralized formalin (2 %), stained with DAPI to a final concentration of 2.5 μg mL^{-1}, and then filtered through a 0.2-μm pore-size black polycarbonate Nucleopore Filter. At least 400 cells per sample were counted by epifluorescence microscopy (Karl Zeiss AX10).

For measurements of the Chl a concentration, 0.5-1L of water from each microcosms were filtered onto Whatman GF/F filters (25 mm in diameter), which were frozen at -20°C until analyses, when they were thawed and placed in centrifuge tubes (15 mL) with 5 mL of acetone (90 %) for 24 h in the dark at 4°C. Next, the samples were centrifuged, and the fluorescence of the supernatant was measured with a fluorimeter (LS 55 Perkin Elmer, USA). A Chl *a* standard (Chl *a* from spinach, Sigma) was used to transform the fluorescence data into Chl *a* concentrations.

4.3.5. Biotic functional variables

Heterotrophic bacterial production (HBP) was determined by ^{3}H-thymidine (S.A= 52 Ci mmol^{-1}, Amersham Pharmacia) incorporation into the bac-

terial DNA (Fuhrman y Azam, 1982). In brief, ^{3}H-thymidine was added to each experimental vial with 1.5 mL of sample (3 replicates and 2 trichloroacetic acid [TCA]-killed controls per treatment) to a final (saturating) concentration of 15.2 nM. Vials were incubated at each experimental temperature for 60 min in darkness. After incubation, the incorporation of thymidine or leucine was stopped by adding 100 μL 100 % TCA). Extraction was performed by adding TCA and keeping the vials in ice for 20 min, after which the precipitate was collected by centrifugation at 14000 rpm for 10 min. Then, vials were rinsed twice with 1.5 mL of 5 % TCA to remove any residual unincorporated radioactivity, and the scintillation liquid (Ecoscint A) was added for subsequent measurement in an autocalibrated scintillation counter (Beckman LS 6000TA) (Smith y Azam, 1992). The conversion factor 1×10^{18} cell mol^{-1} (Bell, 1993) was used to estimate the number of bacteria produced per mol of incorporated thymidine. The factor 2×10^{-14} g C cell^{-1} (Lee y Fuhrman, 1987) was applied to estimate the amount of carbon.

For primary production (PP) measurements, sets of four 50 mL quartz (UVR treatments) or glass (PAR treatments) flasks (three clear and one dark for each of 12 experimental treatments) added with 0.37 MBq of $NaH^{14}CO_3$ [SA: 310.8 MBq mmo^{-1}, DHI] were incubated in situ at 0.5 m under polyethylene plastic (UVR samples) or Plexiglas UF3 cover (PAR samples) for 5 h symmetrically distributed around noon. All flask sets were kept horizontal throughout the incubations. PP calculations were based on the ^{14}C method (Lignell, 1992). In brief, the ^{14}C retained in particulate matter (>0.2 μm; PP) was segregated from the dissolved fraction [<0.2 μm; excreted organic carbon, (EOC)] using 0.2-μm pore-size filters of 25 mm diameter (Nucleopore Whatman); low pressure (<100 mmHg) was applied to minimize cell breakage (more details on laboratory procedure in Carrillo *et al.* (2002).

4.3.6. Data treatment and statistics

The method described by Luo *et al.* (2008) was used to analyze the strength of the interactive effects. The main effects were calculated by sub-

tracting the control value from the treatment value. The two-way interaction between two factors (IE^2) and the three-way interaction among three factors (IE^3) were calculated by using the following equations:

$$IE^2 = (IV^2 - CV) - ME_1 - ME_2 \tag{4.2}$$

$$IE^3 = (IV^3 - CV) - IE^2_a - IE^2_b - IE^2_c - ME_1 - ME_2 - ME_3 \tag{4.3}$$

where IV^2 = value of the two-way interaction, IV^3 = value of the three-way interaction, CV = control value, $ME_{1,2,3}$ = main effect of factors 1, 2, or 3, respectively, and $IE^2_{a,b,c}$ = the three possible combinations of two-way interactions when three factors are studied.

The relative magnitudes of two-factor and three-factor interactions were evaluated and compared between the ecosystems by means of the following equations:

$$I^2 = 100\frac{IE^2}{(|ME_1| + |ME_2|)/2} \tag{4.4}$$

$$I^3 = 100\frac{IE^2}{(|ME_1| + |ME_2| + |ME_3|)/3} \tag{4.5}$$

These equations express the magnitude of the interactions relative to the mean value of the absolute main effects of the factors, where I^2 = relative magnitude of the two-way interactions and I^3 = relative magnitude of the three-way interactions.

Because of the potential opposite sign of the main effect of each factor on the biological response variables, we followed the procedure reported by Crain *et al.* (2008) to determine the nature of the interactions (synergistic or antagonistic). The observed combined effect (non-additive effect) of the three factors ($Factor_{1\times2\times3}$ - control) was compared with their expected additive effect [($Factor_1$ -Control) + ($Factor_2$ - Control) + ($Factor_3$ - Control)]. If the combined impact of the three factors exceeded (i.e. produced a greater stimulus or inhibition) the additive effect, the interaction

was defined as synergistic, and if their combination did not exceed the additive effect (i.e. lesser or opposed effect), the interaction was considered antagonistic. A t-test was used to examine significant differences between the additive effect and the non-additive effect for each variable response and ecosystem. In addition, synergistic and antagonistic interactions were evaluated as positive or negative according to the biological meaning of the interactive effect on the response variable.

The inhibition by UVR as percentage (UVR_{inh}) on the different functional variables was calculated as:

$$\mathrm{UVR}_{inh}(\,\%) = ((PAR - UVR)/PAR) \times 100 \tag{4.6}$$

where UVR and PAR are the mean values in the presence (UVR) or absence (PAR) of ultraviolet radiation for each nutrient or nutrient × temperature treatment. Propagation errors were used to calculate the variance of the UVR effect size.

To determine significant interactions among the radiation and nutrients for sestonic N:P, PA and Chl *a*, a two-way analysis of variance (ANOVA) was used. Experimental data obtained from the split-plot design were analyzed by repeated-measure ANOVA, which was used to test the radiation × nutrients interactive effect on each response variable as main-plot effect; the radiation × nutrients × temperature interaction was tested as sub-plot effect using the multivariate tests of Pillai, Hotelling, and Roy, as recommended by (Schneider y Gurevitch, 2001). When a significant interactive effect of the three factors on the response variable was found, post hoc tests were applied to determine the effect of each main factor alone (Dunne, 2010). The conservative Tukey's post hoc test was used to determine significant differences between treatments, and the Fisher LSD test to assess marginally significant effects. Data were checked for normal distribution with the Kolmorov-Smirnov test and for homoscedasticity with Cochran's and Levene's tests; data were log-transformed or squared when these conditions were not met. Linear regression analysis was used to study the relationship between bacterial variables and DOC and between bacterial variables and EOC. Statistica 7.0 for Windows (Statsoft 2001) was employed for the

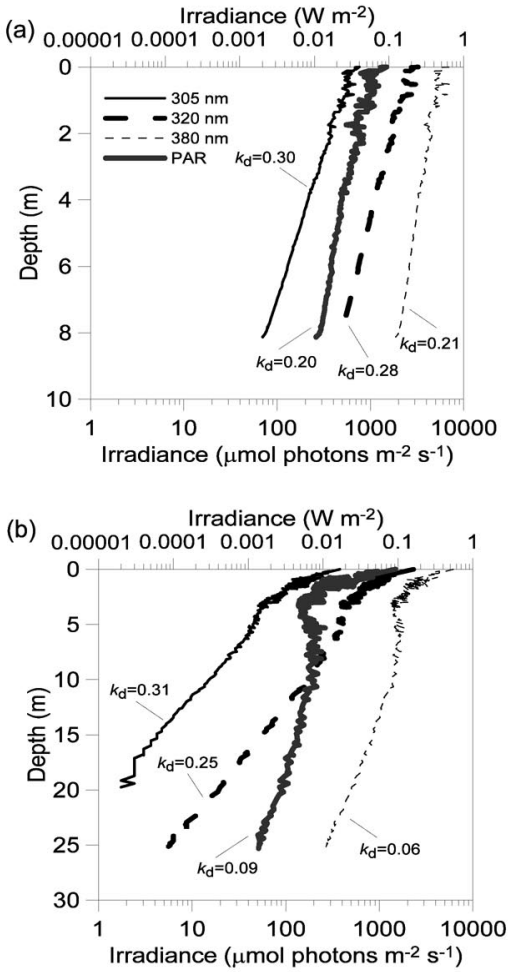

Figura 4.1: Depth profile of the irradiance in the water column at the beginning of the experiment. Irradiance data in the UVR range are expressed in W m^{-2}, PAR is in μmol photons m^{-2} s^{-1}. The diffuse attenuation coefficients (k_d) are also shown. (a) high- mountain lake; and (b) coastal marine ecosystem

Variable	High-Mountain Lake	Coastal Marine Ecosystem
TN (μmol L^{-1})	22.80±7.60	7.30±0.90
TP (μmol L^{-1})	0.11±0.05	1.46±0.55
SRP (μmol L^{-1})	0.06±0.001	0.02±0.006
DIN (μmol L^{-1})	14.60±7.30	0.32±0.08
DIN:TP (μmol)	142.10±27.12	0.21±0.05
DOC (μmol L^{-1})	72.12±16.21	250±120
Chl *a* (μg L^{-1})	3.09±0.04	0.99±0.22
$N:P_{sestonic}$	40.90±7.40	20.30±4.80
PA (cell mL^{-1})$\times 10^3$	4.88±1.61	1.19±1.10
PP (μg C $L^{-1}h^{-1}$)	2.02±0.43	2.99±0.64
BA (cell mL^{-1})$\times 10^5$	5.76±2.130	2.04±0.07
HBP (μg C $L^{-1}h^{-1}$)	0.067±0.005	0.040±0.002

Cuadro 4.1: Mean values of the main chemical and biological variables studied in the water column under the initial conditions of the experiment.TN: total nitrogen; TP: total phosphorus; SRP: soluble reactive phosphorus; DIN: dissolved inorganic nitrogen; DOC: dissolved organic carbon; Chl a: chlorophyll a; N:Psestonic: Nitrogen to Phosphorus ratio of the seston; PA: phytoplanktonic abundance; PP: primary production; BA: bacterial abundance; HBP: heterotrophic bacterial production.

statistical analysis.

4.4. Results

4.4.1. Initial conditions

Figure 4.1 depicts the penetration of solar radiation in the water column in both ecosystems. A higher UVR-flux at surface was recorded in the HML than in the CME, and this difference was >two-fold greater in the UV-B region. Both ecosystems showed low k_d for $UV\text{-}B_{305}$ and $UV\text{-}A_{380}$. Although HML was slightly less transparent than CME, the near surface $UV\text{-}B_{305}$

irradiance was higher in HML (0.035 W m^{-2} nm^{-1}) than in CME (0.010 W m^{-2} nm^{-1}), i.e. UV-B_{305} was 350 % higher in HML than CME. The temperature in the HML was 17.2°C at surface and had a homogeneous vertical profile (difference surface-bottom ≤0.9°C). The temperature in the CME was 21.1°C at surface and decreased by 5°C through the sub-surface layer (0-25 m), with no thermal stratification.

Both ecosystems differed in their nutrient ratios, with higher DIN:TP and sestonic N:P ratios in HML than in CME (Table 4.1).This denotes P limitation of the sestonic fraction in the HML. While a low DIN:TP ratio was observed in the CME, suggesting N limitation, the sestonic N:P ratio was, however, close to the Redfield ratio (Table 4.1). Algal and bacterial abundance and Chl a were 2-4 fold higher in the HML than in the CME (Table 4.1). Non-flagellate phytoplankton were the dominant group in the HML, largely represented by Chlorophyceae (87 %, mainly *Monoraphidium* sp.), whereas flagellates predominated in the CME (74 %, mainly Prasinophyta). Nanoplankton diversity was higher in CME (H': 1.66 bits $ind.^{-1}$) than in the HML (H': 0.9 bits $ind.^{-1}$). Autotrophic picoplankton was found only in the CME, and was composed of *Synechococcus* (2.2×10^4 cell mL^{-1}) and Prochlorococcus (5.8×10^2 cell mL^{-1}).

4.4.2. Interactive effects of UVR and P on microplankton in the high-mountain lake and their modulation by T-shifts

Under P-ambient conditions the sestonic N:P ratio had values higher than 40, as under the initial conditions, in both radiation treatments. P-addition decreased the sestonic N:P ratio (<20) regardless of the radiation treatment (Fig. 4.2a), and radiation × nutrients did not affect the N:P ratio (Table **??**). Under P-ambient and $T_=$ conditions, UVR did not affect PA or Chl *a*, whereas the P-addition stimulated these variables without differences between the radiation treatments (Fig. 4.2b, c). Hence radiation × nutrients had no effect on PA or Chl *a*. For functional variables, UVR inhibited PP under P-ambient and $T_=$ conditions (Fig. 4.3a) but not EOC. P-addition raised the PP value up to 10-fold under PAR treatment

Treatment \ Variable	High-Mountain Lake				Coastal Marine Ecosystem			
	PP	EOC	HBP	BA	PP	EOC	HBP	BA
UVR	20^{a}	ns	-782^{a}	ns	ns	56	-42^{a}	ns
UVR×P_{+}×$T_{=}$	63^{b}	47^{a}	-654^{a}	ns	69^{a}	ns	-71^{b}	ns
UVR×P_{+}×T_{+}	62^{b}	52^{a}	-65^{b}	30^{a}	37^{a}	ns	-50^{ac}	ns
UVR×P_{+}×T_{-}	27^{c}	70^{a}	78^{b}	57^{a}	40^{a}	ns	-47^{ac}	-53

Cuadro 4.2: Percentage of UVR inhibition (UVR_{inh}) on phytoplanktonic and bacterial variables under the indicated treatments. Different superscript letters indicate significant differences based on *post-hoc* test between each two treatments for phytoplanktonic and bacterial variables. PP: primary production; EOC: excreted organic carbon; HBP: Heterotrophic bacterial production; BA: bacterial abundance. ns: not significant, indicates that differences between UVR and equivalent PAR treatment for each nutrient or nutrient × temperature treatment were not found (LSD-test>0.05).

(Fig. 4.3a), triggering an antagonistic UVR × P_{+} effect (Table 4.3, 4.5), and increased the UVR inhibitory effect (Table 4.2). For EOC, radiation × nutrients effect was also antagonistic (Table 4.3, 4.5), with P-addition producing an inhibitory UVR effect on EOC (Table 4.2). The algal composition barely changed with the treatments over the experiment (Fig. 4.2c).

At subplot level, radiation, nutrients and T exerted a significant interactive effect on PP and EOC (Table 4.5). Thus, T-shift ($T_{\pm}$) further decreased the PP and EOC values found under the UVR × P_{+} × $T_{=}$ treatment (Fig. 4.3a), although only the cooling decreased the UVR inhibitory effect generated by P-addition on PP, but not on EOC (Table 4.2). T-shift did not modify the antagonist nature of the UVR × P_{+} × $T_{=}$ on PP and EOC but increased the strength of its interaction on both variables (Table 4.3).

With regard to bacterial community, under $T_{=}$ single and interactive effect of radiation and nutrients (main plot level) significantly stimulated HBP, but not BA (Fig. 4.3b, c; Table 4.5). The stimulatory effect of UVR on HBP (Table 4.2) was increased after P-addition resulting in a (positive)

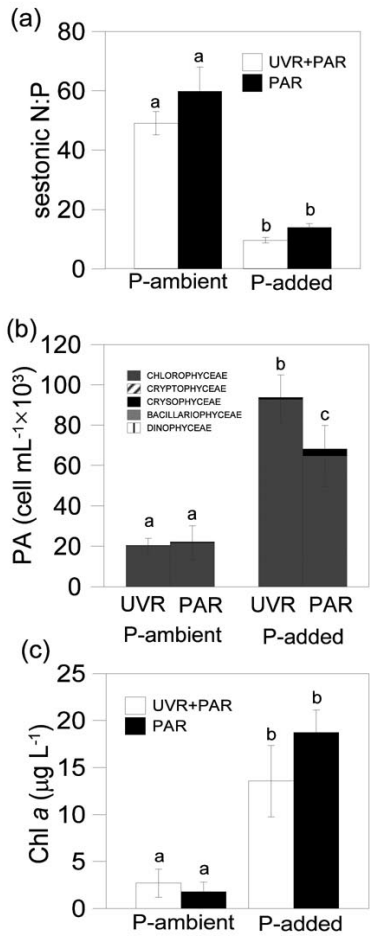

Figura 4.2: Sestonic N:P ratio, phytoplankton abundance (PA) and Chl *a* content under photosynthetically active radiation (PAR) and full sunlight (UVR+PAR) in P-ambient and P-added conditions. Data are expressed as mean values ± SD (n=3). Significant differences among treatments are denoted by different lower case letters

	PP			EOC			HBP			BA		
	$T_=$	T_+	T_-	$T_=$	T_+	T_-	$T_=$	T_+	T_-	$T_=$	T_+	T_-
High-Mountain Lake												
Control	3.37	3.37	3.37	0.12	0.12	0.12	0.21	0.21	0.21	$1.03^{\#}$	$1.03^{\#}$	$1.03^{\#}$
Additive	34.45	34.7	34.8	3.73	3.76	3.86	3.44	3.46	3.46	$1.61^{\#}$	$1.62^{\#}$	$1.82^{\#}$
Non-additive	12.29	6.76	9.66	2.00	0.88	0.77	13.13	3.88	0.19	$0.93^{\#}$	$1.13^{\#}$	$0.88^{\#}$
Interactive effect	A	A	A	A	A	A	S	ns	A	ns	ns	ns
Strength	-64	-81	-72	-46	-76	-80	282	12	-94	-41	-30	-51
Coastal Marine Ecosystem												
Control	2.42	2.42	2.42	9.13	9.13	9.13	1.96	1.96	1.96	$1.73^{\#}$	$1.73^{\#}$	$1.73^{\#}$
Additive	9.35	9.76	9.85	7.19	6.09	5.42	5.80	5.92	5.94	$4.90^{\#}$	$5.43^{\#}$	$5.03^{\#}$
Non-additive	6.15	4.76	5.22	12.50	11.6	15.4	8.50	8.37	7.64	$4.35^{\#}$	$4.26^{\#}$	$4.73^{\#}$
Interactive effect	A	A	A	A	A	A	S	S	S	ns	ns	ns
Strength	-34	-51	-47	73	90	184	47	41	29	-21	-6	-11

Cuadro 4.3: Quantification of additive and non-additive UVR× P_+×T-shift effect on phytoplanktonic and bacterial variables. The strength of interactive effect represents the difference between the non-additive and additive effects divided by additive effect (as percentage). Controls correspond to variable response values in PAR, P-ambient and $T_=$ treatment. A: antagonistic interaction; S: synergistic interaction. ns: not significant, indicates that differences between additive and non-additive effect were not found (t-test, $p>0.05$). PP: primary production; EOC: excreted organic carbon; HBP: heterotrophic bacterial production; BA: bacterial abundance. #: variable response value $\times 10^6$.

synergistic UVR× P_+ (Table 4.3). At subplot level, a significant radiation × nutrients × T effect was found on HBP, but not on BA (Table 4.5). Thus, i) warming (T_+) lowered HBP values found under UVR × P_+ × $T_=$ treatment (Fig. 4.3b), decreasing the UVR stimulatory effect on HBP (Table 4.2), and suppressed the synergistic UVR × P_+ effect generated (Table 4.3); ii) cooling (T_-) reduced HBP values (Fig. 4.3b) and led to a significant inhibitory-UVR effect (Table 4.2). The interaction of UVR× P_+ × T_- resulted in a significant (negative) antagonistic effect on HBP (Table 4.3).

HBP positively correlated with EOC when we considered all data subjected to UVR regardless the others factors levels (r=0.902, p <0.05; Fig. 4.4)

4.4.3. Interactive effects of UVR and P on microplankton in the coastal marine ecosystem and their modulation by T-shifts

Under P-ambient conditions, the N:P ratio was >40, almost two-fold higher than that at initial conditions (Table 4.1 and Fig. 4.5a). By contrast, after P-addition, sestonic N:P ratio remained near to 20 regardless of the radiation treatment, and radiation × nutrients did not affect the N:P ratio (Fig.4.5a; Table **??**). UVR under P-ambient conditions did not have significant effect on PA (Fig. 4.5b). However, P-addition had a stimulatory effect in both radiation treatments, although higher under PAR, which resulted in a UVR inhibitory effect on PA (Fig 5b). Chl *a* (Fig. 4.5c), the algal composition (Fig. 4.5b) or ciliates abundance (Fig. 4.5d) were not affected by UVR, P-addition or their interactive effect (Table **??**).

Under P-ambient and $T_=$ conditions, UVR did not affect PP, and P-addition increased PP regardless the radiation treatment, but more strongly under PAR (Fig. 4.6a).This generated a significant inhibitory UVR effect (Table 4.2) resulting in an antagonistic interaction (Table 4.3). UVR reduced EOC values under P-ambient and $T_=$ conditions and P-addition increased it regardless the radiation treatment, although more strongly under UVR (Fig. 4.6a), eliminating the inhibitory UVR effect on EOC (Table

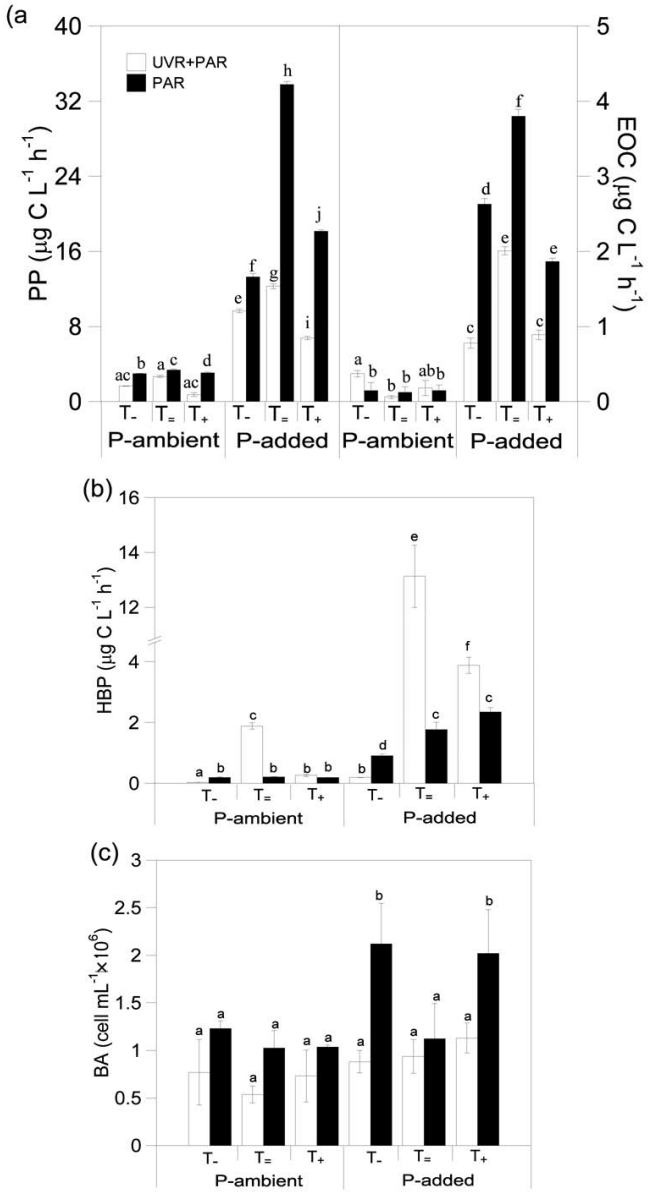
(a)
UVR+PAR
PAR
PP (µg C L-1 h-1)
EOC (µg C L-1 h-1)
T- T= T+ T- T= T+ T- T= T+ T- T= T+
P-ambient P-added P-ambient P-added
(b)
HBP (µg C L-1 h-1)
T- T= T+ T- T= T+
P-ambient P-added
(c)
BA (cell mL-1×10^6)
T- T= T+ T- T= T+
P-ambient P-added

Figura 4.3: Primary production (PP) and excreted organic carbon (EOC), heterotrophic bacterial production (HBP) and bacterial abundance (BA) under photosynthetically active radiation (PAR) and full sunlight (UVR+PAR) in P-ambient and P-added conditions. Data are expressed as mean values ± SD (n=3). Significant differences among treatments are denoted by different lower case letters

4.2) and producing a significant antagonistic effect (Table 4.5, Table 4.3).

At subplot level, radiation, nutrients and T exerted significant interactive effect on PP and EOC (Table 4.5). T-shift did not modify PP values found under UVR × P_+ × $T_=$ treatment (Fig. 4.6a) or the antagonistic nature of their interaction on PP, but increased the strength of this interaction (Table 4.3). Regarding to EOC, only the cooling increased the EOC values found under UVR × P_+ × $T_=$ treatment (Fig. 4.6a). However, T-shift did not modify the UVR effect found under UVR × P_+ × $T_=$ treatment (Table 4.2) or the antagonistic nature of their interaction (Table 4.3).

Our findings on the heterotrophic bacterial community showed that under P-ambient and $T_=$ conditions, UVR enhanced HBP, and P-addition stimulated HBP in both radiation treatments, but its effect was higher under UVR (Fig. 4.6b). As result, P-addition increased the stimulatory UVR-effect on HBP (Table 4.2) and a synergistic UVR × P_+ effect on HBP was generated (Table 4.3). In contrast to HBP, under $T_=$, UVR had no significant effect on BA (Fig. 4.6c), whereas P-addition significantly stimulated BA regardless the radiation treatment (Fig. 4.6c), although radiation × nutrients did not exerted an significant effect Table 4.5).

Finally, T-shift did not change HBP value found under UVR × P_+ × $T_=$ treatment (Fig. 4.6b), but slightly reduced the stimulatory UVR effect on HBP (Table 4.2) and only T_- decreased the strength of the interactive UVR × P_+ effect (Table 4.3). Likewise, T-shift did not affect the BA values found under UVR × P_+ × $T_=$ treatment or the nature of the radiation × nutrients interaction on BA (Fig. 4.6c; Table 4.3).

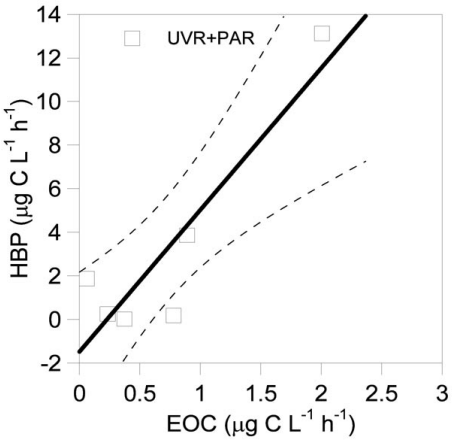

Figura 4.4: Lineal relationship between heterotrophic bacterial production (HBP) and excreted organic carbon (EOC) in the high-mountain lake

HBP directly correlated with EOC when all data were considered regardless the treatment (Fig. 4.7; HBP: r=0.583, $p<0.05$).

4.4.4. Magnitude of two- and three-way interactions

Overall, the relative magnitude of the two and three-way interactions (I^2 and I^3) was lower for PP than for HBP (Fig. 4.8a, b, c). Besides, the I^3 for HBP was always higher in the HML than in the CME, particularly after the warming (>50 fold under the UVR $\times$ P_+ $\times$ T_+, and only 2-fold under the UVR $\times$ P_+ $\times$ T_- treatment, Fig. 4.8b).

4.5. Discussion

This study was designed to assess the vulnerability of aquatic ecosystems that are chronically exposed to UVR and are subjected to the complex

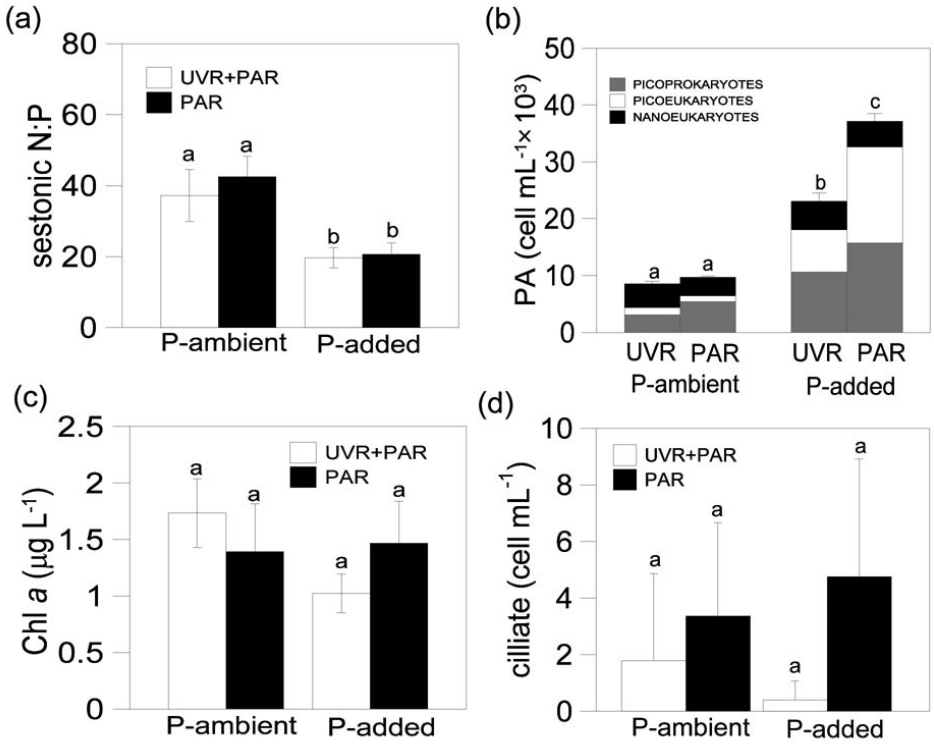

Figura 4.5: Sestonic N:P ratio, phytoplankton abundance (PA), Chl *a* content and ciliates abundances under photosynthetically active radiation (PAR) and full sunlight (UVR+PAR) in P-ambient and P-added conditions. Data are expressed as mean values ± SD (n=3). Significant differences among treatments are denoted by different lower case letters

effects of increased allochthonous nutrient inputs to the Mediterranean region (Escudero, 2005; Marañón, 2010) and temperature extreme events, i.e. global change-related stressors (Luo *et al.*, 2008). Recently, extreme weather events have gained in importance relative to gradual climatic trends as mechanistic drivers of broad ecological responses to climatic change (Parmesan *et al.*, 2000). Therefore, our results contributes the first quantification of the magnitude of the interactive effects of multiple abiotic factors acting at different temporal scales on the abundance and production of bacterial and phytoplankton communities, two key trophic levels in the functioning of aquatic ecosystems. Knowledge of the magnitude of these interactive effects is necessary because the results of single-factor experiments cannot reliably predict the dynamics of organisms or processes in ecosystems in which interactive effects dominate (Luo *et al.*, 2008).

We shall simplify our analysis and discussion of the complex response obtained by focusing on three aspects: (i) the responses of algal and bacterial variables to chronic UVR and pulsed nutrient (P); (ii) the influence of T-shifts on these responses, assessing the strength of the radiation × nutrients × temperature interaction according to the criteria of Crain *et al.* (2008), who quantified for the first time the net effect on a response variable of factors with opposing (inhibitory vs. stimulatory) effects; and (iii) quantification of the relative magnitude of the 2-way or 3-way interactive effects radiation, nutrient and temperature in relation to their single effects.

4.5.1. Responses of algal and bacterial variables to simultaneous action of UVR and P-addition

In support of our study hypothesis, a net positive response of PP and HBP to joint action of UVR and P-addition was obtained in both ecosystems under ambient temperature. This may be the result of two mechanisms :(i) UVR-tolerance based on cell photorepair mechanisms, consistent with previous reports in oligotrophic UVR-stressed ecosystems (Medina-Sanchez *et al.*, 2002; Medina-Sánchez *et al.*, 2006; Xenopoulos y Schindler, 2003); (ii) balanced nutritional status (N:P ratio ∼20; Fig. 3c, d) after the P-pulse, that would strengthen the light-independent mechanisms for repairing cell

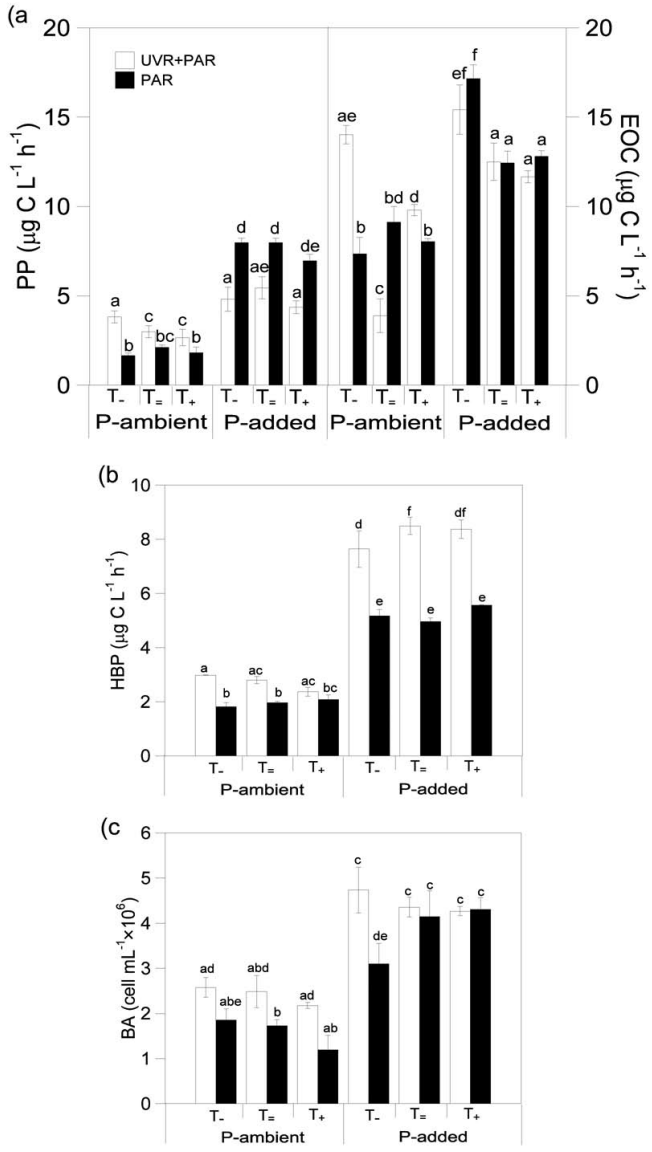
(a
UVR+PAR
PAR
PP (µg C L-1 h-1)
EOC (µg C L-1 h-1)
T- T= T+ T- T= T+ T- T= T+ T- T= T+
P-ambient P-added P-ambient P-added
(b
HBP (µg C L-1 h-1)
T- T= T+ T- T= T+
P-ambient P-added
(c
BA (cell mL-1×10^6)
T- T= T+ T- T= T+
P-ambient P-added

Figura 4.6: Primary production (PP) and excreted organic carbon (EOC), heterotrophic bacterial production (HBP) and bacterial abundance (BA) under photosynthetically active radiation (PAR) and full sunlight (UVR+PAR) in P-ambient and P-added conditions. Data are expressed as mean values ± SD (n=3). Significant differences among treatments are denoted by different lower case letters

damage (e.g. nucleotide excision repair), which are ATP-dependent and carry a higher energetic cost (Kaiser y Herndl, 1997; Heraud *et al.*, 2005). P input can also stimulate the synthesis of phospholipids required to repair chloroplast membranes (Hessen *et al.*, 2002) and enhance the production of photoprotective pigments (Leavitt *et al.*, 1997) in phytoplankton. Furthermore, the increased P availability may prompt a more efficient utilization of DOC either from photolysis (Hessen *et al.*, 1994; Vähätalo *et al.*, 2003) or from algal EOC by bacterioplankton. Although our experimental approach was not focused to evaluate DOM utilization by bacteria, the lack of a significant relationship between EOC and HBP under ambient temperature in any ecosystems suggests that the positive bacterial response to UVR and P-addition might be the outcome of direct effects of P under UVR rather than indirect effects mediated by carbon, in spite of the bacterial dependence on EOC consistently evidenced in HML (Medina-Sanchez *et al.*, 2002; Medina-Sánchez *et al.*, 2004, Chapter III).

The net increase in PP due to P-enrichment was reflected in a greater algal abundance (higher growth) and higher Chl a concentration in the HML, but only in higher algal abundance in the CME. The stimulation of standing-stock variables by P in the HML is consistent with the balanced N:P ratio and reflects the severe algal P limitation, in agreement with previous reports on this ecosystem (Carrillo *et al.*, 2008a)[38]. Noticeably, BA was stimulated by P in CME but not in HML, suggesting a competitive advantage of phytoplankton over bacteria in high-mountain lake (Carrillo *et al.*, 2008a). The absence of ciliate and low abundance of mixotrophs in

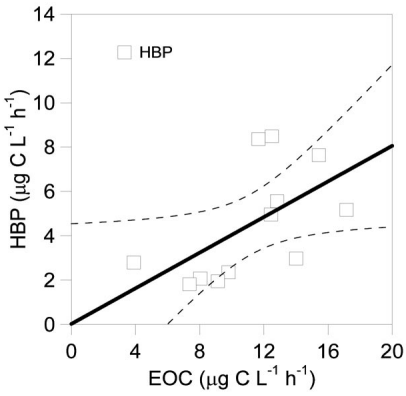

Figura 4.7: Lineal relationship between heterotrophic bacterial production (HBP) and excreted organic carbon (EOC) in coastal marine ecosystem

HML (in this experiment) led us to discard potential bacterivory processes as control mechanism of bacterial abundance in HML, although we cannot rule out a constraint on BA development due to viral lysis (Medina-Sánchez *et al.*, 2013).

Despite the net increase in the PP values under the joint action of UVR and P-addition, P unmasked or accentuated the negative UVR effect (i.e., antagonistic effect) on PP in the CME and HML. This response does not appear to be mediated by grazing on phytoplankton; given that zooplankton (the main grazers on phytoplankton in these ecosystems) were excluded in our experimental setup. This result agrees with reports of the unmasking or negative effect of UVR after nutrient enrichment on PP (Carrillo *et al.*, 2008a; Ogbebo y Ochs, 2008; Korbee *et al.*, 2012), phytoplankton growth (Xenopoulos y Frost, 2003; Doyle *et al.*, 2005), and diversity (Delgado-Molina *et al.*, 2009). Although the study of the physiological mechanisms behind the unmasked UVR effect after P-addition is out of the objectives of the present work, several potential explanations have been proposed: (i) to be the result of higher UVR damage on cell with stimulated growth and

high rates of DNA synthesis under nutrient-replete conditions; (ii) increased C-loss by photorespiration or respiration under high abiotic stress (Gao et al 2012b; Yan and Gao 2012); (iii) an increase in the water –water cycle (Asada, 1999) after P- addition could also drain electrons from PSII without contribution to carbon fixation under UVR . Hence, using the definition of stressor in a mechanistic sense, as reported by Boyd y Hutchins (2012), it can be considered that, under UVR exposure, P-addition rather than P scarcity acts as a stressor on PP by triggering negative UVR effects on phytoplankton (Delgado-Molina *et al.*, 2009).

By contrast, the increased positive (synergistic) effect of UVR on HBP after P-pulse in both ecosystems indicates a higher UVR tolerance of bacterioplankton than phytoplankton, irrespectively of the ecosystem. However, our results show the greater strength of non-additive effects for both algal and bacterial activities in the HML system. This result would be in accord with the lesser environmental predictability in these ecosystems in comparison with the marine environment.

4.5.2. Influence of T-shift on the interaction between UVR and P

Although sudden variations of temperature might seem unrealistic at first glance, previous studies suggested that phytoplankton cells can become trapped in transitional thin surface mixing layers within which not only find exposed to high irradiances but also abrupt temperature changes (Neale *et al.*, 2003; Xenopoulos y Schindler, 2003; Rueda *et al.*, 2007).

Our findings, consistently with the lesser environment predictability of high-mountain ecosystems, showed that T-shift modified the net response of algal and bacterial variables to the UVR $\times$ P_+ interaction more strongly in the HML than CME and T-shift ($T_\pm$) reduced PP values under UVR $\times$ P_+ only in the HML. However, T-shift did not alter the antagonistic nature of the interactive effect of UVR $\times$ P_+ on PP in either ecosystem (i.e., the effect of UVR $\times$ P_+ $\times$ temperature was less than their additive effect), although it increased the strength of this interaction (UVR $\times$ P_+) in the HML. Likewise, T-shift decreased the net response of HBP to the combined

action of UVR and P-input in the HML alone. Based on the above results, UVR and P (by unmasking negative UVR-effect) can be considered stressors for PP but not for HBP. Furthermore, T-shift accentuated inhibitory UVR effect on PP in both ecosystems but only trigged a negative UVR effect on HBP under cooling in HML. The difference in the response of the bacterial community to T-shift between the ecosystems (i.e., no effect in CME and inhibitory effect in HML under UVR $\times$ P_+) may be related to the thermal environment to which the organisms are adapted. Thus, Cooper *et al.* (2001) found that cold-adapted bacteria thrived poorly when subjected to higher temperatures, whereas warm-adapted bacteria did not suffer the same relative decrease when exposed to T-shifts. The contrasting responses of bacterial and phytoplankton variables may be attributable to a higher thermal tolerance of the more diverse bacterial (Coll *et al.*, 2010)[87] and algal community (see results) in the marine environment than in the high-mountain lake (Reche *et al.*, 2005, see results for phytoplankton in the present study). The difference in the response of the bacterial community to T-shift between both ecosystems might be also related with the bacterial dependence on EOC in the HML (see above). Thus, the decrease in EOC under UVR $\times$ P_+ after a change in temperature could promote the subsequent decrease in HBP and this explanation is supported by the direct relationship between C-released by algae and HBP found under UVR in HML, suggesting the role of temperature shift in bacterial dependence on algal carbon in the less unpredictable ecosystem.

4.5.3. Quantification of the magnitude of radiation $\times$ nutrients $\times$ temperature interaction

Whereas Crain *et al.* (2008) gives a objective classification of the nature of the interaction among multiple factors (antagonism or synergism) allowing us to compare among treatments, organisms or ecosystems, Luo et al.'s index offers a further quantification of the relative importance of the 2-way or 3-way interactive effects with respect to the single effects, regardless the nature of the interaction.

The strength of non-additive effects, measured by Crain *et al.* (2008)'s

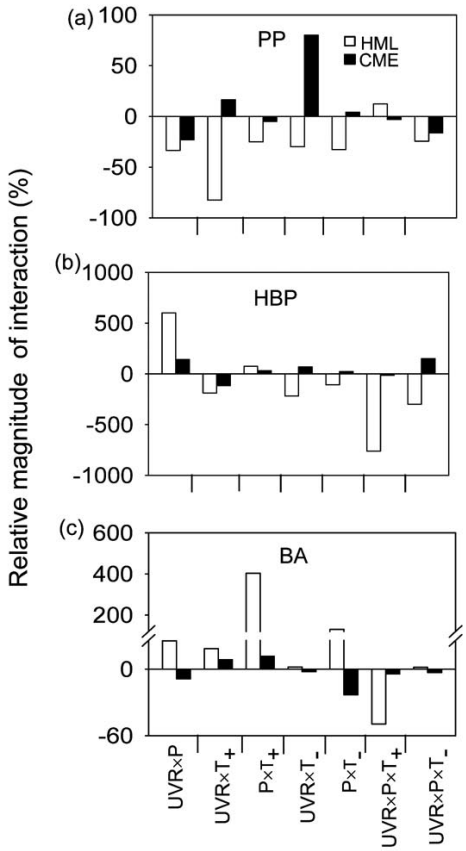

Figura 4.8: Relative magnitude of two- or three-factor interaction among UVR, P-input and T-shifts on primary production (PP), heterotrophic bacterial production and bacterial abundance (BA). Relative magnitude of interactive effects was calculated by using Eqs. 4.4 and 4.5. HML= high-mountain lake; CME= coastal marine ecosystem

procedure, was low in the CME, where the phytoplanktonic and bacterial communities showed very little sensitivity to temperature changes. In the HML, however, temperature-shifts under UVR×P_+ reduced the values of algal and bacterial production in comparison to UVR ×P_+ treatment at ambient temperature. Besides, Luo *et al.* (2008)'s index showed the predominance of the interactive effects (two- and three-way interactions, I^2 and I^3 >100 %) for bacterial functional variables. In addition, higher I^3 values were found in the HML, indicating a stronger interactive effect in comparison to the CME. These findings support our initial hypothesis that abrupt variations in temperature might impair the development of organisms under UVR × P_+, what was verified in the HML ecosystem, probably related to the lower thermal constancy than CME, what may obligate to organisms to metabolic readjustments before growth.

Algal metabolism may serve as a sensitive biosensor of the harmful effects of chronic UVR, given that the strong negative antagonistic effect generated by the three factors of global change, although it was largely attributable to the stronger negative effect of UVR, which counterbalanced the positive effects of P and, to a lesser degree, temperature shifts. This proposal is supported by previous findings (Carrillo *et al.*, 2008a) and by the low values of Luo *et al.* (2008)'s index for PP, indicating the greater importance of the single rather than interactive effects on this biotic compartment. In contrast, bacterioplankton was positively affected by both UVR and P and negatively affected (HML) or unaffected (CME) by temperature-shifts and was highly sensitive to the 3-way interaction, (I^3 values >100 % for the bacterial functional variables, especially in HML). These findings underscore the ability of this biotic compartment to respond to (and reveal) high-order interactions among environmental change stressors, especially when these act on different time scales. This outcome might be related to the greater turnover rate of bacteria than of phytoplankton. Finally, at ecosystems level, our results support the idea that high-mountain lakes are particularly vulnerable to the combined action of global-change stressors and therefore represent useful sentinels of environmental change on local and global scales (Catalan *et al.*, 2006; Williamson *et al.*, 2008).

4.6. Supplementary tables

		N:P$_{sestonic}$		PA		Chl *a*		Ciliates	
High-Mountain Lake									
		$F_{1,8}$	p	$F_{1,8}$	p	$F_{1,8}$	p		
	Radiation	20.19	<**0.01**	3.73	0.08	0.15	0.70		
	Nutrients	609.30	<**0.001**	93.89	<**0.001**	111.50	<**0.001**		
	Radiation×Nutrients	1.96	0.198	4.92	0.057	1.58	0.24		
Coastal Marine Ecosystem									
		$F_{1,8}$	p	$F_{1,8}$	p	$F_{1,8}$	p	$F_{1,8}$	p
	Radiation	1.09	0.326	154.48	<**0.001**	4.20	0.07	2.78	0.133
	Nutrients	44.40	< **0.001**	1201	<**0.001**	2.77	0.13	0.00	1.00
	Radiation×Nutrients	0.54	0.479	117.55	<**0.001**	0.07	0.79	0.61	0.45

Cuadro 4.4: Results of the two-way split-plot analysis of variance of the interactive effect of radiation and phosphorus. Numbers in bold indicate significant interactive effect among the factors. N:Psestonic: Nitrogen to Phosphorus ratio of the seston; PA: phytoplanktonic abundance; Chl *a*: chlorophyll a

		PP		EOC		HBP		BA	
High-Mountain Lake									
Main plot effect		$F_{1,8}$	p	$F_{1,8}$	p	$F_{1,8}$	p	$F_{1,8}$	p
	Radiation	1453	**<0.001**	3669	**<0.001**	464.40	**<0.001**	34.09	**<0.001**
	Nutrients	9280	**<0.001**	22392	**<0.001**	2242	**<0.001**	20.39	**< 0.010**
	Radiation×Nutrients	63.12	**<0.001**	4340	**<0.001**	122.10	**<0.001**	2.77	0.134
Sub-plot effect		$F_{2,7}$		$F_{2,7}$		$F_{2,7}$		$F_{2,7}$	
	T	1260	**<0.001**	283.42	**<0.001**	2353	**<0.001**	15.42	**< 0.01**
	T×Radiation	117.60	**<0.001**	31.88	**<0.001**	1064	**<0.001**	6.65	**<0.050**
	T×Nutrients	175.70	**<0.001**	348.58	**<0.001**	965.40	**<0.001**	4.50	0.055
	T×Radiation×Nutrients	357.80	**<0.001**	35.26	**<0.001**	199.10	**<0.001**	7.96	**<0.050**
Coastal Marine Ecosystem									
Main plot effect		$F_{1,8}$		$F_{1,8}$		$F_{1,8}$		$F_{1,8}$	
	Radiation	0.64	0.447	0.07	0.799	321.70	**<0.001**	33.92	**<0.001**
	Nutrients	823.90	**<0.001**	535.55	**<0.001**	1800	**<0.001**	308.70	**<0.001**
	Radiation×Nutrients	224.40	**<0.001**	21.73	**<0.01**	111.50	**<0.001**	0.81	0.395
Sub-plot effect		$F_{2,7}$		$F_{2,7}$		$F_{2,7}$			
	T	4.05	0.067	41.55	**<0.001**	4.77	**<0.050**	2.29	0.170
	T×Radiation	4.28	0.060	18.71	**<0.001**	12.74	**<0.010**	3.97	0.070
	T×Nutrients	1.94	0.212	38.44	**<0.001**	23.17	**<0.001**	9.93	**<0.01**
	T×Radiation×Nutrients	13.11	**<0.01**	37.25	**<0.001**	9.50	**<0.050**	8.59	**<0.05**

Cuadro 4.5: Results of the repeated three-way split-plot analysis of variance of the interactive effect of radiation, phosphorus, and temperature. Numbers in bold indicate significant interactive effect among the factors from repeated-measures ANOVA (main-plot effect) or from multivariate tests of Pillai, Hotelling and Roy (sub-plot effect). PP: primary production; EOC: excreted organic carbon; HBP: heterotrophic bacterial production; BA: bacterial abundance

Capítulo 5

Long-term interactive effects of UVR and repeated P inputs in a microplanktonic community of a mid-altitude Mediterranean lake

5.1. Abstract

The cumulative effects of nutrient input interact with other factors of global change such as ultraviolet radiation (UVR) and may alter the functioning of aquatic ecosystems. We performed a long term in situ experiment in a oligotrophic lake (La Conceja lake, Spain), with the following treatments: full sunlight (UVR+ PAR, >280 nm) vs. UVR exclusion (PAR only, >400 nm); ambient vs. phosphorus (P) addition simulating two natural P pulses (30 μg P L^{-1}). Abundance and biomass of the components of the microplankton community (<45μm; autotrophic nanophytoplankton, autotrophic picoplankton, mixotrophs, ciliates, and heterotrophic bacterioplankton) were studied, as well as metabolic variables of phytoplankton [primary production (PP), excretion of organic carbon (EOC)], bacterioplankton [heterotrophic bacterial production (HBP)] and mixotrophs and ciliates through bacterivory (%HBP_b). We found that the interactive effect of cumulative P pulses and UVR resulted in a UVR inhibitory effect on PP but stimulatory on HBP over the long term. Despite this different UVR effect on phyto and bacterioplankton, the joint action of UVR and P pulses increased the values of functional and structural variables in both compartments, although more strongly after the second than the first P pulse, evidencing an adaptation to repeated P pulses. Bacterioplankton responses were mediated by a dual biotic control through EOC excretion and bacterivory. Under UVR, after cumulative P pulses, the decrease in % %HBP_b enabled an increase in bacterial abundance concomitantly with the stimulation of HBP. However, microplanktonic biomass in this ecosystem remained dominated by autotrophs. Bacterivory, hence, proved to be the most plausible explanation of the scant development of the bacterial compartment, implying a deviation from the general pattern established for oligotrophic ecosystems.

5.2. Introduction

Microplankton comprises the majority of aquatic biomass and is the responsible for the bulk of productivity and nutrient cycling in aquatic systems (Paerl *et al.*, 2003; Fouilland y Mostajir, 2010). In oligotrophic ecosystems the microplanktonic biomass has been long considered to be dominated by heterotrophic organisms (Gasol *et al.*, 1997; Biddanda *et al.*, 2001; Duarte *et al.*, 2013). However, evidence of deviation from this pattern has been detected in ecosystems subjected to high ultraviolet radiation (UVR) flux and with low dissolved organic matter (DOM) content or colorless waters, which showed autotrophic biomass dominance (Elser *et al.*, 2003; Medina-Sánchez *et al.*, 2004; Duarte *et al.*, 2005; Zubkov y Tarran, 2008; Straškrábová *et al.*, 2009). Alterations in the physical chemical conditions of the water masses, with effects on any of the components of the microbial trophic web, could have far reaching consequences for the dominance of autotrophic or heterotrophic metabolism and, hence, for the general functioning of the ecosystem and carbon cycle. Global change encompasses different changes in the environmental conditions. Particularly, in the Mediterranean region, the expected higher frequency of droughts (Baldi *et al.*, 2006; Stocker *et al.*, 2013; Giorgi y Lionello, 2008; Garcia-Herrera *et al.*, 2014), together with land use change and heavy rain episodes are increasing the input of dissolved organic matter (DOM) and inorganic nutrients to freshwater systems (Solomon *et al.*, 2007a; Moss *et al.*, 2012). Due to the nature of the nutrient sources, the Mediterranean aquatic ecosystems are subjected to nutrient pulses, i.e. episodes of increased resource availability that combine low frequency, large magnitude and short duration (Yang *et al.*, 2008). Because of the pulsed resource dynamics have been found in a wide diversity of systems, ecologists are becoming increasingly aware of the importance of pulsed nutrient subsidies in shaping community function and structure in aquatic ecosystems (Svensen *et al.*, 2002; Nowlin *et al.*, 2008; Weber y Brown, 2013). These resource pulses can have different effects depending on the trophic state of the system, since resource pulses could have stronger stimulatory effects during scarcity periods than during higher availability periods (Yang *et al.*, 2008). Similarly, organisms' response to nutrient pulses

can be mediated by the adaptations to previous nutritional environments, in such a way that pulses with similar magnitude could result in different final responses (Plaetzer *et al.*, 2005; Aubriot *et al.*, 2011). Moreover, in oligotrophic ecosystems the nutrient and DOM inputs might be crucial for coping with the negative effects of the expected increase in incident UVR (Manney *et al.*, 2011; Stocker *et al.*, 2013). Thus, nutrients, besides altering the structure and functioning of the microbial planktonic web (Christoffersen *et al.*, 2006; Medina-Sánchez *et al.*, 2013; Calbet *et al.*, 2014), might enhance mechanisms of reparation of UVR damage (Häder y Sinha, 2005, and references therein). On the other hand, higher DOM content could act as a sunscreen against the direct effect of incident ultraviolet radiation on organisms (Williamson *et al.*, 2010).

It is increasingly acknowledged that the interaction among factors affected by global change is more complex than predicted from the additive effect of single factors (Crain *et al.*, 2008; Christensen *et al.*, 2006). Thus, the interactive effects depend, among others, on the type of organism, the response variables, the biotic interactions and the period of study. Thus, under UVR and P addition, in high mountain lakes, changes in community structure have been evidenced, such as a long term decrease in mixotroph abundance against a development of a low diversity algal community dominated by strict autotrophs (Delgado-Molina *et al.*, 2009) or a transitory development of heterotrophic microbial food web (bacteria, heterotrophic nanoflagellates, ciliates, and viruses) at moderate P concentrations to be replaced by the algal community which became the dominant microplankton (Medina-Sánchez *et al.*, 2013). With regard to functional variables, at short term nutrient addition (under non fluctuating UVR regime) can attenuate the UVR inhibitory effect on primary production (Medina-Sánchez *et al.*, 2006; Helbling *et al.*, 2013, PP;) and on heterotrophic bacterial production (HBP), and even when bacteria are also exposed to a mixing regime (Chapter II). However, the interaction between UVR and nutrients can shift over time, and an exacerbation of the UVR inhibitory effect has been found under nutrient addition on PP (Carrillo *et al.*, 2008a; Korbee *et al.*, 2012) and on HBP (Ogbebo y Ochs, 2008) at longer temporal scales. Therefore, long term analyses of multiple factor interactions are being demanded (Darling

y Côté, 2008), especially when factors act at different temporal scales (i.e. chronic, e.g. UVR; pulsed, e.g. nutrient pulses).

Notably, the interaction among abiotic factors can condition the type of relationship among organisms, a topic still scarcely dealt with in the literature. The commensalistic algal bacterial relationship mediated by the excreted organic carbon (EOC), evidenced in numerous studies (Aota y Nakajima, 2001; Carrillo *et al.*, 2002; Morán *et al.*, 2002; Duarte *et al.*, 2005), can be reinforced by higher UVR exposure in upper water layer through increased EOC (Carrillo *et al.*, 2002; Medina-Sanchez *et al.*, 2002; Korbee *et al.*, 2012), which stimulates UVR resistant bacterial growth (Carrillo *et al.*, 2002; Xenopoulos y Schindler, 2003). However, this commensalistic relationship can shift to competition for inorganic nutrients in oligotrophic environments subjected to P inputs (Villar-Argaiz *et al.*, 2002) or to mutualism based on mixotrophic metabolism under UVR (Rojo *et al.*, 2012) and under the joint action of UVR and P inputs (Medina-Sánchez *et al.*, 2004). The dominant relationship will determine the direction of the carbon flux in the system and the corresponding higher development of the grazing or the microbial food web.

From above, the aim of this study was to contribute to an understanding of how an interactive effect of global change factors on algae microbial loop can alter the pathways of energy and nutrient mobilization, affecting the predominance of the heterotrophic vs. autotrophic compartments. For this study a model oligotrophic ecosystem (La Conceja) was selected because the commensalistic algal bacterial relationship has been demonstrated to be sensitive to short term changes in UVR exposure linked to stratification (Chapter I), making this ecosystem ideal to study this topic. We hypothesize that cumulative P pulses under UVR may lead to a shift in the long term microplankton community composition, with a higher development of bacterial and bacterivorous compartment (mixotrophic algae and ciliates) against the weakening of the strict autotrophic compartment.

5.3. Methods

5.3.1. Study site

This study was performed in La Conceja lake, situated at 37°/2° W and 850 m.a.s.l. in Lagunas de Ruidera Natural Park (Spain) with a depth of 14 m (Rojo *et al.*, 2012). It was considered a P limited and N enriched Mediterranean oligotrophic system with concentrations of total phosphorus (TP) of $<10\mu$g P L^{-1} and total nitrogen (TN) of $\sim$15 mg N L^{-1} (Álvarez y Cirujano, 2007; Rojo *et al.*, 2012). Because nitrogen (N) is the most common limiting nutrient of crop production in terrestrial ecosystems and is used in large quantities, La Conceja lake is polluted by nitrate inputs through groundwater, originating mainly from agricultural irrigation (Alvarez-Cobelas *et al.*, 2006). Moreover, TP has increased during the last 30 years mainly due to the impact of tourism and land use changes (Álvarez y Cirujano, 2007). Thus, the lake has a high N:P ratio (Álvarez y Cirujano, 2007) which results in a P limitation for planktonic community growth. The lake is monomictic with a well defined stratification which begins in March April and ends in October (Álvarez y Cirujano, 2007). The dissolved organic carbon (DOC) ranged between 2.5 and 3.5 mg C L^{-1} and the Secchi disk depth between 6-8 m (Rojo *et al.*, 2012).

5.3.2. Experimental design

A long term in situ experiment was conducted in La Conceja lake in 2009: from 11^{th} to 28^{th} in July. The experiment had a 2×2 factorial design in triplicate with two radiation treatments, (i) UVR+PAR (>280 nm; treatment UVR) and (ii) exclusion of UVR (>400 nm; treatment PAR); and two nutrient treatments, (i) ambient nutrient concentration (P- ambient) and (ii) P- addition (P-added). Each treatment used a mesocosm consisting of a cylinder (70 cm in diameter, 1.5 m deep and a total volume of 600 liters) which was tied and fixed with a weight at the base to maintain a vertical position. The mesocosm depth was chosen according to the depth of the lake reached by >1 % UV-B of the surface. For the UVR treatments, clear polyethylene plastic (which transmits $\sim$60 % [280

nm] and >80 % [400 700 nm]) was used. A modified polyethylene which blocks the UVR (transmitting <1 % [≤380nm] and 85 % [≥400nm]) was used for the PAR treatments (Souza *et al.*, 2010). The optical features of this polyethylene were checked before the experiment using a double-bean spectrophotometer (Shimadzu UV2450). Each mesocosm was covered with a cap made in UVR-opak 395 filter (Ultraphan, Difegra) or polyethylene for the PAR and UVR treatments, respectively. Besides determining the regime of radiation, the cap avoided possible uncontrolled P inputs inside the mesocosm. Each mesocosm was filled with water filtered through 45μm pore size (in order to remove the zooplankton) from the photic layer receiving >1 % UV-B. The mesocosms were supported by means of a rack constructed with polyvinyl chloride pipe. The racks were secured to a buoy attached to an anchor rope.

P added treatments were made by adding KH_2PO_4 in two consecutive pulses at the initial day and at day 11 of the experiment (Fig. 5.1) to a final concentration of 30 μg P L^{-1} each, to maintain the P availability over the course of the experiment. We added no nitrogen (N) to the enclosures because La Conceja, due to the N loads from fertilization in agriculture, is a N-enriched system and N was not limiting even in P-added enclosures (DIN:TP >400, see Table 5.1). Samples for organic and inorganic analysis were taken at day one (T1) and two (T2) after first P pulse and at day 12 (T3) and 17 (T4) after the second P pulse (Fig. 5.1). To simplify the presentation and understanding of the results and discussion, hereafter we use "first period" of the experiment to refer to the times T1 and T2, and "second period" of the experiment to refer to the times T3 and T4. The temporal structure of the experiment is shown in Fig. 5.1.

5.3.3. Physical analyses

Vertical profiles of solar radiation and temperature in the water column were recorded around noon with a BIC compact 4-channel underwater radiometer (Biospherical Instruments Inc., CA, USA), which has three channels in the UVR region of the spectra (305, 320 and 380 nm) and one broad-band channel for PAR (400-700 nm). This radiometer also has tem-

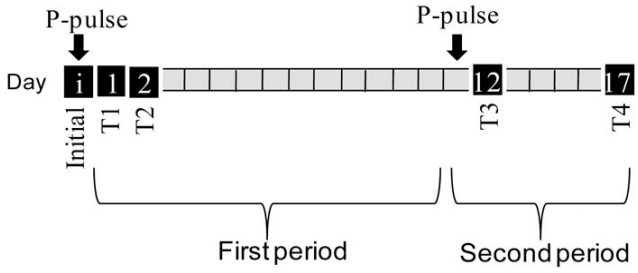

Figura 5.1: Temporal structure of the experiment.

perature and depth sensors. Diffuse attenuation coefficients for downward irradiance (k_d) were determined from the slope of the linear regression of natural logarithm of downwelling irradiance vs. depth for each wavelength range considered. A large sample size (pairs of irradiance and depth values, n >200) was used and a good fit (R^2> 0.95) was found for all regressions.

5.3.4. Chemical variables

Before the sampling, the water column was gently mixed over the entire length of each mesocosm and then integrated depth samples were taken using a plastic bucket (5 L).

Water samples were collected in triplicate from the lake and each mesocosm to determine total nitrogen (TN), dissolved inorganic nitrogen (DIN), total phosphorus (TP), and total dissolved phosphorus (TDP). TN was determined by the ultraviolet (UV) spectrophotometric screening method (APHA, 1992). DIN was considered the sum of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+), which in turn was determined by UV-spectrophotometric screening, sulfamide and phenol-hypochlorite techniques, respectively. TP and TDP were analyzed by means of the acid molybdate technique after persulfate digestion (APHA, 1992). P particulate (P_{part}) was calculated as the difference between PT and PDT.

Chlorophyll *a* (Chl *a*) was measured fluorimetrically by gridding fil-

ters (Whatman GF/F glass-fiber filters, 25 nm in diameter) with pigments (concentrated by filtering 300 ml at <100 mm Hg) and extraction of the pigments in 90 % acetone kept in the dark at 4°C for 24 h. A Chl *a* standard (Chl *a* from spinach, Sigma) was used to transform the fluorescence data into Chl *a* concentrations.

Dissolved organic carbon (DOC) values were determined by filtering the samples through pre-combusted (2h at 500°C) glass-fiber filters (Whatman GF/F) and acidifying them with HCl. Samples were then measured in a total organic carbon analyzer (TOC-V CSH/CSN Shimadzu).

5.3.5. Biological analysis

The method described by Uthermöl (1958) was followed to determine nanophytoplankton abundance (ANP abundance; >3μm) and heterotrophic ciliate abundance in samples preserved in 250 mL brown glass bottles and fixed with alkaline Lugol's reagent (1 % vol vol^{-1}). A volume of sample (50-100 mL) was allowed to settle for 48 h in a Uthermöhl chamber (Hydro-Bios GmbH, Germany) and species were identified, enumerated and measured using an inverted microscope (Leitz, Labovert) for abundance and cell biovolume determination. Cell shape was assessed through approximation to a geometrical shape (Hillebrand *et al.*, 1999) and converted to nanophytoplanktonic biomass (ANP biomass) and ciliates biomass by using the conversion factors of Rocha y Duncan (1985) and Vaqué *et al.* (1997), respectively.

Autotrophic picoplankton (APP; <3μm) was fixed with formaldehyde at a final concentration of 2 %. Samples for APP abundance (APP abundance) determination were processed in less than one day after their collection by filtering through 0.2 μm black polycarbonate membrane (Millipore) filters and counted by autofluorescence observation with a Nikon epifluorescence microscope at 1.250× magnification and with appropriate filters (Rodrigo *et al.*, 2003). Bacterial abundance was determined by staining with 4'-6- diamidino-2-phenylindole (DAPI) to a final concentration of 2.5 μg ml^{-1} (Porter y Feig, 1980). Water samples were previously fixed with neutralized formalin (2 %) at the moment of sample collection. After the

DAPI staining the samples were filtered through a 0.2-μm pore-size polycarbonate black Nucleopore Filter. At least 400 cells per sample were counted by epifluorescence light microscopy (Karl Zeiss AX10).

To count and measure the bacteria and APP cells, we used photographs taken of the preparations where the cells were counted using Image J software (National Institutes of Health, USA). For volume determination, the volume of cocci was approximated to a sphere whereas bacillus and filamentous forms were approximated to an ellipse.Bacterial biomass was estimated with the conversion factor of Posch *et al.* (2001) and APP biomass using that of Menden-Deuer y Lessard (2000).

Heterotrophic bacterial production (HBP) was determined by incorporating ^{3}H-thymidine (S.A= 52 Ci mmol^{-1}, Amersham Pharmacia) into the bacterial DNA (Fuhrman y Azam, 1982; Smith y Azam, 1992). ^{3}H-thymidine was added to each experimental vial with 1.5 ml of water sample (3 replicates and 2 blanks for each treatment) to a final concentration of 15.2 nM. The final concentration was saturating and was determined by a previous experiment using increasing concentrations of ^{3}H-thymidine (data not shown). Vials were incubated in situ for one hour in darkness at 0.5 m deep. After incubation, the incorporation of ^{3}H-thymidine was stopped with the addition of 100 μL 100 % thrichloroacetic acid (TCA). Blanks were killed with 100 μL 100 % TCA before the addition of the radiotracer. Precipitation was prompted by adding 5 % TCA and keeping the sample on ice for 20 min. The precipitate was collected by centrifugation (1600g for 10 min). Then, vials were rinsed twice with 1.5 ml of TCA 5 % to remove any residual unincorporated radioactivity. Finally, scintillation liquid (Ecoscint A) was added for subsequent measurement in an autocalibrated scintillation counter (Beckman LS 6000TA). The conversion factor 1.5×10^6 cell mol^{-1} (Bell, 1993) was used to estimate the number of bacteria produced per mol of incorporated thymidine and the factor 2×10^{-14} g C cell^{-1} (Lee y Fuhrman, 1987) was applied to estimate the amount of carbon.

To estimate the primary production (PP), we added with 0.37 MBq of $NaH^{14}CO_3$ (SA: 310.8 MBq mmol^{-1}, DHI Water Environment, Germany) to sets of four 50 mL-quartz (UVR treatments) or -glass (PAR treatments) flasks (three clear and one dark for each experimental treatment) and incu-

bated them in situ at 0.5 m under polyethylene plastic (UVR samples) or under Ultraphan 395 filter (PAR samples) for 5 h symmetrically distributed around noon. All the flasks were horizontally held during the incubations. PP calculations were based on the ^{14}C method (Lignell, 1992). Total organic carbon (TOC) was measured from 4 mL subsamples collected before filtration. The ^{14}C retained in particulate matter >3μm (nanoplanktonic primary production, PP_n) was segregated from particulate matter 3-0.2μm (picoplanktonic primary production, PP_p) using 3μm-pore-size filters of 25 mm in diameter (Nucleopore Whatman). PP_p was segregated from the dissolved fraction (<0.2 μm; excreted organic carbon, EOC) by using 0.2 μm-pore-size filters of 25 mm diameter (Nucleopore Whatman). Low pressure (<100 mmHg) was applied to minimize cell breakage.

Bacterivory was assessed as the proportion of HBP incorporated by the bacterivorous fraction (heterotrophic and mixotrophic) by using ^{3}H-thymidine incorporation (Medina-Sánchez *et al.*, 2004). ^{3}H-thymidine was added to each experimental vial with 24 ml of water sample (3 replicates and 1 blank per treatment) to a final concentration of 15.2 nM. The samples incubated in situ were subjected to the different light treatments. The incorporation of ^{3}H-thymidine incorporation was stopped with the addition of neutralized formaldehyde to a final concentration of 3.4 %. Likewise, blanks were killed with neutralized formaldehyde before the radiotracer was added. After incubations, 1.5 ml was taken from the sample in order to assess the total HBP (HBP_t) and the rest of the water was filtered by 3-μm pore-size Nucleopore filter. This filter was used to quantify the HBP incorporated by the bacterivorous fraction (HBP_b), whereas the sample filtered through 3 μm was used to quantify the remnant HBP (not incorporated by bacterivores). The filters containing the HBP_b fraction were rinsed twice with 1.5 ml of acetone to dissolve them. Then all fractions were subjected to a similar procedure as the HBP (see above).

To calculate the percentage of HBPt incorporated by the bacterivores ($\%HBP_b$), we used the following equation:

$$\%\mathrm{HBP}_b = HBP_b/HBP_t \times 100 \tag{5.1}$$

To assess the bacterivore abundance (BV abundance) and biomass (BV biomass) we considered the ciliates and, the potential mixotrophic groups in the water samples to be potential bacterivores (BV): Cryptophyceae, Chrysophyceae, and Dinophyceae. The groups Chlorophyceae, Bacillariophyceae and Zygnemophycea were considered to be strict autotrophic nanophytoplankton. We also calculated the bacterial grazing rate as the number of bacteria consumed per bacterivorous cell. For this, HBP_b was expressed as the number of bacteria produced (see above HBP protocol) divided by BV abundance in each treatment.

5.3.6. Data treatment and statistics

The percentage of inhibition (UVR_{inh}) on the different variables by UVR was calculated as:

$$\mathrm{UVR}_{inh}\ (\%) = 100[(PAR - UVR)]/PAR \tag{5.2}$$

where PAR and UVR represent the value of the variable in samples under the PAR and UVR treatments, respectively. We used propagation errors to calculate the variance of the inhibition due to UVR.

To determine significant interactions among the radiation treatment and P addition at each different date, two-way analyses of variance (ANOVA) were used. Furthermore, a t-test for dependant samples was performed in order to compare the effect size of the first P-pulse (day 1) to the second P-pulse (day 12) on microbial variables. Effect of P-pulses were assessed by comparing P-added with non-added treatments at each date. When a significant interactive effect of the factors on a response variable was found, a *post hoc* Fisher LSD test was used to determine significant differences among treatments.

Stepwise multiple-regression analyses were performed to assess the relative strength of abiotic (i.e. TDP, P_{part}) and biotic controls (i.e. $\%HBP_b$, BV abundance, EOC) regulating the trophic web (via bacterial abundance and HBP, as dependent variables) under P-added conditions. Linearity and orthogonality among independent variables were verified by a previous

	Initial conditions
TN (μmol L^{-1})	1065±3
TP (μmol L^{-1})	0.15±0.01
DIN (μmol L^{-1})	758±126
DIN:TP (μmol)	4519±1108
DOC (μmol L^{-1})	156±9
Chl *a* (μg L^{-1})	1.29±0.03
PA (cell mL^{-1})	1.4
PB (μg C L^{-1})	78.6
APPA (cell mL^{-1})$\times 10^3$	2.0
APPB (μg C L^{-1})	0.54
BA (cell mL^{-1})$\times 10^5$	2.5
BB (μg C L^{-1})	2.04

Cuadro 5.1: Mean values of the main chemical and biological variables studied in the water column under the initial conditions of the experiment.TN: total nitrogen; TP: total phosphorus; DIN: dissolved inorganic nitrogen; DOC: dissolved organic carbon; Chl *a*: chlorophyll *a*; PA: nanophytoplanktonic abundance; PB: nanophytoplanktonic biomass; APPA: autotrophic picoplankton abundance; APPB: autotrophic picoplankton biomass; BA: bacterial abundance; BB: bacterial biomass; PP_n: nanoplanktonic primary production; PP_p: picoplanktonic primary production; HBP: heterotrophic bacterial production.

correlation analysis and controlled by specifying 0.6 as the minimum acceptable tolerance (Stat-Soft Inc, 2005). The F values entering the multiple-regression model were established on the basis of the number of independent variables and cases. Statistica 7.0 for Windows (Statsoft 2001) was used for the statistical analysis.

5.4. Results

5.4.1. Initial conditions and dynamics of physical variables during the experimental periods

Under the initial experimental conditions , La Conceja lake had a high TN but low TP concentration, which resulted in a high DIN:TP ratio, the lake being limited by P (Table 5.1). The microbial community was composed by Bacillariophyceae, which constituted 46.28 % of the total ANP abundance, Chlorophyceae 28 %, Chrysophyceae the 7.1 %, Dinophyceae the 2.7 %, Cryptophyceae the 1 %, and other small non-identified flagellates which were 14.5 % of total density. Therefore, the potential plastidic bacterivores (see Methods) represented the 25.3 % of total ANP abundance, i.e. 3.7×10^2 cell mL^{-1} which meant 18 % of phytoplanktonic biomass (15.51μg C L^{-1}); whereas ciliates, also potential bacterivores, had an abundance of 13 cell mL^{-1} (2.81μg C L^{-1}). Thus, potential bacterivorous (mixotrophs and ciliates) accounted 15.37μg C L^{-1} (18 % of microplanktonic biomass).

Regarding the physical conditions during the experiment, UV-B was strongly attenuated in the upper water layers of the lake as indicated by the high values of k_d (Fig. 5.2) and, for example, 1 % of the surface energy at 305 nm reached only ca. 1.5 m deep. By contrast, up to 2 % of surface irradiance of both UVR_{380} and PAR reached the bottom of the lake (14 m). The vertical profile of temperature was stable over the experiment with a thermal discontinuity at 2-4 m (variation $\sim$1°C), and a thermocline at 8-10 m (variation >2°C; Fig. 5.2).

5.4.2. Dynamics of chemical characteristics and structure of the microbial community in mesocosm experiment

TN (1078 μmol N L^{-1}), DOC (170 μmol C L^{-1}) and DIN:TP (>2000 molar ratio) were quite similar among treatments (ANOVA $p>0.05$) over the experiment (t-test $p>0.05$; Table 5.2). Under P-ambient treatments, TP, TDP and P_{part} values were steady over the experiment and among radiation treatments (t-test>0.05; Table 5.2). However, under P-added condi-

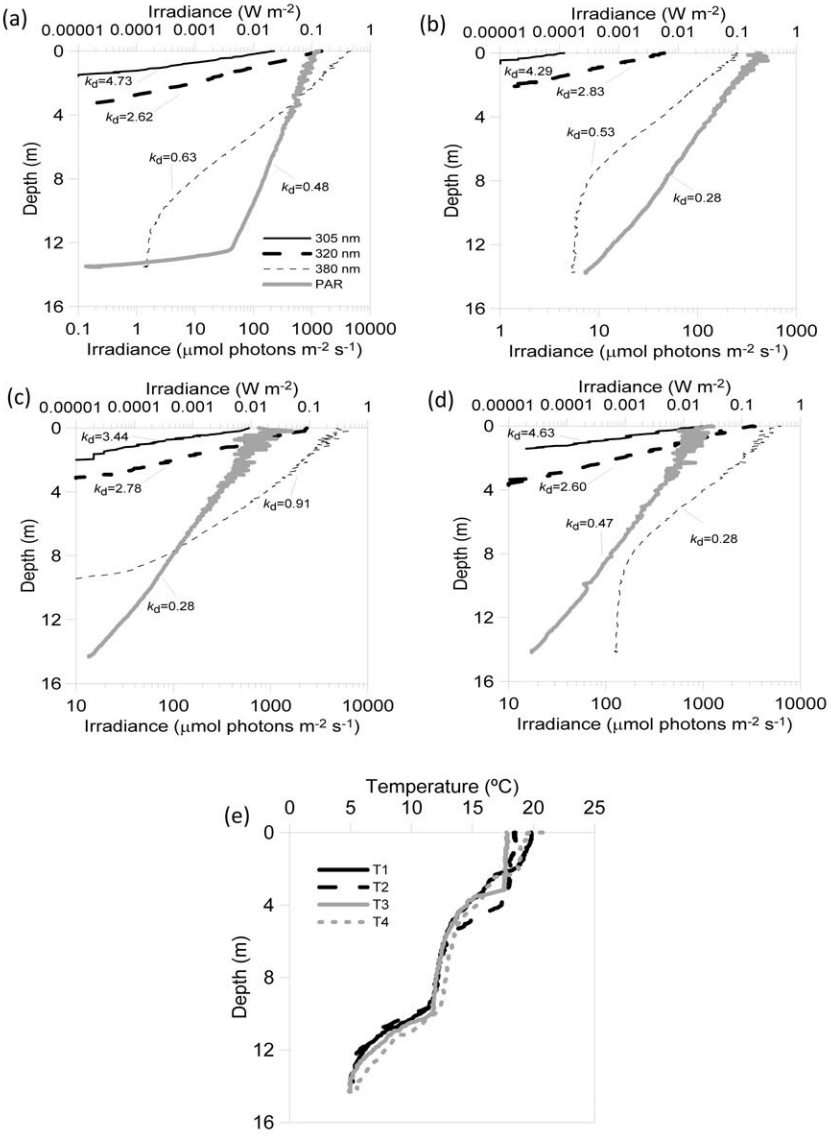
(a)
Irradiance (W m-2)
0.00001 0.0001 0.001 0.01 0.1 1
Depth (m)
kd=4.73
kd=2.62
kd=0.63
kd=0.48
305 nm
320 nm
380 nm
PAR
0.1 1 10 100 1000 10000
Irradiance (μmol photons m-2 s-1)
(b)
Irradiance (W m-2)
0.00001 0.0001 0.001 0.01 0.1 1
Depth (m)
kd=4.29
kd=2.83
kd=0.53
kd=0.28
1 10 100 1000
Irradiance (μmol photons m-2 s-1)
(c)
Irradiance (W m-2)
0.00001 0.0001 0.001 0.01 0.1 1
Depth (m)
kd=3.44
kd=2.78
kd=0.91
kd=0.28
10 100 1000 10000
Irradiance (μmol photons m-2 s-1)
(d)
Irradiance (W m-2)
0.00001 0.0001 0.001 0.01 0.1 1
Depth (m)
kd=4.63
kd=2.60
kd=0.47
kd=0.28
10 100 1000 10000
Irradiance (μmol photons m-2 s-1)
(e)
Temperature (ºC)
0 5 10 15 20 25
Depth (m)
T1
T2
T3
T4

Figura 5.2: Depth profile of the irradiance (a, b, c, d) and temperature (e) in the water column at the different sampling dates. Irradiance data in the UVR range are expressed in W m^{-2} , PAR is in μmol photons m^{-2} s^{-1}. (a) T1, (b) T2, (c) T3 and (d) T4.

tions, we found higher values of TP during the second period of the experiment (T3 and T4) as compared with the first period (T1 and T2), whereas the opposite was found for TDP (higher values at T1 and T2; Table 5.2).

Microplanktonic biomass was dominated by autotrophic organisms (> 50 % of total biomass) over the experiment regardless of the radiation and nutrient treatment (Fig. 5.3), rising >90 % in the P-added treatments (Fig. 5.3b, d). The fraction >3μm (ANP) accounted for about >80 % of the autotrophic biomass in all treatments whereas the fraction <3μm (APP) constituted around 0.5 % under P-ambient conditions and up to 20 % in P-added treatments at T4 (Fig. 5.3). Nanophytoplankton was dominated by Bacillariophyceae (>50 % of nanophytoplankton abundance and biomass) until T3, when it was replaced by Chlorophyceae, particularly in the P-added treatments (>65 % of nanophytoplankton abundance and biomass; Fig. 5.4). Bacillariophyceae was represented by *Cyclotella kutzingiana* and *C. ocellata* whereas Chlorophyceae was dominated mainly by *Nanochloropsis* sp.

Potential mixotrophic algae represented up to 10 % (in P-ambient treatments) or 32 % (in P-added treatments) of the total microplanktonic biomass until T3 (Fig. 5.3). These potential mixotrophic groups were composed by Chrysophyceae (*Dynobrion* sp. and *Ochromonas* sp.), Dinophyceae (*Ceratium* sp., *Gymnodiniun* sp., *Katodinium* sp. and *Peridinium* sp.), Cryptophyceae (*Cryptomonas* sp. and *Plagioselmis* sp.) and other small non-identified flagellates. Mixotrophs constituted >28 % of ANP abundance at T1 and T2, and <10 % of ANP abundance at the end of the experiment (T4) (Fig. 5.4).

Ciliates were present in all the treatments, with higher abundance in the

Date	Treatment	TN (μmol N L^{-1})	TP (μmol P L^{-1})	TDP (μmol P L^{-1})	P_{part} (μmol P L^{-1})	DIN (μmol N L^{-1})	DIN/TP molar	DOC (μmol C L^{-1})
T1	UVR	1048±18	0.15±0.02	0.166±0.05	0.04±0.06	1015±191	6411±691	145±2
	UVR+P	1050±4	1.18±0.26	0.74±0.17	0.52±0.09	929±80	853±150	127±2
	PAR	1047±24	0.21±0.02	0.115±0.05	0.032±0.07	980±19	4393±1054	143±4
	PAR+P	1044±6	1.05±0.04	0.64±0.07	0.46±0.02	1176±25	1104±36	144±3
T2	UVR	1046±11	0.18±0.02	0.14±0.02	0.03±0.02	823±249	5705±1327	128±9
	UVR+P	1074±2	1.26±0.26	0.46±0.08	0.89±0.35	979±23	835±165	125±5
	PAR	1061±5	0.15±0.03	0.14±0.03	0.01±0.01	1049±140	6052±1762±	132±3
	PAR+P	1073±4	1.09±0.12	0.34±0.05	0.74±0.17	746±354	470 ±55	129±2
T3	UVR	1121±18	0.15±0.01	0.15±0.02	0.02±0.01	1247 ±37	7742±1033	139±8
	UVR+P	1103±36	2.34±0.13	0.22±0.02	2.1±0.13	1233±37	519±41	180±13
	PAR	1134±11	0.68±0.91	0.13±0.01	0.55±0.90	1281±13	8873±510	168±6
	PAR+P	1091±2	2.35±0.32	0.22±0.02	2.1±0.30	1209±62	480±68	202±8
T4	UVR	1104±24	0.31±0.05	0.15±0.02	0.18±0.04	817±76	2785±577	134±12
	UVR+P	1086±47	0.60±0.12	0.17±0.01	0.41±0.12	1159±17	2061 ±444	154±9
	PAR	1099±11	0.37±0.17	0.10±0.01	0.25±0.18	1121±61	3213±1224	137±5
	PAR+P	1073±17	0.50±0.03	0.18±0.03	0.34±0.04	991±187	1995±161	181±18

Cuadro 5.2: Mean values of the main chemicalvariables studied under the different experimental treatments.TN: total nitrogen; TP: total phosphorus; TDP: total dissolved phosphorus; P_part: particulate phosphorus; DIN: dissolved inorganic nitrogen; DOC: dissolved organic carbon

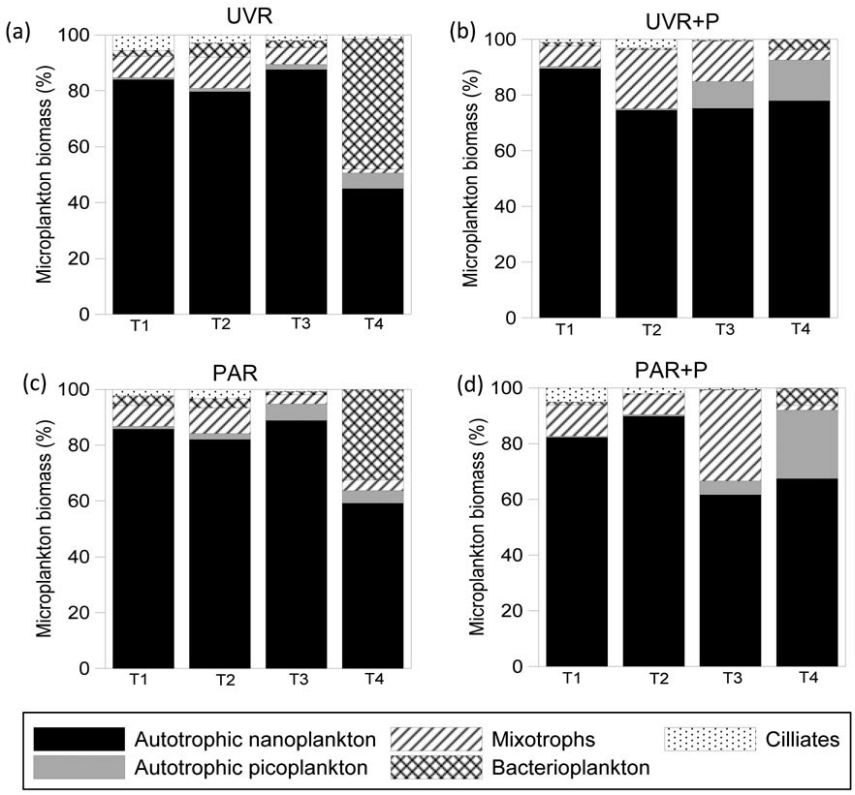

Figura 5.3: Biomass variation of each fraction of the microplantonic community over the experiment.

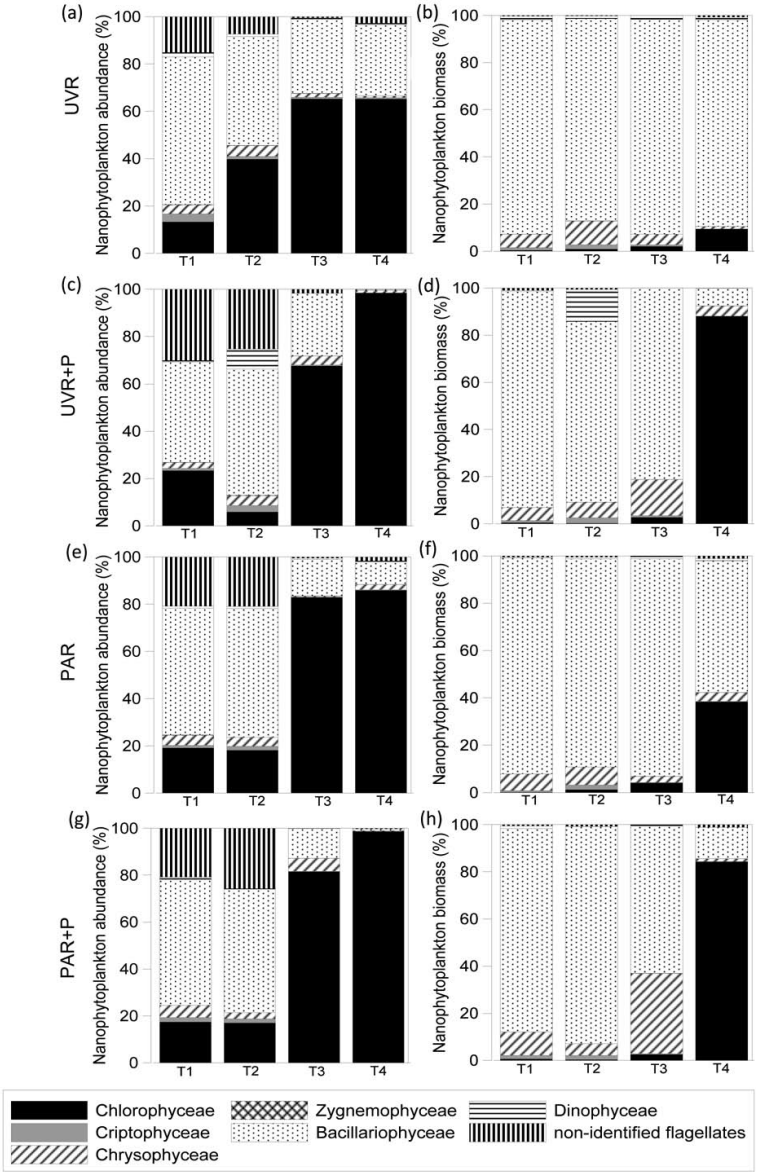
(a)
UVR
Nanophytoplankton abundance (%)
(b)
Nanophytoplankton biomass (%)
(c)
UVR+P
Nanophytoplankton abundance (%)
(d)
Nanophytoplankton biomass (%)
(e)
PAR
Nanophytoplankton abundance (%)
(f)
Nanophytoplankton biomass (%)
(g)
PAR+P
Nanophytoplankton abundance (%)
(h)
Nanophytoplankton biomass (%)
0
20
40
60
80
100
T1
T2
T3
T4
Chlorophyceae
Criptophyceae
Chrysophyceae
Zygnemophyceae
Bacillariophyceae
Dinophyceae
non-identified flagellates

Figura 5.4: Changes in nanophytoplanktonic composition as abundance (left panels) and as biomass (right panerls) over the experiment.

P-added treatments, but they were reduced at T4, disappearing under UVR in P-added conditions and under PAR in P-ambient conditions (Fig. 5.3). The bacterivorous assemblage (both mixotrophs and ciliates) accounted for 4-25 % of the total microplanktonic biomass (Fig. 5.3) whereas heterotrophic nanoflagellates represented a negligible fraction. Bacterioplankton accounted for 1-3 % of the total microplanktonic biomass under P-ambient conditions, but this percentage increased remarkably at T4 (up to 30-40 %, Fig. 5.3a, c). P addition decreased the proportion of bacterioplankton at all sampling dates (Fig. 5.3b, d).

5.4.3. Interactive effects of solar radiation and P-pulses on microplanktonic community structure

Under P-ambient conditions, UVR decreased APP abundance and APP biomass at intermediate times (T2 and T3, Table 5.3) and BV abundance and BV biomass at T4; Table 5.3). Contrarily, UVR increased abundance and biomass of ciliates at T1 (Table 5.3).

The first P-pulse stimulated ANP abundance, APP abundance, BV abundance; (LSD test, $p<0.05$; Fig. 5.5a, c, f), and the biomass of autotrophic picoplankton (APP biomass; Fig. fig:memoria5d). The second P-pulse also stimulated these variables as well as the biomass of the nanophytoplankton (ANP biomass) and bacterivorous assemblages (BV biomass) (LSD test, $p<0.05$; Fig. fig:memoria5b, d, f). Noticeably, in agreement with our first hypothesis, the stimulus was higher after the second than the first P-pulse on abundance (t-test; ANP abundance: $p=0.06$; APP abundance: $p<0.05$; BV abundance: $p<0.01$; Fig. fig:memoria5a, c, f) and on biomass (ANP biomass, BV biomass, APP biomass; t-test, $p<0.05$; Fig. fig:memoria5b, d, f).

	T1		T2		T3		T4	
Variable	-P	+P	-P	+P	-P	+P	-P	+P
PA	ns	ns	ns	ns	ns	97	ns	ns
PB	ns	ns	ns	ns	ns	58	ns	-62
APPA	ns	-34	46	ns	69	ns	ns	ns
APPB	ns	-34	46	ns	69	ns	ns	ns
BA	ns	ns	ns	ns	ns	ns	ns	ns
BB	ns	ns	ns	ns	ns	ns	67	67
CA	-204	75	ns	ns	ns	59	-	-
CB	-204	83	ns	ns	ns	59	-	-
BVA	ns	ns	ns	ns	ns	63	37	-77
BVB	ns	60	-3	-51	ns	62	43	5
PP_n	62	22	40	41	ns	87	ns	22
PP_p	93	-274	62	42	ns	-1095	ns	79
EOC	55	-60	56	43	ns	-16	ns	63
HBP	46	-24	ns	-15	ns	ns	ns	-13

Cuadro 5.3: Percentage of UVR inhibition (UVR_{inh}) on structural and functional variables under the indicated treatments and dates. ns: not significant, indicates that differences between UVR and equivalent PAR treatment for each nutrient treatment were not found (LSD test>0.05). PA: nanophytoplankton abundance; PB: nanophytoplankton biomass; APPA: autotrophic picoplankton abundance; APPB: autotrophic picoplankton biomass; BA: bacterial abundance; BB: bacterial biomass; CA: cilliate abundance; CB: cilliate biomass; BVA: bacterivorous abundance; BVB: bacterivorous biomass; PP_n: nanoplanktonic primary production; PP_p: picoplanktonic primary production; EOC: excreted organic carbon; HBP: heterotrophic bacterial production. -P: P-ambient; +P:P-added.

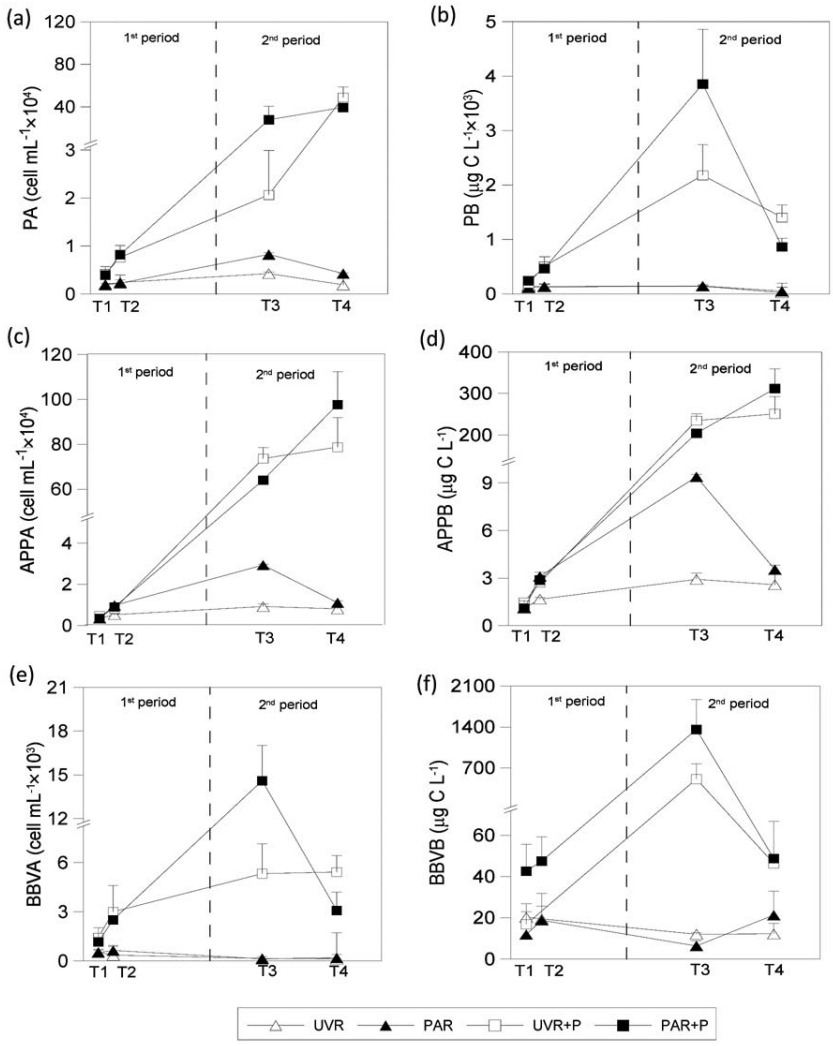
(a)
PA (cell mL-1×10⁴)
1st period
2nd period
T1 T2 T3 T4
(b)
PB (µg C L-1×10³)
(c)
APPA (cell mL-1×10⁴)
(d)
APPB (µg C L-1)
(e)
BBVA (cell mL-1×10³)
(f)
BBVB (µg C L-1)
UVR
PAR
UVR+P
PAR+P

Figura 5.5: Temporal responses of abundance (left panels) and biomass (right panels) of different components of the microplanktonic community over the experiment. PA: nanophytoplankton abundance; PB: nanophytoplankton biomass; APPA: autotrophic picoplankton abundance; APPB: autotrophic picoplankton biomass; VA: bacterivorous abundance; BVB: bacterivorous biomass. Symbols represent mean values and error bars represent standar deviations (SD) (n=3).

UVR and P had significant interactive effect only on nanophytoplankton after the second P-pulse (ANP abundance at T3; ANP biomass at T4; Table 5.5S1, 5.6S2), inhibiting UVR on ANP-abundance but stimulating ANP biomass (Table 5.3). For the bacterivorous assemblage, UVR, and P had a significant interactive effect on BV-abundance at T3, and on their biomass from T1 to T3 (Table 5.5S1, 5.6S2), resulting in an inhibitory UVR effect on both variables at T3 (Table 5.3), but stimulatory on BV biomass at T2 (Table 5.3).

Regarding the autotrophic picoplankton, a significant interactive effect between UVR and P was found on APP biomass at T1 and T3 (Table 5.6S2), resulting in a stimulatory UVR effect on both dates (Table 5.3). On the contrary, no significant interactive effect between the two factors was found on bacterial abundance or biomass, although both variables increased under both radiation treatments at T4 (Fig. 5.6a, b).

5.4.4. Interactive effects of solar radiation and P-pulses on the microplanktonic community function

Under ambient P conditions, UVR exerted an inhibitory effect on HBP over the short term (T1; Table 5.3) and on PP_n and PP_p (T1, T2; Table 5.3). P addition had a stimulatory effect on HBP (LSD test, $p<0.001$; Fig. 5.7a) and on both fractions of PP (LSD test; $PP_n= p<0.05$; $PP_p= p<0.001$; Fig. 5.7b, c) regardless of the radiation treatment, with a significantly higher

effect after the second than after the first P-pulse for all the variables, although only under PAR for PP_n (t-test, $p<0.05$; Fig. 5.7b).

P interacted with UVR by different ways among variables and time (Table **??**S3). Thus, (i) first P-pulse reversed the inhibitory UVR effect on HBP over the short term (T1), resulting in a stimulatory UVR effect (Table 5.3). This effect remained after the second P-pulse (T4), although this was transiently masked at T3 (Table 5.3; Fig. 5.7a). (ii) The first P-pulse attenuated the inhibitory UVR effect on PPn (T1), but the second P-pulse (T3) accentuated it (Table 5.3; Fig. 5.7b). (iii) P addition generated an early stimulatory UVR effect on PPp after each P-pulse (i.e. at T1 and T3) but it turned inhibitory later after the pulses (i.e. at T2 and T4; Table 5.3; Fig. 5.7c).

For EOC, under P-ambient conditions, UVR decreased EOC rate only at T1 (Table 5.3) and P addition increased EOC under all experimental conditions (LSD test, $p<0.001$; Fig. 5.7d). An interactive effect between UVR and P was detected (Table **??**S3), because P addition reversed the negative UVR effect on EOC at T1, but resulted in a late UVR inhibitory effect after both P-pulses (T2 and T4; Table 5.3; Fig. 5.7d).

Bacterivory, estimated as percentage of HBP-incorporated by bacterivores is depicted in Fig. 5.8a. During the first period, values of $\%HBP_b$ were greater than 60 % regardless of the treatment, which matched a mean bacterial grazing rate of 3.4 bacteria$\times$bacterivores^{-1}h^{-1} (Fig. 5.8a, b). However, during the second experimental period, because of a lower abundance of the bacterivores, $\%HBP_b$ was reduced to reach only 22 % in the P-added treatments at T4, with values of $\%HBP_b$ significantly lower than those of P-ambient treatments (Tukey's test, $p<0.05$), despite that the bacterial grazing rate was significantly higher in the P-added treatments (Fig. 5.8a, b).

5.4.5. Regulation of trophic web by biotic and abiotic controls

The only variable that explained the BA variance in La Conceja was $\%HBP_b$, suggesting that bacterioplankton was directly controlled by predation (Ta-

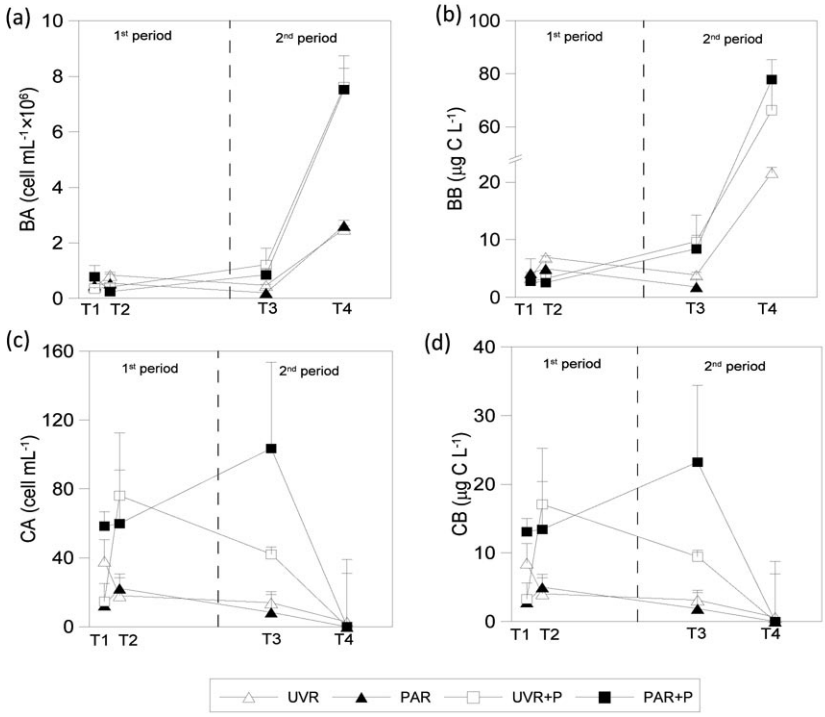

Figura 5.6: Temporal responses of abundance (left panels) and biomass (right panels) of bacterioplankton and ciliates over the experiment. BA: bacterial abundance; BB: bacterial biomass; CA: ciliates abundance; CB: ciliates biomass. Symbols represent mean values and error bars represent standar deviations (SD) (n=3).

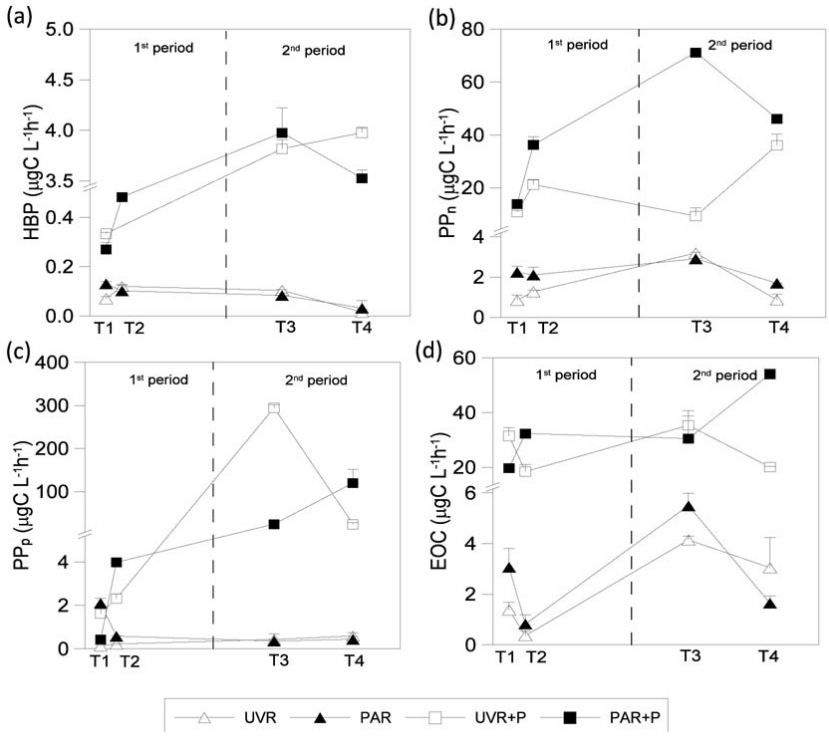

Figura 5.7: Temporal responses of functional variables of phyto- and bacterioplankton over the experiment. HBP: heterotrophic bacterial production; PP_n: nanophytoplankton primary production; PP_p: autotrophic picoplankton production; EOC: Excreted organic carbon. Symbols represent mean values and error bars represent standar deviations (SD) (n=3).

ble 5.4). In turn, %HBP_b was controlled by PDT, which explained 41 % of its variance (Table 5.4). Also, HBP was explained by EOC, which explained ca. 16 % of its variance, and by P_{part}, which contributed an additional 14 % (Table 5.4).

5.5. Discussion

This study contributes experimental evidence that cumulative P-pulses alter not only the effects that UVR exert on microplanktonic metabolism, abundance and biomass, but also the regulation of microbial trophic web, via predatory control by bacterivores. Despite the high DOC content of La Conceja lake, which could act as protection against UVR influence (Williamson *et al.*, 2010), interactive effects of UVR and P addition on phytoplanktonic and bacterial metabolism at long-term were consistent with those found in low-DOC aquatic systems (very transparent to UVR) over the middle term (photoinhibition of PP, photostimulation of HBP, and higher production values; Chapter IV), but not with those at short-term (Chapter II, III). This suggests that transparency of the water column is not a major factor modulating UVR and nutrients interactive effects on microbial food-web, but they were dependent of the temporal scale of analysis.

Under P-ambient conditions, UVR had a common photoinhibitory effect on functional variables in the early (first) period (T1). However, this photoinhibition was not accompanied by the same effect on abundance or biomass and therefore affected only the physiology of the microbial community. Our results are in line with those of previous experiments with manipulation of factors (Reche *et al.*, 2009, Chapter IV) showing higher sensitivity of functional variables compared to abundance, this highlighting the role of functional variables as early indicators of environmental perturbations. Notably, the UVR photoinhibition on HBP was no longer detected after T1, whereas the suppression of the UVR photoinhibition on PP was delayed until the second period. These findings evidence a higher capacity of bacteria than algae for a rapid acclimatization to UVR (Ruiz-González *et al.*, 2013).

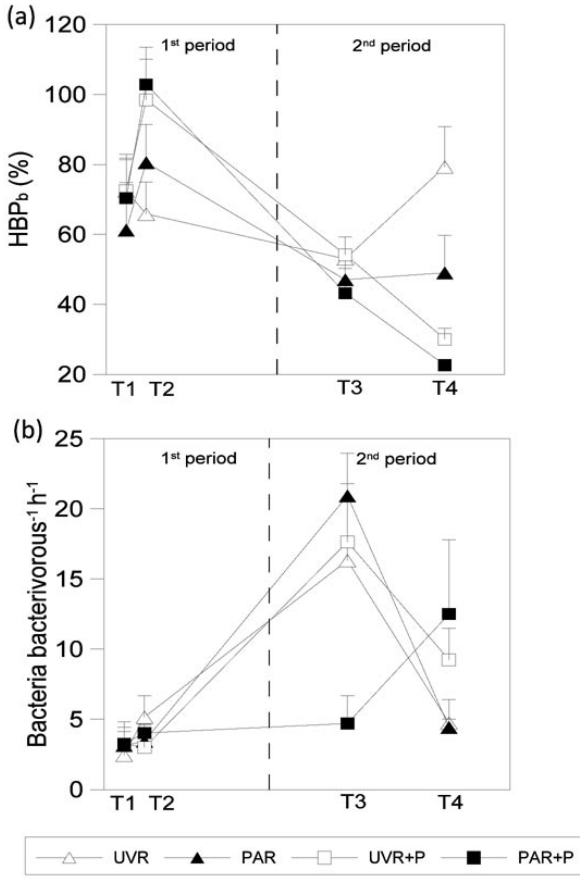

Figura 5.8: Temporal responses of the percentage of bacterivory (a) and bacterial grazing rate (b) over the experiment. Symbols represent mean values and error bars represent standar deviations (SD) (n=3).

In nature, frequently no obvious separation between pulse events and background resource variability in time or space is possible (Yang *et al.*, 2008). Thus, the cumulative nature of the repeated P-pulses in our experimental design did not allow a clear distinction between the P-input and the effect time. However, from our experimental design, we can infer the response of microbial community to a single P-pulse as well as to cumulative P-pulses by comparing the P-added treatment with control treatments after either the first or the second P-pulse, respectively.

The higher magnitude of the stimulation by P-pulses on bacterial and autotrophic picoplankton production than autotrophic nanoplankton production coincides with the higher capacity of the picoplanktonic organisms for the uptake of nutrients compared with nanoplankton due to their higher surface:volume ratio and higher growth rates of the former (Cotner y Biddanda, 2002; Solić *et al.*, 2010, and references therein). Surprisingly, the P-pulse caused a stronger stimulus on the microbial community production (PP and HBP) than after the first one, irrespectively of the radiation conditions, which also resulted in a stronger stimulus of the abundance and biomass. In the case of bacterioplankton, only after the two cumulative P-pulses did the increase in HBP translate as increased BA over the long term, although this increase could also be mediated by a reduction in bacterivory (see below).

The fact that organisms did not respond in the same way after a single P-input as after repeated P-inputs suggests a sort of "memory" or "learning", based on previous phosphate accumulations (Plaetzer *et al.*, 2005; Falkner y Falkner, 2003; Aubriot *et al.*, 2011) or changes in the community composition, which caused the response to identical resource pulses to be different.

5.5.1. Modulation of the UVR effect by P-pulses on phytoplankton and bacterioplankton

Despite the common positive cumulative effect of P-pulses on phyto- and bacterioplankton, P-pulses altered the phytoplanktonic and bacterial response to UVR in different ways. Thus, P addition slightly alleviated the

Dependent variable	Independent vars. entered	b	Multiple R^2	R^2 change	df1	df2	$F_{df1,df2}$	p
BA	$\%HBP_b$	-0.85	0.73	0.73	1	22	62	<0.001
HBP	EOC	0.39	0.16	0.16	1	22	5	<0.05
	P_{part}	0.37	0.31	0.14	1	21	4.39	<0.05
$\%HBP_b$	TDP	0.64	0.41	0.41	1	22	15.5	<0.001

Cuadro 5.4: Results of multiple stepwise regression analysis between (i) bacterial abundance (BA) and resources (C, P), heterotrophic bacterial production (HBP) and bacterivory [bacterivorous abundance (BVA), percentage of bacterivory ($\%HBP_b$)]; (ii) HBP and resources (C, P); (iii) $\%HBP_b$ and P and BVA.

UVR effect on PP_n early (T1) but not later (T2) or after the repeated P-pulses (second period), which even resulted in a negative higher UVR effect which also translated as abundance and biomass. With regard to PP_p, P-pulses were beneficial over the short term (i.e. immediately after their input) because they resulted in an stimulatory UVR effect on PP_p. However, this effect became inhibitory over the long term. These results agree with those of previous long-term experiments testing the interactive effect of UVR and the P-pulse in a high-mountain lake where high P addition unmasked an inhibitory UVR effect on structural nanophytoplanktonic variables (Carrillo *et al.*, 2008a). Contrarily, P addition resulted in a generalized stimulatory UVR effect in HBP, which persisted after the repeated P-pulse (T4), this underlying the major tolerance of bacterioplankton to UVR, enhanced under P addition. A similar UVR stimulatory effect on HBP has previously been found after a single P-pulse (30 μg P L^{-1}) at the middle term, but this effect disappeared over the long term, when dissolved inorganic P was depleted (Medina-Sánchez *et al.*, 2013).

Notably, the positive response of PP to P-input resulted in unmasked deleterious effects of UVR. This could be due to the harmful UVR effect exerted firstly on cell components associated with photosynthetic activity, as was found by Carrillo *et al.* (2008a). By contrast, the positive response of HBP to P addition concomitant with a stimulatory UVR-effect could be the outcome of the enhancement of mechanisms for repairing UVR damage (Kaiser y Herndl, 1997), probably more efficient in bacterioplankton (Carrillo *et al.*, 2002; Medina-Sanchez *et al.*, 2002).

5.5.2. Joint effect of UVR and P-pulses on ecological interactions within microplankton

Heterotrophic biomass did not match the development of total microplankton biomass after the first or second P-pulse. The positive net response of HBP to P-pulses under UVR, besides the use of P, suggested that bacterioplankton more efficiently utilized the carbon released (EOC) from stressed algae. This interpretation is supported by the fact that P_{part} and EOC explained up to 31 % of HBP variance, agreeing with the bacterial

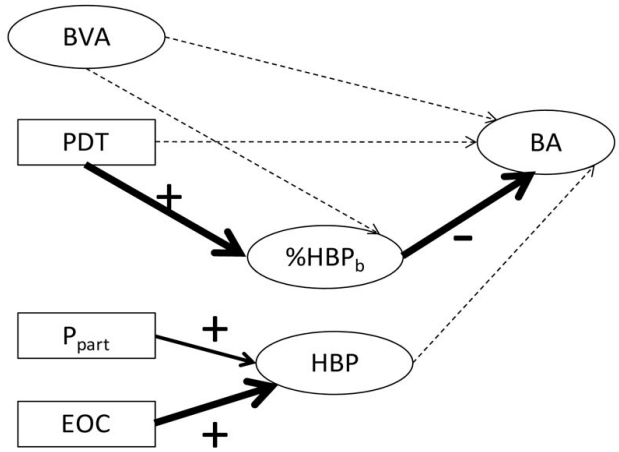

Figura 5.9: Graphical representation of stepwise analysis results. Dash arrows represent non-significant relationship. Thickness of the solid arrows represent the proportional magnitude of the significant lineal relationship. (+): positive lineal relationship. (-): negative lineal relationship.

dependence on EOC evidenced in other oligotrophic ecosystems (Medina-Sánchez *et al.*, 2004, Chapter III). The lack of correspondence between bacterial function and structure, suggests a further biotic control on bacterioplankton, and might be the result of the high mortality rate caused by predatory control (Jugnia *et al.*, 2006, and references therein). The results of the stepwise regression analyses support the contention that, under P-added conditions, bacterivory ($\%HBP_b$) exerted a major control on BA (Fig. 5.9). This predatory control appeared to be in turn modulated positively by TDP. Thus, under P-added conditions, the highest values of TDP coincided with the highest values of $\%HBP_b$ during the first period of the experiment (Fig. 5.9). The high $\%HBP_b$ values could prevent an increase in BA by the stimuli on HBP, due to a higher bacterial loss. In this regard, previous studies have reported that protists can enhance their bacterivory capability with increased P availability, because of a stimulus of HBP (Isaksson *et al.*, 1999). At the end of the experiment, the lowest $\%HBP_b$ indicate an alleviation of the predatory control through bacterial loss (coinciding with the lowest PDT values) and, accordingly, ciliates were substantially reduced, and mixotrophic organisms constituted a minor fraction of nanophytoplankton community.

Finally, our results show a dominance of autotrophic compartment in La Conceja lake, deviating from the general pattern established for oligotrophic waters (Gasol *et al.*, 1997; Biddanda *et al.*, 2001; Caston *et al.*, 2009; Duarte *et al.*, 2013). This pattern remained even after the joint action of UVR and cumulative P-pulses, contrary to our hypothesis. A dual control exerted by algae has been previously proposed as a plausible mechanism responsible of the dominance of microplankton biomass by (mixotrophic) algae against the heterotrophic microbial compartment in high-mountain oligotrophic lakes (Medina-Sánchez *et al.*, 2004). In La Conceja, a dual biotic control on bacterioplankton exerted by algae through bottom-up (providing EOC as resource) and top-down (bacterivory by mixotrophs together with ciliates) proved to be the most plausible explanation of the meager development of heterotrophic bacterial compartment deviating from the general pattern for oligotrophic aquatic ecosystems. Hence, these biotic controls may be responsible of algal dominance found in many colorless-water oligotrophic

ecosystems.

5.6. Supplementary tables

				PA		APPA		BA		CA		BVA	
T1		df1	df2	F	p	F	p	F	p	F	p	F	p
	Radiation	1	8	0.129	0.728	21.517	**<0.01**	3.0928	0.110	2.996	0.122	0.482	0.507
	Nutrients	1	8	18.840	**<0.01**	25.795	**<0.001**	0.77326	0.404	4.517	0.066	15.261	**<0.01**
	Radiation×Nutrients	1	8	0.012	0.917	36.251	**<0.001**	1.651	0.234	42.919	**<0.001**	0.359	0.566
T2													
	Radiation	1	8	0.008	0.929	18.201	**<0.01**	7.3168	**<0.05**	0.188	0.676	1.962	0.199
	Nutrients	1	8	98.950	**<0.001**	2.987	0.122	18.915	**<0.01**	11.742	**<0.01**	94.979	**<0.001**
	Radiation×Nutrients	1	8	0.676	0.435	8.094	**<0.05**	0.704	0.450	0.536	0.485	3.723	0.090
T3													
	Radiation	1	8	16.718	**<0.01**	90.400	**<0.001**	2.7026	0.138	0.307	0.595	28.100	**<0.01**
	Nutrients	1	8	56.734	**<0.001**	4858	**<0.001**	13.111	<0.01	38.923	**<0.001**	127.364	**<0.001**
	Radiation×Nutrients	1	8	10.718	**<0.05**	146.500	**<0.001**	0.064	0.807	4.362	0.070	28.507	**<0.001**
T4													
	Radiation	1	8	0.919	0.365	4.8	0.059	0.0005	0.982	4.000	0.081	63.609	**<0.001**
	Nutrients	1	8	159.130	**<0.001**	1496.800	**<0.001**	57.040	**<0.001**	4.000	0.081	677.222	**<0.001**
	Radiation×Nutrients	1	8	2.756	0.135	0.110	0.746	0.026	0.875	4.000	0.081	0.309	0.594

Cuadro 5.5: Results of the two-way analysis of variance of the interactive effect of radiation and phosphorus at each date of experimentation. Numbers in bold indicate significant interactive effect among the factors. PA: nanophytoplanktonic abundance; APPA: autotrophic picoplankton abundance; BA: bacterial abundance; CA: ciliate abundance; BVA: bacterivorous abundance

			PB		APPB		BB		CB		BVB	
	df1	df2	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
T1												
Radiation	1	8	0.258	0.625	21.517	**<0.01**	4.473	0.067	3.962	0.082	8.677	**<0.05**
Nutrients	1	8	18.776	**<0.01**	25.795	**<0.01**	1.194	0.306	2.288	0.169	3.491	0.099
Radiation×Nutrients	1	8	3.416	0.102	36.251	**<0.001**	2.350	0.164	40.571	**<0.001**	13.440	**<0.01**
T2												
Radiation	1	8	0.066	0.804	18.202	**<0.01**	2.932	0.125	0.188	0.676	188.946	**<0.001**
Nutrients	1	8	41.874	**<0.001**	2.987	0.122	15.243	**<0.01**	11.742	**<0.01**	8.477	**<0.05**
Radiation×Nutrients	1	8	0.212	0.658	8.094	**<0.05**	0.683	0.433	0.536	0.485	5.890	**<0.05**
T3												
Radiation	1	8	3.149	0.114	90.418	**<0.001**	1.122	0.320	0.819	0.392	31.306	**<0.01**
Nutrients	1	8	367.568	**<0.001**	4858.135	**<0.001**	15.644	**<0.01**	41.795	**<0.001**	6.407	**<0.05**
Radiation×Nutrients	1	8	3.463	0.100	146.536	**<0.001**	0.060	0.812	4.963	0.056	6.581	**<0.05**
T4												
Radiation	1	8	13.830	**<0.01**	4.803	0.060	65.164	**<0.001**	4.000	0.081	14.426	**<0.01**
Nutrients	1	8	180.877	**<0.001**	1496.797	**<0.001**	1.633	0.237	4.000	0.081	0.506	0.497
Radiation×Nutrients	1	8	19.622	**<0.01**	0.112	0.746	0.419	0.535	4.000	0.081	0.168	0.693

Cuadro 5.6: Results of the two-way analysis of variance of the interactive effect of radiation and phosphorus at each date of experimentation. Numbers in bold indicate significant interactive effect among the factors. PB: nanophytoplanktonic biomass; APPB: autotrophic picoplankton biomass; BB: bacterial biomass; CA: ciliate biomass; BVB: bacterivorous biomass

				HBP		PP_n		PP_p		EOC		$\%HBP_b$	
T1		df1	df2	F	p	F	p	F	p	F	p	F	p
	Radiation	1	8	0.049	0.830	83.841	**<0.001**	15.989	**<0.01**	5.544	**<0.05**	1.079	0.329
	Nutrients	1	8	592.151	**<0.001**	2033	**<0.001**	0.929	0.360	804.887	**<0.001**	0.515	0.494
	Radiation×Nutrients	1	8	55.939	**<0.001**	11.642	**<0.01**	263.487	**<0.001**	42.800	**<0.001**	0.496	0.501
T2													
	Radiation	1	8	19.867	**<0.01**	64.313	**<0.001**	2203	**<0.001**	49.987	**<0.001**	3.682	0.091
	Nutrients	1	8	1568	**<0.001**	1952	**<0.001**	214.325	**<0.001**	806.579	**<0.001**	30.555	**<0.001**
	Radiation×Nutrients	1	8	7.3120	**<0.05**	0.098	0.762	10.615	**<0.05**	48.758	**<0.001**	1.081	0.329
T3													
	Radiation	1	8	0.829	0.380	6255	**<0.001**	1241	**<0.001**	49.987	**<0.001**	8.347	**<0.05**
	Nutrients	1	8	2516	**<0.001**	6797	**<0.001**	1279	**<0.001**	806.579	**<0.001**	0.208	0.660
	Radiation×Nutrients	1	8	1.393	0.270	6366	**<0.001**	1241	**<0.001**	48.758	**<0.001**	0.736	0.416
T4													
	Radiation	1	8	61.190	**<0.001**	17.919	**<0.01**	65.6540	**<0.001**	5.755	**<0.05**	9.264	**<0.05**
	Nutrients	1	8	17886	**<0.001**	966.433	**<0.001**	1640	**<0.001**	605.036	**<0.001**	37.317	**<0.001**
	Radiation×Nutrients	1	8	70.750	**<0.001**	12.829	**<0.01**	84.449	**<0.001**	44.456	**<0.001**	3.411	0.102

Cuadro 5.7: Results of the two-way analysis of variance of the interactive effect of radiation and phosphorus at each date of experimentation. Numbers in bold indicate significant interactive effect among the factors. HBP: heterotrophic bacterial production; PP_n: nanoplanktonic primary production; PP_p: picoplanktonic primary production; EOC: excreted organic carbon; $\%HBP_b$: percentage of bacterivory

Capítulo 6

Síntesis

The main assumption of this thesis has been considered oligotrophic aquatic ecosystems of the Mediterranean Region as a "hot spot" against alterations of abiotic factors related to global change. The analysis of the responses of their biotic communities to experimental manipulation of multiple abiotic factors has allowed us to estimate their vulnerability to global change, so that these systems can be used as excellent tools for monitoring climate change at the regional scale. Factors considered in this Thesis were: (i) increased availability of nutrients resulting from regional changes (atmospheric inputs) or local (human activity), (ii) increases or fluctuations in the intensity of ultraviolet radiation, which is related to indirect effects of global warming (increased exposure to UVR) or (iii) abrupt changes in temperature associated with an increased frequency of heat waves.

The complexity in determining the nature of the non-additive effects generated by these stressors, as well as the quantification of the interaction strength has led us to use standardized indices in order to systematize the quantification of the differences between the observed interactive effect and the expected interactive effect (established from the individual effects of the factors). Based on this difference, we have established the magnitude of the interaction. Moreover, we have followed the standard rate of Luo *et al.* (2008) for three-factor interactions when our goal was to make a

comparative analysis of ecosystems affected by similar stressors. Then, we summarize the set of results obtained with a new, more inclusive approach that emphasizes the role of altitudinal and optical gradients as powerful predictors of the magnitude of the inhibitory effect of UVR on algal and bacterial metabolisms. We then analyze the magnitude of the inhibitory effect of UVR and how this is modified by alterations in the intensity and fluctuation of UVR, emperature and pulse of nutrients to finalize summarizing the consequences of metabolic changes in the producer-decomposer commensalistic relationship.

How does UVR affect phytoplankton and bacterioplankton depending on the intensity and exposition to UVR of the ecosystems?

The inhibitory effect of UVR on phytoplankton in our ecosystem model was positively related to the gradient of altitude and negatively with gradient transparency of water bodies. Due to the increase in the proportion of incident UVR with altitude, phytoplankton of high mountain lakes was more photoinhibited than that from ecosystems located at lower altitudes (Fig. 6.1). Also, the phytoplankton from ecosystems with higher content in dissolved organic carbon (DOC), more opaque to UVR, experienced less photoinhibition, and even in the Enol Lake it was weakly stimulated by UVR (Fig. 6.2). Bacterioplankton, however, did not follow a clear pattern of response along gradients of altitude or transparency, suggesting the existence of other "factors" as determinants of the net response to UVR, such as its interaction with other components of the microbial food web.

Contrary to the established paradigm (Williamson *et al.*, 2010), organisms from more opaque ecosystems suffered higher damage after an increase in the intensity of the UVR irradiance than organisms from more transparent ecosystems to UVR (Chapter I, II). This result is especially relevant in the current context of global warming which predicts the thinning of the epilimnetic layer (stratification) and greater exposure of organisms to UVR. Although it has been reported that this situation can be coun-

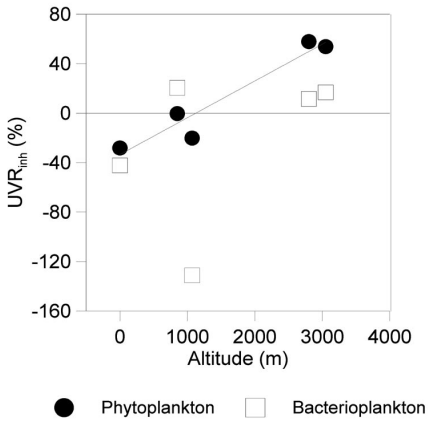

Figura 6.1: Relationship between the percentage of photoinhibition (UVR$_{inh}$ on organisms and altitude of the studied ecosystems

teracted by an increase in the speed of wind and an intensification of the epilimnetic mixing (Helbling *et al.*, 2013), our results showed that in opaque lake, fluctuating mean irradiance increased UVR damage on phytoplankton and bacterioplankton (Chapter II). The magnitude of the inhibitory effect of fluctuating UVR on bacterioplankton was more accentuated in opaque lakes, as result of both direct and indirect effects of UVR, the latter mediated by the availability of carbon from algal origin. Our results, therefore, indicate the existence of an inverse relationship between the magnitude of the photoinhibition exerted by UVR and the optical gradient established in the set of studied oligotrophic ecosystems.

How do temperature changes affect the effect of UVR?

In addition to the indirect effects of an increase in temperature, ma-

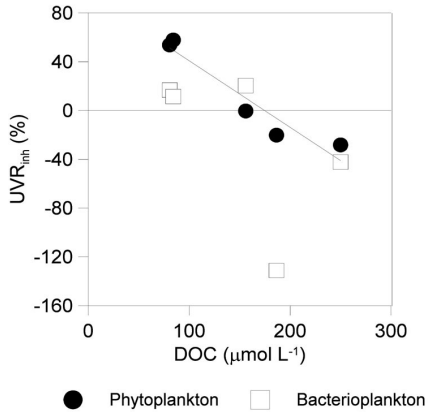

Figura 6.2: Relationship between the percentage of photoinhibition (%UVR$_{inh}$ on organisms and dissolved organic carbon (DOC) content of the studied ecosystems

nifested in greater stratification of the water bodies, the temperature has a direct action by accelerating the metabolism of organisms, which can modify the response to UVR of phyto-and bacterioplankton. Our results show that microorganisms of high mountain ecosystems were most affected by variations in temperature than those of coastal marine ecosystems (Chapter IV). Surprisingly, between high-mountain lakes there were also different vulnerability to increased temperature, so that small variations in its transparency to UVR and/or average temperature, related to exposure/geographical orientation, have resulted in contrasted responses of primary producer to UVR. Thus an increase in temperature had a greater effect on phytoplankton in lakes with highest mean temperature of the water column, than in colder lakes, while the UVR effect on bacterioplankton did not change with increased temperature (Chapter III).

How does the nutrient input affect UVR effect? Importance of the temporal scale.

Nutrient availability, due to its relevance in the damage/repair balance, plays a key role in the net response of organisms to UVR. In Mediterranean oligotrophic ecosystems, pulsed nutrient inputs (Ex. heavy rain, dust storms) can buffer the harmful effects of UVR and even stimulate the growth of plankton communities at long-term scales. Therefore, it has been a priority in this Thesis to evaluate the response of the components of the microbial food web on different time scales. This approach has allowed us to establish the regulatory role on the microbial dynamics of these pulsed events that, although of short duration, persist over time in the ecosystem (Yang *et al.*, 2008).

1. At short term (less than 24 hours), the input of nutrients consistently emphasized the inhibitory effect of UVR on primary production (PP). However, the effect of UVR on bacterial heterotrophic production (HBP) ranged from inhibitor to stimulator between ecosystems, depending on the magnitude of the direct stimulatory effects of the supply of nutrients and the indirect effect generated by this nutrient input through changes in trophic interactions (Chapter II, III).

2. At mid-term (days), a pulse of nutrients generated similar responses of algal and bacterial communities between ecosystems. A single P pulse increased both PP and HBP and enhanced an UVR inhibitor effect on PP but stimulator of HBP (Chapters IV and V).

3. At long-term (weeks) and repeated nutrient pulses, responses and effects kept similar to those found at mid-term on PP and HBP(Chapter V).

Analysis of changes in the structure of the microbial network shows a predominance of autotrophic compartment over the bacterial heterotrophic one under the joint action of UVR and cumulative P-pulses. The mismatch

between the function and structure of the bacterial community was the result of a high rate of bacterivory. Hence, we interpret that a dual control on bacterioplankton exercised by algae through resources (EOC) and predation (bacterivory by mixotrophs and ciliates) were the dominant forces responsible for these oligotrophic ecosystems algae (Chapter V).

Effect of UVR under different global change scenarios

The joint action of epilimnetic vertical mixing and nutrient input, produced a greater photoinhibition of phytoplankton in opaque clear that ecosystems ecosystems (Chapter II). By contrast, the harmful effect of UVR after a nutrient pulse and fluctuating radiation on bacterioplankton, was higher in opaque ecosystems than in the clearest ones (Chapter II). This difference in sensitivity between algae and bacteria was mainly determined by the indirect effects generated after the addition of nutrients on algal carbon excretion, which has implications for the commensal algae-bacteria interaction. Similarly, we found different sensitivity of phytoplankton and bacterioplankton under the simultaneous action of a pulse of nutrients and increased temperature. At short term, in the presence of nutrients, the increase in temperature reduced the inhibitory effect of UVR on PP, but instead resulted in an increase in UVR damage on HBP (Chapter III).

A more prolonged exposition to the effect of nutrient inputs (mid-term) and a sudden change in temperature had a similar effect on phytoplankton and bacterioplankton, although the response differed between ecosystems depending on their basal temperature regime. Abrupt changes in temperature produced a significant effect on organisms from cold ecosystems with higher thermal amplitude, such as high-mountain lakes.

Algal-bacterial relationship under different scenarios of global change

The predominance of a commensalistic algal-bacterial relationship has

been described in many oligotrophic freshwater and marine water. The carbon excreted by algae was enough to meet the bacterial carbon demands in all ecosystems studied (Chapters I-IV)] underlining the autotrophic nature of most of the oligotrophic Mediterranean region (**?**). The equilibrium of this commensalistic relationship was extremely sensitive to changes in factors of global change and under certain scenarios, reaching to produce a weakening of the connection commensal algae, bacteria, i.e. bacterial demands carbon were not covered completely by EOC, sensu Morán *et al.* (2002), in different global change scenarios:

1. Under a scenario of stratification of the water column and increased irradiance of UVR, EOC excretion exceeded the bacterial C demands reinforcing the commensalistic relationship between producer and decomposers in clear ecosystems, whereas the opposite occurred in opaque ecosystems (Chapter I) (Fig. 6.3).

2. The input of nutrients to the stratified epilimnion and a constant mean UVR irradiance resulted in a decoupling of the commensalistic algal-bacterial relationship in the clearest (La Caldera) and darkest (Lake Enol) lakes, but reinforced this relationship in the lake with intermediate transparency (Las Yeguas) (Chapter II, III) (Fig. 6.3).

3. Nutrient input and fluctuating radiation resulted in a decoupling of the commensalistic algal-bacterial relationship in opaque ecosystems, but reinforcement in clear ecosystems (Chapter II) (Fig. 6.3).

4. The joint action of a pulse of nutrients and an increase in temperature resulted in a decoupling of the commensalistic relationship between algae and bacteria in colder ecosystems but this relationship was reinforced in warmer ecosystems (Chapter III) (Fig. 6.3).

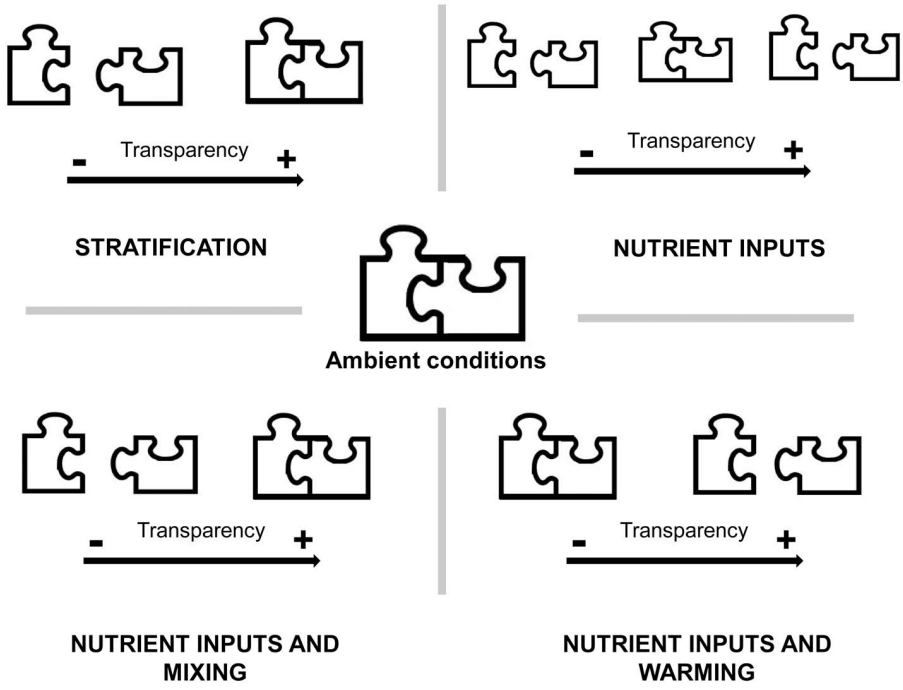

Figura 6.3: Algal-bacterial commensalistic relationship under different global change scenarios

El principal supuesto de esta tesis doctoral ha sido considerar que los ecosistemas acuáticos oligotróficos de la Región Mediterránea son enclaves "hot spot" frente a alteraciones de factores abióticos que se relacionan con el cambio global. El análisis de las respuestas de sus comunidades bióticas a la manipulación experimental de múltiples factores abióticos nos ha permitido estimar su vulnerabilidad frente al cambio global, por lo que estos sistemas pueden ser utilizados como excelentes herramientas para el seguimiento de cambio climático a escala regional. Los factores considerados en esta Tesis fueron: (i) incremento en la disponibilidad de nutrientes, resultado de cambios regionales (entradas atmosféricas) o locales (actividad antrópica); (ii) incrementos en la intensidad o fluctuación de la radiación ultravioleta, que se relaciona con efectos indirectos del calentamiento global (mayor exposición a UVR) o (iii) cambios bruscos en la temperatura relacionados con un incremento en la frecuencia de olas de calor.

La complejidad en la determinación de la naturaleza de los efectos no aditivos generados por estos factores de estrés, así como de de la cuantificación de su fuerza de interacción nos ha llevado a utilizar índices normalizados con objeto de sistematizar la cuantificación de las diferencias entre el efecto interactivo observado y el efecto interactivo esperado (establecido a partir de los efectos individuales de los factores). En base a esta diferencia hemos establecido la magnitud de la interacción. Más aun, hemos seguido el índice normalizado de Luo *et al.* (2008), para interacciones de tres factores cuando nuestro objetivo fue hacer un análisis comparativo entre ecosistemas afectados por similares factores de estrés. A continuación sintetizamos el conjunto de resultados obtenidos con un nuevo enfoque más integrador que enfatiza el papel de los gradientes altitudinales y ópticos como potentes predictores de la magnitud del efecto inhibidor de UVR sobre los metabolismos algal y bacteriano. Después analizamos la magnitud del efecto inhibidor de UVR y como se modifica tras alteración en la intensidad y fluctuación de la UVR, temperatura y pulso de nutrientes, para finalizar resumiendo las consecuencias de los cambios metabólicos en la interacción comensalista productor-descomponedor

¿Cómo afecta la UVR a fitoplancton y bacterioplancton en función de la intensidad y exposición a UVR de los ecosistemas?

El efecto inhibidor de UVR sobre el fitoplancton en nuestros ecosistemas modelo se relacionó positivamente con el gradiente de altitud y negativamente con el gradiente de transparencia de las masas de agua. Debido al incremento de la proporción de UVR incidente con la altitud, el fitoplancton de lagos de alta montaña resultó más fotoinhibido que el de ecosistemas situados a menor altitud (Fig. 6.1). Asimismo, el fitoplancton de ecosistemas con mayor contenido en carbono orgánico disuelto (DOC), más6.22). El bacterioplancton, por el contrario, no siguió un patrón de respuesta nítido a lo largo de los gradientes de altitud o transparencia, sugiriendo la existencia de otros "factores" determinantes de su respuesta neta a UVR, como es su interacción con otros componentes de la red trófica microbiana.

Contrariamente al paradigma establecido (Williamson *et al.*, 2010), los organismos de ecosistemas más opacos sufrieron una mayor daño tras un incremento en la intensidad de la irradiancia de UVR, que organismos de ecosistemas transparentes a UVR (Capítulo I, II). Este resultado adquiere especial relevancia en el contexto actual de calentamiento global que predice el adelgazamiento de la capa epilimnética (estratificación) y mayor exposición de los organismos a UVR. Aunque ha sido descrito que esta situación puede ser contrarrestada por un incremento de la velocidad del viento e intensificación la mezcla epilimnética (Helbling *et al.*, 2013), nuestros resultados evidenciaron que en lagos opacos la irradiancia media fluctuante incrementó el daño de UVR sobre el fitoplancton y bacterioplancton (Capítulo II). La magnitud del efecto inhibidor de la UVR fluctuante sobre el bacterioplancton fue más acentuada en lagos opacos, resultado tanto de efectos directos como indirectos de UVR, estos últimos mediatizados por la disponibilidad de carbono de origen algal. Nuestros resultados, por tanto, indican la existencia de una relación inversa entre magnitud de la fotoinhibición ejercida por UVR y el gradiente óptico establecido en el conjunto de ecosistemas oligotróficos estudiados.

¿Cómo afectan los cambios de temperatura al efecto de UVR?

Además de los efectos indirectos de un incremento de la temperatura, que se manifiestan en la mayor estratificación de los cuerpos de agua, la temperatura ejerce una acción directa acelerando el metabolismo de los organismos, lo que puede modificar la respuesta a UVR del fito- y bacterioplancton. Nuestros resultados muestran que los microorganismos de ecosistemas de alta montaña resultaron más afectados por las variaciones en la temperatura que los de ecosistemas costeros marinos (Capítulo IV). Sorprendentemente, entre lagos de alta montaña también hubo distinta vulnerabilidad al incremento de la temperatura, de forma que pequeñas variaciones en su transparencia a UVR y/o temperatura media, relacionada con su exposición/orientación geográfica, han dado lugar a respuestas contrastadas de los productores primarios frente a UVR. Así, un incremento de temperatura ejerció un efecto mayor sobre el fitoplancton en lagos con temperatura media de la columna de agua más alta (lagos cálidos), que en lagos más fríos, mientras que el efecto de UVR sobre el bacterioplancton no varió con el incremento de temperatura (Capítulo III). Estos resultados modificaron la interacción alga-bacteria

¿Cómo afecta la entrada de nutrientes al efecto de UVR? Importancia de la escala temporal.

La disponibilidad de nutrientes, debido a su relevancia en el balance daño/reparación juega un papel clave en el resultado neto que UVR ejerce sobre los organismos. En los ecosistemas oligotróficos mediterráneos, los aportes pulsados de nutrientes (Ej. lluvias torrenciales, tormentas de polvo) pueden amortiguar el efecto dañino de UVR e incluso estimular el crecimiento de las comunidades planctónicas sobre escalas temporales largas. Por ello, ha sido un objetivo prioritario en esta Tesis Doctoral evaluar la respuesta de los componentes de la red trófica microbiana sobre distintas escalas temporales. Esta aproximación, nos ha permitido establecer el papel regulador sobre la dinámica microbiana de estos eventos pulsados, que aunque de corta duración, persisten a lo largo del tiempo en el ecosistema (Yang *et al.*, 2008).

1. A corto plazo (menos de 24 horas), la entrada de nutrientes consistentemente acentuó del efecto inhibidor de UVR sobre la producción primaria (PP). Sin embargo, el efecto de UVR sobre producción heterotrófica bacteriana (HBP) varió desde inhibidor a estimulador entre ecosistemas, dependiendo de la magnitud de los efectos estimuladores directos por el aporte de nutrientes y del efecto indirecto generado por dicho aporte a través de cambios en las interacciones tróficas (Capítulo II, III).

2. A medio plazo (días), un pulso de nutrientes generó similares respuestas de comunidades algales y bacterianas entre ecosistemas. Un pulso único de P incrementó tanto la PP como la HBP y potenció un efecto inhibidor de UVR sobre la PP, pero estimulador sobre HBP (Capítulos IV y V).

3. A largo plazo (semanas) y con pulsos repetidos de nutrientes, se mantuvieron similares respuestas y efectos a los encontrados a medio plazo sobre PP y HBP (Capítulo V).El análisis de cambios en la estructura de la red microbiana muestra un predominio del compartimento autotrófico sobre el heterotrófico bacteriano por la acción conjunta de UVR y pulsos acumulados de fósforo. La falta de correspondencia entre la función y la estructura de la comunidad bacteriana, fue el resultado de una elevada tasa de bacterivoría. De aquí interpretamos que un control dual sobre el bacterioplancton ejercido por las algas a través de los recursos (EOC) y por la depredación (bacterivoría por mixótrofos y ciliados) fueron las fuerzas responsables de la dominancia de algas estos ecosistemas oligotróficos (Capítulo V).

Efecto de UVR en diferentes escenarios de cambio global

(i) La acción conjunta de mezcla vertical epilimnética y entrada de nutrientes, produjo un mayor fotoinhibición sobre el fitoplancton en ecosistemas claros que en ecosistemas opacos (Capítulo II). Por el contrario, el

efecto dañino de UVR tras un pulso de nutrientes y radiación fluctuante sobre bacterioplancton fue mayor en ecosistemas opacos que en los ecosistemas claros (Capítulo II). Esta diferencia de sensibilidad entre algas y bacterias estuvo principalmente determinada por los efectos indirectos generados tras la adición de nutrientes sobre la excreción de carbono algal, lo que tiene implicaciones en la interacción comensalista alga-bacteria. Del mismo modo, encontramos distinta sensibilidad del fitoplancton y bacterioplancton bajo la acción simultánea de un pulso de nutrientes y un incremento de temperatura. A corto plazo, en presencia de nutrientes, el incremento de temperatura redujo el efecto inhibidor de UVR sobre PP, pero por el contrario resultó en un incremento en el daño de UVR sobre HBP (Capítulo III).

Una exposición más prolongada al efecto de una entrada de nutrientes (medio plazo) y un cambio brusco de temperatura tuvo similar efecto sobre fitoplancton y bacterioplancton, aunque la respuesta difirió entre ecosistemas dependiendo de su régimen basal de temperatura. Los cambios bruscos de temperatura produjeron un efecto significativo sobre organismos de ecosistemas con mayor amplitud térmica y más fríos como es el caso de lagos de alta montaña.

Interacción alga-bacteria bajo diferentes escenarios de cambio global.

El predominio de una relación comensalista alga-bacteria ha sido descrita en numerosos ecosistemas oligotróficos de agua dulce y marinos. El carbono excretado por las algas fue suficiente para suplir las demandas de carbono bacteriano en todos los ecosistemas estudiados (Capítulos I-IV) subrayando el carácter autotrófico de la mayoría de los ecosistemas oligotróficos de la región mediterráneas (Medina-Sánchez *et al.*, 2010). El equilibrio de esta relación comensalista se mostró extremadamente sensible a alteraciones de factores de cambio global y bajo determinados escenarios, llegándose a producir un debilitamiento de la relación comensalista alga-bacteria, es decir las demandas bacterianas de carbono no fueron cubiertas

completamente por EOC, sensu Morán *et al.* (2002), en distintos escenarios de cambio global:

1. En un escenario de estratificación de la columna de agua e incremento en la intensidad de la irradiancia de UVR, la excreción de EOC excedió las demandas de C bacterianas reforzándose la relación comensalista entre productores y descomponedores en ecosistemas claros, mientras que lo opuesto ocurrió en ecosistemas opacos (Capítulo I) (Fig. 6.3).

2. La entrada de nutrientes al epilimnion estratificado y una irradiancia media de UVR constante produjo un desacople de la relación comensalista alga-bacteria en el lago más claro (La Caldera) y más oscuro (Enol) pero reforzó la interacción en el lago de transparencia intermedia (Yeguas) (Capítulo II, III) (Fig. 6.3).

3. La entrada de nutrientes y radiación fluctuante resultó en un desacople de la relación comensalista alga-bacteria en ecosistemas opacos, pero en un reforzamiento de las misma en ecosistemas claros (Capítulo II) (Fig. 6.3).

4. La acción conjunta de un pulso de nutrientes y un incremento de temperatura produjo un desacople de la relación comensalista entre algas y bacterias, en ecosistemas más fríos pero reforzó dicha interacción en ecosistemas más cálidos (Capítulo III) (Fig. 6.3).

Capítulo 7

Conclusiones

1. Phytoplankton was more sensitive to the negative effects of UVR than bacterioplankton. The interaction among UVR and other abiotic factors was mainly antagonistic at short-term, but synergistic at long-term, accentuating the inhibitory UVR effect on phytoplankton and the stimulatory UVR effect on bacterioplankton.

2. Algae and bacteria were more damaged in opaque lakes then in clear lakes under conditions of increase in mean UVR irradiance or fluctuating radiation regime. Nutrient input reversed this response pattern, but only in phytoplankton.

3. An increase in nutrient availability and temperature attenuated the inhibitory effect of UVR on primary production, but increased this effect on heterotrophic bacterial production. Warming negatively affected primary and bacterial production, particularly in ecosystems with higher thermal amplitude and lower climatic predictability, as high-mountain lakes.

4. Commensalistic algal-bacterial relationship was weakened under simultaneous disturbances of UVR exposure regime and nutrient input in opaque lakes, but those of temperature and nutrient input in clear lakes.

5. High-mountain lakes were ecosystems especially vulnerable to a global change scenario characterized by nutrient inputs, thinning of the epilimnion depth or increase in temperature.

1. El fitoplancton se mostró más sensible a los efectos negativos de UVR que el bacterioplancton. La interacción de UVR con otros factores abióticos mayoritariamente fue de carácter antagónico a corto plazo, mientras que a largo plazo tuvo naturaleza sinérgica, acentuando el efecto inhibidor de UVR sobre fitoplancton o su efecto estimulador sobre bacterias.

2. En un régimen de radiación fluctuante o de incremento en la intensidad de la irradiancia de UVR, algas y bacterias fueron más dañadas en lagos opacos que en lagos claros. La entrada de nutrientes invirtió este patrón de respuesta sólo del fitoplancton.

3. Un incremento de temperatura y nutrientes atenuó el efecto inhibidor de UVR sobre PP, pero lo incremento sobre HBP. El calentamiento tuvo un efecto mas negativo sobre la producción primaria y bacteriana en ecosistemas con mayor amplitud térmica y una menor predictibilidad climática, como los lagos de alta montaña.

4. La interacción comensalista alga-bacteria se debilitó con perturbaciones simultaneas en el régimen de exposición a UVR y entradas de nutrientes en lagos oscuros, y de temperatura y nutrientes en los lagos claros.

5. En un escenario de cambio global caracterizado por entradas de nutrientes, reducción de la profundidad del epilimnion o incremento de temperatura, los lagos de alta montaña fueron los ecosistemas más vulnerables

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