ESTUDIO DE LA RED TRÓFICA MICROBIANA EN LAGUNAS SOMERAS: AUTOTROFIA *VERSUS* HETEROTROFIA

Núria Àvila Izquierdo

Universitat
de Girona

Tesis Doctoral

Estudio de la red trófica microbiana en lagunas someras: autotrofia *versus* heterotrofia

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Memoria presentada para optar al título de doctor por la Universidad de Girona

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El Dr. Xavier de Quintana Pou del Instituto de Ecología Acuática de la Universidad de Girona, y la Dra. Rocío López-Flores del Dpto. de Ciencias Agrarias y del Medio Natural de la Universidad de Zaragoza,

Declaran:

Que el trabajo titulado *"Estudio de la red trófica microbiana en lagunas someras: autotrofia versus heterotrofia"*, que presenta **Núria Àvila Izquierdo** para la obtención del título de doctora, se ha realizado bajo nuestra dirección.

Y para que así conste y tenga los efectos oportunos, firmamos el presente documento.

Dr. Xavier de Quintana Pou   Dra. Rocío López-Flores

Girona, diciembre de 2016

Diuen que una imatge val més que mil paraules...

Sé que em deixo molta gent, podria fer pàgines i pàgines amb persones amb les quals he compartit grans records al llarg d'aquests anys i que sempre m'han donat un cop de mà. Moltes gràcies a tots i totes per acompanyar-me al llarg d'aquest camí acadèmic i personal. Sobretot als "aiguamollerus", gràcies per ensenyar-me a entendre i a estimar encara més aquest "petit món". He après molt i he gaudit moltíssim d'aquesta gran experiència.

Només em resta dir...AIGUAMOLLERA per sempre.

PUBLICACIONES DERIVADAS DE LA TESIS DOCTORAL

Según el Acuerdo del Comité Ejecutivo de la Escuela de Doctorado de la Universidad de Girona, en la sesión 10/2012, de 12 de diciembre de 2012, la tesis fue redactada como compendio de los siguientes artículos de investigación:

Artículo 1

Àvila, N., R. López-Flores, D. Boix, S. Gascón, & X. D. Quintana, 2016a. Environmental factors affecting the balance of autotrophs versus heterotrophs in the microbial food web of temporary ponds. Hydrobiologia. Springer International Publishing, 9 July, pages 1–17.

<div align="center">

HYDROBIOLOGIA
FI: 2.051, Rango: 33/104 (Q2)

</div>

Artículo 2

Àvila, N., R. López-Flores, X. D. Quintana, & L. Serrano, 2016b. The microbial food web in the Doñana marshland: Influence of trophic state and hydrology. Estuarine, Coastal and Shelf Science Academic Press 180: 150–159.

<div align="center">

ESTUARINE COASTAL AND SHELF SCIENCE
FI: 2.335, Rango: 24/104 (Q1)

</div>

Artículo 3

Àvila, N., R. López-Flores & X. D. Quintana. Microbial food web composition in Mediterranean coastal aquatic ecosystems under extreme drought conditions.

<div align="center">

ENVIADO

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A: Autótrofos
AL: Albera
ANCOVA: Análisis de covarianza
Bacil: Bacilariofitos
Ca^{2+}: Calcio
Chl-a: Clorofila-a
Chlor: Clorófitos
Chrys: Crisófitos
Cl$^-$: Cloro
Cond: Conductividad
Crypt: Criptófitos
Cyano: Cianobacterias
DIN: Nitrógeno inorgánico disuelto
Dinop: Dinoflagelados
DOC: Carbono orgánico disuelto
DÑ: Parque Nacional de Doñana
ECOFRAME: Ecological quality and functioning of shallow lake ecosystems with respect to the needs of the European Water Framework Directive
Eugle: Euglenófitos
Eut: Eutróficas
EW: Aiguamolls de l'Empordà
F: Área de inundación de la laguna
F: Agua dulce
g440: absorbancia gilvin a 440nm
GG: Giara di Gesturi
GU: Clots de Guils
H: Heterótrofos
Hapto: Haptófitos
HUFA: Ácidos grasos altamente insaturados
K$^+$: Potasio
MEMs: Moran's eigenvector maps
Mg^{2+}: Magnesio

MOD: Materia orgánica disuelta
Na$^+$: Sodio
NAD: Datos no disponibles
NH$_4^+$: Amonio
NMDS: Análisis multidimensional no-métrico
NO$_3^-$: Nitrato
NO$_2^-$: Nitrito
Nt: Nitrógeno total
Norg: Nitrógeno orgánico
Oligo: Oligotróficas
O$_2$%: Porcentaje de oxígeno disuelto
PO$_4^{3-}$: Fosfato
Porg: Fósforo orgánico
Pt: Fósforo total
PCA: Análisis de componentes principales
SO$_4^{2-}$: Sulfato
SRP: fosforo inorgánico disuelto
SWALE: Shallow wetland lake function and restoration in a changing European environment
T: Marea
Temp: Temperatura
TN: Nitrógeno total
TP: Fósforo total
TOC: Carbono orgánico total
RDA: Análisis de redundancia
UTM: Universal transversal de Mercator
Xanth: Xantófitos
W: Superficie de llanura de inundación
Wzoo: Biomasa del zooplancton
WFD: Directiva marco del agua

DISSCUSIÓN GENERAL

CAPÍTULO 3

RESUMEN

Los microorganismos autótrofos (A) y heterótrofos (H) son dos unidades funcionales de la red trófica microbiana de los ecosistemas acuáticos que desempeñan un papel clave en el ciclo de los nutrientes y el flujo de carbono además de influir en la estructura, composición y dinámica de las redes tróficas acuáticas. En lagunas someras, el patrón hidrológico es uno de los factores más importantes, que determina la disponibilidad de nutrientes y la composición funcional de la comunidad microbiana y por tanto de la estrategia trófica dominante dentro de la red trófica microbiana. El principal objetivo que se plantea en esta tesis, es la descripción de la estructura de la red trófica microbiana en lagunas someras con diferentes condiciones de circulación del agua (tasas de renovación) y de estado trófico (concentración de nutrientes). Se analiza así la abundancia relativa de microrganismos A y H en la red trófica microbiana y su relación con diferentes variables ambientales en distintas tipologías de lagunas, buscando la máxima representatividad de estas variables en condiciones de estabilidad hídrica. Para este estudio se han escogido seis sistemas, cuatro sistemas de lagunas someras de interior (Capítulo 1: Parque natural de la Albera, Clots de Guils de Cerdanya, Vilanova de Milfontes, Giara di Gesturi) y dos de marismas y lagunas costeras (Capítulo 2: Parque Nacional de Doñana y Capítulo 3: Aiguamolls de l'Empordà).

Los resultados de esta tesis muestran que las lagunas de interior se encuentran dominadas por una estrategia heterótrofa (balance A/H siempre negativo) donde el DOC es la única variable ambiental relacionada significativamente con este balance. Cuando las concentraciones de DOC aumentan, el balance disminuye sugiriendo que el DOC favorece un aumento de la producción bacteriana. Una cuenca de recepción bien forestada y en buen estado de conservación parece ser la causa principal de que los aportes a estas lagunas sean mayoritariamente de DOC y no de nutrientes inorgánicos.

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Las marismas de Doñana presentan una red trófica microbiana dominada por organismos con una estrategia autótrofa (balance A/H siempre positivo). Aun y así, se observan pequeñas variaciones en el balance A/H debidas principalmente a la distribución espacial de las masas de agua con respecto al origen del agua y a las consecuentes diferencias en la tasa de renovación. Concretamente, aquellas lagunas que se encuentran más aisladas de las entradas de agua son las que presentan una proporción relativamente mayor de organismos heterótrofos. Por el contrario, las lagunas más cercanas a las entradas de agua reciben una proporción más alta de nutrientes inorgánicos y son dominadas por organismos autótrofos.

Las lagunas y marismas del Empordà presentan una hidrología muy variable donde se engloban lagunas confinadas, oligohalinas y estuarios de ríos. En condiciones de máximo estiaje, tanto las lagunas oligohalinas como los estuarios presentan una red trófica microbiana dominada por una estrategia heterótrofa (bacterioplancton). En cambio, las lagunas confinadas se encuentran dominadas por autótrofos. Este balance A/H es similar al visto en las lagunas más aisladas de Doñana. Sin embargo, en este caso, el confinamiento excesivo que se da en estas lagunas limita el crecimiento de los organismos autótrofos al uso de los nutrientes reciclados, favoreciendo las estrategias autótrofas de organismos K.

El hecho de que un sistema esté dominado por una estrategia trófica u otra depende principalmente del régimen hidrológico del sistema y de los factores relacionados con este, como son sobre todo la distribución espacial, el gradiente renovación de agua-confinamiento, la permanencia del agua y la concentración de nutrientes. En todos los sistemas estudiados, el efecto *top-down* del zooplancton sobre el balance A/H fue poco significativo.

RESUM

Els microorganismes autòtrofs (A) i heteròtrofs (H) són dues unitats funcionals de la xarxa tròfica microbiana dels ecosistemes aquàtics que tenen un paper clau en el cicle dels nutrients i el flux del carboni a més d'influir en l'estructura, composició i dinàmica de les xarxes tròfiques aquàtiques. En llacunes someres, el patró hidrològic és un dels factors més importants, que determina la disponibilitat de nutrients i la composició funcional de la comunitat microbiana i per tant de l'estratègia tròfica dominant dins de la xarxa tròfica microbiana. El principal objectiu que es planteja en aquesta tesi, és la descripció de l'estructura de la xarxa tròfica microbiana en llacunes someres amb diferents condicions de circulació de l'aigua (taxes de renovació) i d'estat tròfic (concentració de nutrients). S'analitza així l'abundància relativa de microorganismes A i H a la xarxa tròfica microbiana i la seva relació amb diferents variables ambientals en diferents tipologies de llacunes, buscant la màxima representativitat d'aquestes variables en condicions d'estabilitat hídrica. Per aquest estudi s'han escollit sis sistemes, quatre sistemes de llacunes someres d'interior (Capítol 1: Parc natural de l'Albera, Clots de Guils de Cerdanya, Vilanova de Milfontes, Giara di Gesturi) i dos de maresmes i llacunes costaneres (Capítol 2: Parc Nacional de Doñana i Capítol 3: Aiguamolls de l'Empordà).

Els resultats d'aquesta tesi mostren que les llacunes d'interior es troben dominades per una estratègia heteròtrofa (balanç A/H sempre negatiu) on el DOC és l'única variable ambiental relacionada significativament amb aquest balanç. Quan les concentracions de DOC augmenten, el balanç disminueix suggerint que el DOC afavoreix un augment de la producció bacteriana. Una conca de recepció ben forestada i en bon estat de conservació sembla ser la causa principal de que les aportacions a aquestes llacunes siguin majoritàriament de DOC i no de nutrients inorgànics.

Les maresmes de Doñana presenten una xarxa tròfica microbiana dominada per organismes amb una estratègia autòtrofa (balanç A/H sempre positiu). Tot i així, s'observen petites variacions en el balanç A/H degudes principalment a la distribució espacial de les masses d'aigua en funció a l'origen de l'aigua i a les conseqüents diferències en la taxa de renovació. Concretament, aquelles llacunes que es troben més aïllades de les entrades d'aigua són les que presenten una proporció relativament més gran d'organismes heteròtrofs. Per contra, les llacunes més properes a les entrades d'aigua reben una proporció més alta de nutrients inorgànics i són dominades per organismes autòtrofs.

Les llacunes i aiguamolls de l'Empordà presenten una hidrologia molt variable on s'inclouen llacunes confinades, oligohalines i estuaris de rius. En condicions de màxim estiatge, tant les llacunes oligohalines com els estuaris presenten una xarxa tròfica microbiana dominada per una estratègia heteròtrofa (bacterioplàncton). En canvi, les llacunes confinades es troben dominades per autòtrofs. Aquest balanç A/H és similar al vist en les llacunes més aïllades de Doñana. No obstant això, en aquest cas, el confinament excessiu que es dóna en aquestes llacunes limita el creixement dels organismes autòtrofs a l'ús dels nutrients reciclats, afavorint les estratègies autòtrofes d'organismes K.

El fet que un sistema estigui dominat per una estratègia tròfica o una altra depèn principalment del règim hidrològic del sistema i dels factors relacionats amb aquest, com són sobretot la distribució espacial, el gradient renovació d'aigua-confinament, la permanència de l'aigua i la concentració de nutrients. En tots els sistemes estudiats, l'efecte *top-down* del zooplàncton sobre el balanç A/H va ser poc significatiu.

Autotrophic (A) and heterotrophic (H) microorganisms are two essential functional units in the microbial food web of aquatic ecosystems. They play a key role in nutrient cycling and carbon flow and influence the structure, composition and dynamics of aquatic food webs. In shallow lakes, the hydrological pattern is one of the most important factors that determine the availability of nutrients and the functional composition of the microbial community, and therefore the dominant trophic strategy in the microbial food web. The main objective of this thesis is to describe microbial food web structures in shallow lakes with different water circulation (turnover rate) and trophic (nutrient concentration) conditions. We analyzed the relative abundance of A and H microorganisms in the microbial food web and its relationship with environmental variables in different lagoon typologies, and sought the maximum representation of environmental variables under conditions of water stability. For this study, six groups of lagoons were chosen: four groups of temporary ponds (Chapter 1: Albera Natural Park, Clots de Guils de Cerdanya, Vilanova de Milfontes and Giara di Gesturi) and two marshes and coastal lagoons (Chapter 2: National Park of Doñana and Chapter 3: Aiguamolls de l'Empordà).

Results of this study show that temporary ponds are dominated by a heterotrophic strategy (A/H balance always negative) where DOC is the only environmental variable significantly related to this balance. When DOC concentrations increase, the balance A/H decreases, suggesting that DOC favors an increase of bacterial production. A well-forested and well-preserved catchment area seems to be the origin of DOC dominance over inorganic nutrients in allochthonous contributions.

The marshes of Doñana present a microbial food web dominated by organisms with an autotrophic strategy (A/H balance always positive). Even so, there are small variations in the A/H balance due mainly to the spatial distribution of waterbodies

with respect to the water source and, consequently, the turnover rate variations. Specifically, those lagoons that are most isolated from water inputs are those with a relatively higher proportion of heterotrophic organisms. On the other hand, lagoons closest to the water inputs receive a higher proportion of inorganic nutrients and are more dominated by autotrophic organisms.

Empordà lagoons and marshes present a very variable hydrology that includes confined lagoons and oligohaline lagoons and river estuaries. Under extreme conditions, both oligohaline lagoons and estuaries present a microbial food web dominated by a heterotrophic strategy (bacterioplankton). In contrast, the confined lagoons are dominated by autotrophs. This A/H balance is similar to that found in the more isolated lagoons of Doñana. However, under maximum confinement conditions, the growth of autotrophic organisms is limited to the use of recycled nutrients, favoring autotrophic strategies of K organisms.

The dominance of system by one trophic strategy or another depends mainly of the hydrological pattern of the system and the factors related to it, such as spatial distribution, water-confinement gradient, water permanence and nutrient concentration. In all systems studied, the top-down effect of zooplankton on the A/H balance was insignificant.

INTRODUCCIÓN GENERAL

1. Concepto de red trófica microbiana (Microbial food web).

2. Microorganismos que forman la red trófica microbiana.

3. La disponibilidad de alimento (control bottom-up) y el balance autótrofos *versus* heterótrofos en la red trófica microbiana.

4. La presión de depredación (control top-down) y el balance autótrofos *versus* heterótrofos en la red trófica microbiana.

5. Funcionamiento de la red trófica microbiana en lagunas someras.

6. Área de estudio.
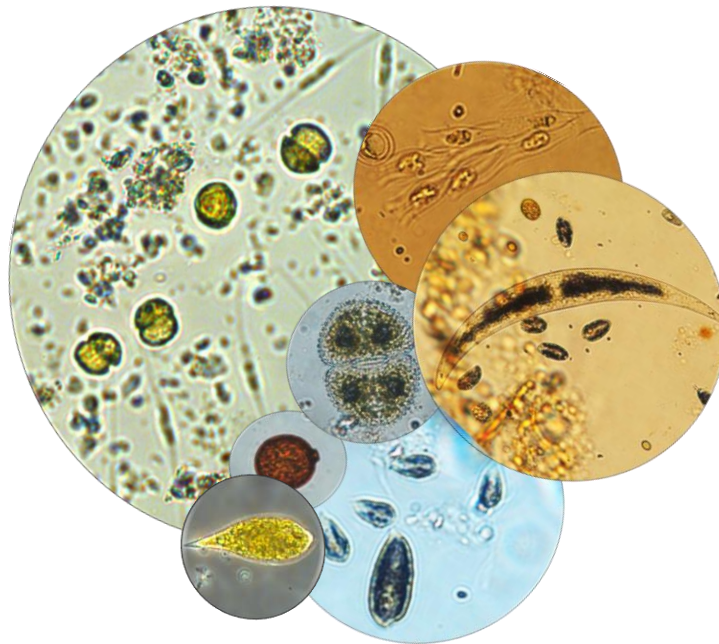
1. **Concepto de red trófica microbiana (*Microbial food web*)**

La red trófica microbiana de los ecosistemas acuáticos está formada por microorganismos que generan una compleja trama de interacciones, las cuales intervienen de forma significativa en los ciclos biogeoquímicos del carbono y de los nutrientes (Sherr & Sherr, 2000; Falkowski et al., 2004). Su importancia en estos ecosistemas se debe a que, como nivel basal de la red trófica, las alteraciones que se producen en este compartimento se ven reflejadas en el resto de niveles y, por tanto, su estudio ha sido de gran interés para entender el funcionamiento global de estos sistemas (Sherr & Sherr, 1988; Cotner & Biddanda, 2002; Litchman et al., 2015).

El esquema clásico de la cadena trófica en ecosistemas acuáticos planctónicos mostrado por Steele (1974) consideraba que la transferencia de energía tenía lugar fundamentalmente a través de 3 niveles; desde el fitoplancton (productores primarios), al zooplancton y de estos a los peces planctívoros. Aunque la existencia de otros microorganismos ya era reconocida (Vernadsky, 1945), no se les había atribuido ningún papel trófico, como era el caso de las bacterias en los ecosistemas acuáticos. Estas ya eran consideradas por su papel en la descomposición de la materia orgánica, pero sin participar de forma significativa en los flujos de materia y energía a través del ecosistema.

Pomeroy (1974) fue uno de los primeros en mostrar el papel de los microorganismos heterótrofos consumiendo el material disuelto y particulado y por tanto, en exponer que esa visión clásica de la cadena trófica representaba solo una pequeña parte del flujo de energía que se daba en los ecosistemas acuáticos. Sin embargo, esta idea no ganó un amplio reconocimiento hasta que la abundancia de bacterias marinas se demostró mediante microscopía de epifluorescencia (Ferguson & Rublee, 1976; Hobbie et al., 1977), y la producción heterotrófica bacteriana se mostró elevada en comparación con la producción primaria (Hagström et al., 1979; Fuhrman & Azam, 1980, 1982). Posteriormente, Azam y colaboradores (1983) formalizaron el

concepto de "bucle microbiano" (*microbial loop)* el cual está formado por un conjunto de microorganismos heterótrofos (procariotas; bacterias, y eucariotas; protozoos flagelados y ciliados). Estos dan lugar a una vía alternativa que explica cómo el flujo de carbono en los sistemas acuáticos pasaría desde los productores primarios hasta los niveles tróficos superiores. Eso comportó una serie de descubrimientos que expresaban la importancia de la participación de estos microorganismos en los flujos de materia y energía y que dieron lugar a un cambio en la interpretación de la estructura y el funcionamiento de la cadena trófica en los sistemas acuáticos (Boenigk & Arndt, 2002; Cotner & Biddanda, 2002).

El descubrimiento de otras interacciones entre los microorganismos ha ido haciendo más complejo este concepto, pasando a llamarse de forma genérica "red trófica microbiana" (*microbial food web)*. Aunque ambas expresiones "red trófica microbiana" y "bucle microbiano" se suelen usar como sinónimos en la literatura, lo cierto es, que el bucle microbiano es un componente integrado dentro de la red trófica microbiana (Sherr & Sherr, 1988), la cual incluye todos los organismos unicelulares procariotas y eucarióticas, tanto autótrofos como heterótrofos. Por tanto, hay que diferenciar cuando se habla de un concepto o de otro. Rassoulzadegan (1993) propone usar bucle microbiano para designar el sistema casi cerrado de bacterias heterótrofas y zooflagelados herbívoros, y utilizar el término red trófica microbiana para un sistema más abierto que también incluye el picoplancton y nanoplancton autótrofos, tal como se ha utilizado a lo largo de esta tesis.

Legendre y Rassoulzadegan (1995) sugieren que tanto la cadena clásica como la red trófica microbiana son dos situaciones extremas que forman parte de un continuo de caminos tróficos. Estos dos extremos no están aislados, sino que coexisten en condiciones naturales de forma directa o indirecta (Sanders et al., 1989; Riemann & Christoffersen, 1993; Auer et al., 2004). Como se muestra en la Figura 1, las bacterias heterótrofas utilizan como fuente de energía tanto la MOD (materia orgánica disuelta) liberada mediante la excreción del fitoplancton, resultado de su

metabolismo y su lisis celular, como a la MOD de origen alóctono; sin embargo, también necesitan de nutrientes inorgánicos para su crecimiento (Azam et al., 1983). Las bacterias mineralizan esta MOD dando lugar a una fuente de nutrientes para los productores primarios (fitoplancton). A su vez, los flagelados y ciliados heterótrofos o mixótrofos pueden incorporar carbono ingiriendo picoplancton autótrofo y/o heterótrofo. Todos estos son consumidos posteriormente por el zooplancton y luego, este a su vez, por peces planctívoros; y, de esta manera, la MOD liberada por el fitoplancton llegaría a los niveles tróficos superiores, conectando la cadena trófica clásica con el bucle microbiano (Azam et al., 1983; Sarmento, 2012).
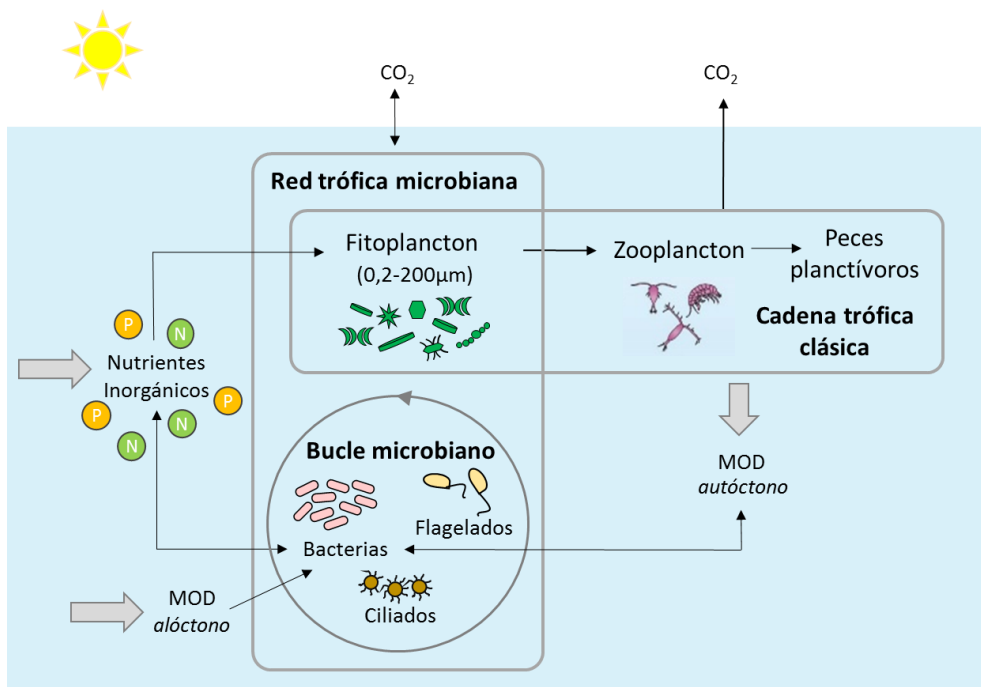
Figura 1. Representación esquemática de la red trófica planctónica en sistemas acuáticos incorporando la cadena trófica clásica, la red trófica microbiana y el bucle microbiano (elaboración propia adaptado de Sherr & Sherr (1988) y Capblancq (1990)).

2. Microorganismos que forman la red trófica microbiana

Los microorganismos planctónicos que forman el nivel basal de lagos y océanos, componentes de la red trófica microbiana, integran un conjunto de grupos taxonómicos muy variados y con papeles ecológicos muy diversos. Estos pueden clasificarse según su taxonomía, su fisiología, o su tamaño (Callieri & Stockner, 2002; Litchman & Klausmeier, 2008; Litchman et al., 2015). En relación a la funcionalidad del organismo, una de las clasificaciones más utilizadas es el tipo de estrategia trófica, la cual se puede dividir de forma genérica en tres categorías: autótrofos (que poseen pigmentos fotosintéticos e incorporan carbono atmosférico mediante fotosíntesis), heterótrofos (que utilizan compuestos orgánicos para su metabolismo) y mixótrofos (que presentan ambos tipos de alimentación, autótrofa y heterótrofa). Esta última estrategia se muestra en un gran número de especies que se extienden sobre una gran variedad de grupos (Stoecker et al., 2009; Flynn & Hansen, 2013). Su presencia se ha observado en sistemas marinos y costeros, tanto eutróficos como oligotróficos (Isaksson, 1998; Jones, 2000; Pitta & Giannakourou, 2000; López-Flores et al., 2006a; Burkholder et al., 2008; Hartmann et al., 2012), así como en sistemas de agua dulce (Sanders, 1991).

Algunos autores han relacionado la estrategia trófica de los microorganismos con su composición de ácidos grasos, proteínas, etc. Estos estudios muestran que los organismos heterótrofos (eucariotas y procariotas) y mixótrofos son más deseables para sus depredadores que los organismos autótrofos debido a su mayor calidad nutritiva, y que por tanto, suponen una mayor transferencia de energía a los niveles tróficos superiores (Elser et al., 2000; Picard & Lair, 2000; Müller-Navarra, 2008; de Carvalho & Caramujo, 2014). Bajo condiciones de escasez, el zooplancton puede mantenerse alimentándose sólo de heterótrofos, concretamente bacterias (Picard & Lair, 2000), sin embargo, aunque estas aportan proteínas, son deficitarias en HUFA (ácidos grasos altamente insaturados; Müller-Navarra, 2008), por lo que a largo plazo, los depredadores requieren de la combinación de presas de distintas

estrategias tróficas. Por tanto, el balance entre organismos autótrofos (A) y heterótrofos (H) además de jugar un papel clave en el ciclo de nutrientes y el flujo de carbono (Biddanda et al., 2001; Cotner & Biddanda, 2002) también puede influir en la estructura, composición y dinámica de las redes tróficas (Del Giorgio et al., 1999). Por esta razón, a lo largo de esta tesis nos hemos centrado en la relación A *versus* H utilizando esta clasificación, donde cada estrategia trófica, además, se ha dividido en función del tamaño celular del microorganismo (Tabla 1).

Tabla 1. Clasificación de los microorganismos que forman la red trófica microbiana en relación a su estrategia trófica, tamaño y tipo de célula. Los organismos mixótrofos estarían incluidos dentro de diversos grupos.

Estrategia trófica	Grupo	Tamaño
Autótrofos	Picofitoplancton	0.2-2 µm
	Nanofitoplancton	2-20 µm
	Microfitoplancton	20-200 µm
	Cianobacterias	5-100 µm
Heterótrofos	Bacterioplancton	0.2-2 µm
	Ciliados	20-200 µm
	Flagelados	20-200 µm

3. La disponibilidad de alimento (control *bottom-up*) y el balance autótrofos *versus* heterótrofos en la red trófica microbiana

Los microorganismos A y H, como hemos visto, son dos unidades funcionales fundamentales en la cadena trófica microbiana de los ecosistemas acuáticos (Biddanda et al., 2001; Biddanda & Cotner, 2002). La abundancia relativa de estos en la red trófica microbiana ha sido analizada por algunos investigadores en lagos profundos y océanos (Del Giorgio & Gasol, 1995; Gasol et al., 1997). Estos estudios determinaron que el equilibrio entre organismos A y H está relacionado con el estado trófico y que esta relación A:H aumenta a lo largo de un gradiente de productividad.

Concretamente en sistemas oligotróficos, se observan las llamadas pirámides invertidas de biomasa (Odum & Barrett, 1971; Wetzel, 2001) donde la biomasa de H puede igualar o exceder a la biomasa de A. Los primeros estudios determinaron que estas pirámides invertidas podían darse gracias a una mayor tasa de renovación de los organismos A (Odum & Barrett, 1971; O'Neil & DeAngelis 1981). Otra de las posibles explicaciones está relacionada con la abundancia relativamente creciente de nutrientes orgánicos disueltos en sistemas oligotróficos, concretamente con las aportaciones alóctonas de carbono (DOC). Las bacterias y heterótrofos eucariotas (ciliados y flagelados) en estos sistemas tienen a su disposición tanto suministros de carbono producidos por el fitoplancton como materia orgánica alóctona (Del Giorgio et al., 1997, 1999). Estudios de Cotner y Wetzel (1992) observan que el bacterioplancton consume unas cantidades más elevadas de compuestos orgánicos de fósforo que el fitoplancton, gracias a la producción de enzimas que rompen estas moléculas. Esto beneficia a las bacterias ante organismos A o de otros H en sistemas oligotróficos (Cotner & Biddanda, 2002), dado que los nutrientes orgánicos disueltos no son asimilables para estos últimos.

En el caso de los sistemas eutróficos, donde la disponibilidad de nutrientes inorgánicos es más elevada, los organismos A dominarían el sistema (Del Giorgio & Gasol, 1995; Gasol et al., 1997). Con elevadas concentraciones de nutrientes por ejemplo durante pulsos de entrada de agua, el fitoplancton es más eficiente en la absorción de nutrientes inorgánicos (Cotner & Biddanda, 2002). Por otro lado, la disminución de la biomasa bacteriana en estos sistemas puede ser explicada por el hecho de que el carbono orgánico se encuentra menos disponible para las bacterias en estos sistemas debido a una reducción relativa de la exudación del fitoplancton y el aumento de la sedimentación (Gasol & Duarte, 2000). El DOC se acumula durante las floraciones del fitoplancton, sugiriendo que el metabolismo de las bacterias heterótrofas no es tan rápido como la producción de fitoplancton, o que éste DOC no es susceptible al ataque de las bacterias (Sherr & Sherr, 1996). Otros estudios determinan que la variación de la biomasa autótrofa es controlada

principalmente por la entrada de nutrientes inorgánicos, mientras que la variación de la biomasa heterótrofa está más influenciada por las entradas alóctonas de carbono orgánico (Garnier & Benest, 1990; Revilla et al., 2000). En este sentido, el hecho de que la comunidad esté dominada por una estrategia más autótrofa o menos dependerá de la ratio de nutrientes inorgánicos: orgánicos y también, del origen de esos nutrientes, sea reciclaje interno o aporte alóctono.

4. La presión de depredación (control *top-down*) y el balance autótrofos *versus* heterótrofos en la red trófica microbiana

Así como la disponibilidad de alimento y energía condiciona la abundancia relativa de cada estrategia trófica dentro de la red trófica microbiana, la estructura del nivel basal también puede estar modelada por la depredación. La presión selectiva de depredación (*top-down*) ejercida sobre los organismos A, sobre todo por parte del zooplancton, se ha determinado que puede controlar la biomasa de A y provocar cambios en la composición de la comunidad (Cyr & Curtis, 1999). La interacción del fitoplancton con los depredadores, se ve afectada por diferentes rasgos como el tiempo de encuentro-captura, el tiempo de procesamiento, y si son o no comestibles. Algunas especies producen toxinas que pueden afectar o producir rechazo a los depredadores (Teegarden, 1999; Huisman et al., 2006; Schultz & Kiørboe, 2009). Además, el fitoplancton también presenta adaptaciones anti-depredación como el tamaño, morfología, formación de mucílago extracelular o una elevada relación C: nutrientes (Reynolds, 2006).

Las interacciones en cascada entre diferentes niveles tróficos se definen como un efecto recíproco depredador-presa que modifican la abundancia, la biomasa, o la productividad de una comunidad, de una población o de un nivel trófico a través de más de un eslabón de una cadena trófica (Carpenter et al., 1985; Carpenter & Kitchell, 1996). Este efecto de interacción en cascada de la depredación sobre la estructura de la red trófica puede disminuir, especialmente en los niveles

superiores, con el aumento de los nutrientes (McQueen et al., 1986). Benndorf (1987, 1995), observó un mayor control en cascada trófica de la biomasa de fitoplancton cuando las concentraciones de nutrientes en el agua eran bajas o moderadas. Sin embargo, las investigaciones de Carpenter et al. (2001) demostraron que el efecto de cascada trófica se observa también en sistemas con altas concentraciones de nutrientes. Se ha comprobado que, en humedales, el control *top-down* puede llegar a producirse exclusivamente cuando el alimento está asegurado (López-Flores et al., 2006a). Por otra parte, la presencia elevada de microfitoplancton mixótrofo tiene una gran influencia sobre la tasa de depredación, llegando a ser considerados los depredadores más importantes sobre el picoplancton (Unrein et al., 2007).

Dentro de la red trófica microbiana, el control *top-down* que se produce en el seno del bucle microbiano es más evidente. La disminución de la abundancia de los organismos H (sobre todo bacterias) en sistemas eutróficos puede ser causada por el aumento de la presión de depredación sobre las bacterias como consecuencia de la presencia de células más grandes y de crecimiento rápido (Sanders et al., 1992). Estos autores vieron como la presión de los depredadores sobre la biomasa bacteriana, principalmente por protozoos flagelados, aumentaba en los sistemas más productivos. Conty et al. (2007) analizaron las relaciones entre protozoos ciliados o flagelados y el bacterioplancton en lagunas someras. Estos observaron que en las lagunas más eutróficas, la elevada depredación de protozoos sobre las bacterias no sólo controlaba su biomasa, sino que contribuían al desarrollo de bacterias con un tamaño más grande y más resistentes a la depredación. Otros han propuesto que la disminución del bacterioplancton es debida a la lisis viral, destino cada vez más probable de las bacterias en sistemas eutróficos (Weinbauer & Peduzzi, 1995). Esta teoría fue apoyada por Gasol y Duarte (2000), que mostraron una correlación más fuerte entre las bacterias y los virus que entre las bacterias y los nanoflagelados.

5. Funcionamiento de la red trófica microbiana en lagunas someras

La investigación de la red trófica microbiana en aguas leníticas se ha centrado inicialmente en los lagos profundos, aun siendo los lagos someros numéricamente más abundantes (Wetzel, 2001). De manera que, en un principio se llegó a generalizar el conocimiento de los sistemas someros con tan solo el estudio de los lagos profundos. Sin embargo, estudios posteriores determinaron que ambos difieren en varios aspectos importantes como son su estructura trófica y funcionamiento y, por lo tanto, el conocimiento obtenido tras el estudio de lagos profundos no podía ser trasferido en su totalidad a las lagunas someras (Jeppesen, 1998; Scheffer, 2004).

Las lagunas someras son sistemas de poca profundidad, esta puede variar de menos de 70 cm a 10 metros según la fuente (Alonso, 1998; Jeppesen et al., 2000). Esta característica hace que, en verano, a diferencia de los lagos profundos, las lagunas someras no presenten estratificación térmica, sino que la masa de agua tiende a estar completamente mezclada como consecuencia del efecto del viento. Esta mezcla constante genera un estrecho contacto entre el sedimento y el agua provocando un rápido reciclaje de nutrientes y una mayor productividad (Jeppesen et al., 1997). La elevada disponibilidad de luz en la columna de agua favorece que la producción primaria en estos sistemas esté dominada por macrófitos sumergidos (Knoppers, 1994; Valiela et al., 1997; Demarty & Prairie, 2009). La presencia de estos macrófitos tiene diferentes efectos sobre la red trófica microbiana, sobre todo en el crecimiento del fitoplancton: crean refugio para su principal depredador el zooplancton (Scheffer, 2004); disminuyen la disponibilidad de luz (Cattaneo et al., 1998); compiten eficazmente en la absorción de nutrientes, especialmente nitrógeno (Ozimek et al., 1993); impiden la resuspensión del sedimento promoviendo la transparencia del agua pero también la escasez de nutrientes (Carpenter & Lodge, 1986; Wilcock et al., 1999; Stanley et al., 2003); y algunas especies de macrófitos pueden liberar sustancias alelopáticas al medio que pueden resultar tóxicas para el fitoplancton (Gross et al., 1996).

Fitoplancton y macrófitos tienden a excluirse. El cambio de un estado de aguas claras dominado por vegetación acuática, a un estado turbio dominado por fitoplancton es lo que se conoce como Teoría de Estados Estables Alternativos propuesta por Scheffer et al. (1993). Esta teoría describe algunos aspectos clave del funcionamiento de los sistemas acuáticos someros y su particularidad al experimentar cambios repentinos. El modelo asume que el paso de un estado a otro es debido a los niveles de nutrientes y la turbidez, relacionada ésta última, de forma inversa, con la disponibilidad de luz en la columna de agua.

Debido al menor volumen de los ecosistemas someros, la concentración de nutrientes y materia orgánica en la laguna suele estar altamente relacionada con las características y la superficie de la cuenca hidrológica (López-Flores et al., 2009). Por el contrario, las altas tasas de desnitrificación registradas es estos ecosistemas (López-Flores et al., 2014), suponen una vía de escape del nitrógeno, llegando a ser este limitante en determinadas situaciones. Estas características se intensifican aún más con la influencia del clima mediterráneo. La composición, estructura y la regulación de la red trófica microbiana en las lagunas someras mediterráneas son en gran parte desconocidas. Ante esta falta de información sobre su funcionamiento, muchos de los procesos y características de estas lagunas se han generalizado con los estudios realizados en las lagunas del norte y centro de Europa (Conty et al., 2007). Sin embargo, varios proyectos europeos sobre lagunas someras como SWALE (Shallow wetland lake function and restoration in a changing European environment) y ECOFRAME (Ecological quality and functioning of shallow lake ecosystems with respect to the needs of the European Water Framework Directive) (Moss et al., 2003), concluyeron que las lagunas someras mediterráneas presentaban peculiaridades únicas, con una estructura y un funcionamiento significativamente diferente a las del norte de Europa.

La particularidad del clima Mediterráneo es la marcada estacionalidad, con períodos de inundación y circulación del agua, seguidos de períodos de confinamiento estival que conlleva la desecación del sistema (Quintana & Moreno-Amich, 2002; García

Novo and Marín Cabrera, 2007). Esta fuerte estacionalidad, por tanto, provoca importantes variaciones en la lámina de agua que tienen efectos directos en el tamaño del sistema, en la disponibilidad de luz, en la dinámica de las poblaciones y en las interacciones tróficas (Fernández-Aláez et al., 2002). Además, la ratio de lluvias anuales respecto la evapotranspiración potencial toma valores que van de 0,12 a 1, dando períodos de falta de agua o sequía severa en muchos ambientes Mediterráneos durante buena parte del año (Alvarez Cobelas et al., 2005). La sequía provoca un fuerte estrés hídrico en estos sistemas (Beklioglu et al., 2007) dando lugar a lo que se llama "confinamiento", situación caracterizada por la baja entrada de energía y nutrientes en la laguna (Guelorget & Perthuisot, 1983), donde el marcado estiaje y la ausencia de mareas o conexión con ríos conllevan el aislamiento de las masas de agua y la sucesiva disminución de agua- concentración de nutrientes (Quintana et al., 1998; Gascón et al., 2006). Esta característica hidrológica, con un período de inundación entre otoño y primavera, y un período seco en verano, determina el funcionamiento ecológico de estos ecosistemas lénticos, en los que la duración del hidroperíodo (ciclo de inundación y secado) y la renovación pueden afectar la composición del agua, la dinámica de nutrientes, el contenido de contaminantes y la estructura de la comunidad acuática (López-Flores et al., 2003; Boix et al., 2004; Quintana et al., 2006).

En sistemas someros, la fuente de nutrientes inorgánicos y de materia orgánica para los organismos es principalmente alóctona (Wetzel, 1992). Las entradas alóctonas aumentan durante las precipitaciones. Después, durante el confinamiento, la materia orgánica se acumula y los nutrientes inorgánicos se agotan y sólo el reciclaje interno puede proporcionar disponibilidad de recursos (Charpy & Charpy-Roubaud, 1990; Quintana et al., 1998; Quintana & Moreno-Amich, 2002; López-Flores et al., 2006a). Por tanto, la disponibilidad de nutrientes (*bottom-up*) y a su vez la composición de la comunidad planctónica viene determinada principalmente por el patrón hidrológico (López-Flores et al., 2006a, 2006b; Beklioglu et al., 2007). La frecuencia y la intensidad del flujo (gradiente circulación-confinamiento), el origen

de las entradas de agua (gradiente de salinidad) y la concentración de nutrientes (gradiente de eutrofia) puede generar variaciones en la composición funcional y taxonómica de la comunidad microbiana y por tanto en la estrategia trófica dominante dentro de la red trófica microbiana.

HIPÓTESIS Y OBJETIVOS GENERALES
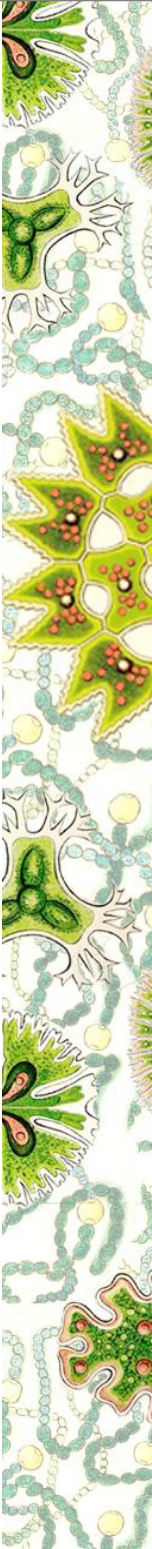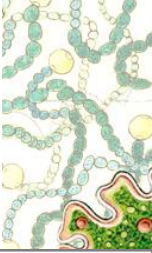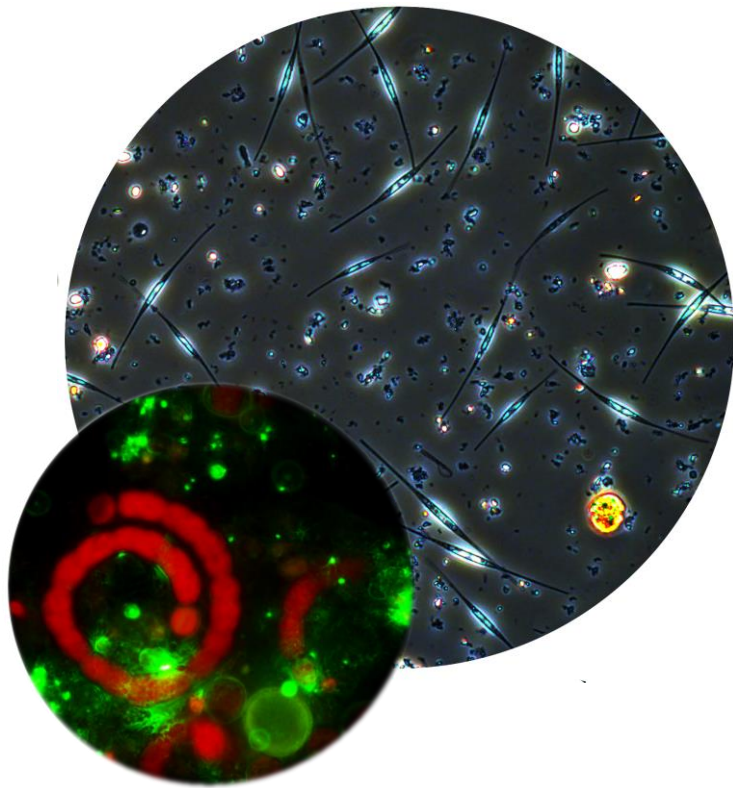
En el marco de esta tesis se plantean las siguientes hipótesis:

Hipótesis 1: En sistemas oligotróficos la abundancia relativamente creciente de nutrientes orgánicos disueltos, concretamente aportaciones alóctonas de carbono (DOC), favorecen la dominancia de organismos H. En el caso de sistemas eutróficos, donde la disponibilidad de nutrientes inorgánicos es más elevada, los organismos A dominan el sistema.

Hipótesis 2: Durante el aislamiento y confinamiento de las lagunas, la materia orgánica alóctona se acumula y los nutrientes inorgánicos se agotan. Esta situación favorece la dominancia de organismos H o mixótrofos, a diferencia de lo que ocurre en sistemas con elevada conectividad, con un flujo relativamente constante de nutrientes inorgánicos, en los que dominan los organismos A.

Hipótesis 3: En condiciones de eutrofia, donde el nitrógeno inorgánico es especialmente escaso a causa de la desnitrificación, este se vuelve un factor limitante para la producción autótrofa. En esta situación se espera que dominen las cianobacterias si la concentración de fósforo es alta, mientras que otros grupos, como por ejemplo criptófitos y dinoflagelados predominan generalmente en bajas concentraciones de fósforo inorgánico.

Hipótesis 4: En ambientes donde los nutrientes son escasos la depredación por parte del zooplancton es un factor secundario, y solo cuando la disponibilidad de nutrientes está asegurada, el control *top-down* se convierte en factor principal.

Por tanto, teniendo en cuenta estas hipótesis el principal objetivo que se plantea en esta tesis es la descripción de la estructura de la red trófica microbiana en lagunas someras con diferentes condiciones de circulación del agua y de estado trófico tanto

en lagunas someras de interior como en lagunas y marismas costeras. Se escogen así distintas tipologías de masas de agua con diferentes tasas de renovación del agua, gradiente de salinidad y concentración de nutrientes (nivel trófico) buscando la máxima representatividad de estas variables en condiciones de estabilidad hídrica.

De forma más concreta, los objetivos específicos para los tres capítulos de esta tesis son:

1. Identificar los factores ecológicos que condicionan la dominancia de A o H en humedales situados en entornos forestados no alterados por la actividad humana (capítulo 1).

2. Determinar la contribución de los diferentes grupos que componen la red trófica microbiana a lo largo de un gradiente hidrológico en una llanura aluvial costera, como la de las marismas de Doñana. Determinar la dominancia de A o H a lo largo de este gradiente (capítulo 2).

3. Evaluar si existen diferencias en la composición y en la dominancia de A o H en masas de agua costeras mediterráneas sometidas a diferencias en el grado de confinamiento, desde estuarios hasta lagunas confinadas, durante los períodos de flujo mínimo (capítulo 3).

4. Analizar las diferencias en la composición de la red trófica microbiana en cada una de estas condiciones (capítulos 1, 2 y 3).

ÁREA DE ESTUDIO

A lo largo de esta tesis, se han realizado muestreos en diferentes tipos de sistemas someros que incluyen cuatro sistemas de lagunas de interior y dos sistemas de marismas y lagunas costeras (Figura. 2). Todos los sistemas presentan algún tipo de protección legal, y se encuentran dentro de la región mediterránea con diferentes características locales (Tabla 2).

Lagunas someras de interior (Capítulo 1)
1. Parque natural de la Albera (AL)
2. Clots de Guils de Cerdanya (GU)
3. Vilanova de Milfontes (VM)
4. Giara di Gesturi (GG)

Marismas y lagunas costeras (Capítulo 2 y 3)
5. Parque Nacional de Doñana (DÑ)
6. Aiguamolls de l'Empordà (EW)

Figura 2. Mapa de situación donde se llevaron a cabo los diferentes muestreos de campo a lo largo de esta tesis.

Tabla 2. Datos de muestreo y características locales medidas en cada localidad. (Oligo; oligotróficas, Eut; eutróficas). Se muestran la media y el coeficiente de variación (%), en paréntesis.

	Lagunas temporales				Lagunas Costeras	
	AL	**GU**	**GG**	**VM**	**DÑ**	**EW**
País	España	España	Italia	Portugal	España	España
Tipo de laguna	tierra baja	montaña	tierra baja	tierra baja	marismas	marismas
Fecha de muestreo	Feb-Mar 12	Jun-12	Abr-12	Abr-13	Jul-10	Jul-Ag 10
Altitud (m)	200	2100	600	55	0	0
Num. lagunas	11	10	11	11	16	17
Profundidad (cm)	85 (72)	34 (70)	26 (48)	65 (40)	50	-
Estado trófico	Oligo	Oligo	Oligo	Oligo	Eut	Eut
Conectividad	Baja	Baja	Baja	Baja	Elevada	Intermedia

Environmental factors affecting the balance of autotrophs versus heterotrophs in the microbial food web of temporary ponds

Núria Àvila , Rocio López-Flores, Dani Boix,
Stéphanie Gascón and Xavier D. Quintana

Abstract

The relative abundance of autotrophic (A) and heterotrophic (H) organisms in the microbial food web of temporary ponds and its relationship to environmental variables were analysed. Four localities (43 temporary ponds) were selected for study. They were located in unaltered, protected basins without intensive agriculture or strong human pressure. Because the supply of allochthonous organic matter is expected to be higher than inorganic nutrient inputs in temporary ponds, we hypothesized that the microbial food web in these unaltered basins was dominated by heterotrophic organisms. Our results showed that the log A:H biomass ratio (*A/H*) was always negative, indicating strong dominance by heterotrophic organisms in these ponds. Moreover, *A/H* was negatively related to DOC concentrations, but this relationship was weaker in the locality with the highest DOC concentration. No significant relationships were found between *A/H* and the other environmental variables analysed, namely inorganic nutrients, fulvic acids, chlorophyll-*a* concentrations, macrophyte biomass or total zooplankton biomass. However, when the different groups of zooplankton were considered separately, *A/H* correlated negatively with cladoreran biomass and positively with harpacticoid biomass. Thus, temporary ponds differ from deeper ecosystems, in which the greater importance of autochthonous energy inputs leads to microbial food web that is more dominated by autotrophs.

Keywords

The microbial food web
in the Doñana marshland:
Influence of trophic state and hydrology

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Xavier D. Quintana and Laura Serrano

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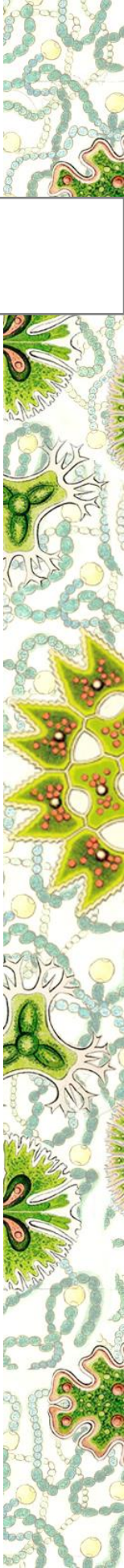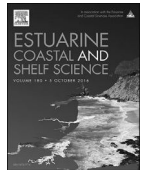
The microbial food web in the Doñana marshland: Influence of trophic state and hydrology

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ABSTRACT

We investigated the composition of the microbial food web in the marshland of Doñana National Park (SW Spain). We analysed factors affecting the predominance of autotrophic (A) or heterotrophic (H) microorganisms in a set of 16 marshland water bodies that differ in their hydrological pattern. Autotrophic organisms were predominant in the Doñana marshland, with autotrophs between 0.3 and 25.3 times higher than heterotrophs in biomass. The variance partitioning analysis using the log A:H biomass ratio (*A/H*) as a response variable revealed that water body spatial position accounted for the largest portion of total variance (16% of unique effects), followed by environmental variables (13%), with a shared variation of 24%. Zooplankton biomass had no significant influence on *A/H* ratio. The two first axes of RDA analysis were related to soluble reactive phosphate (SRP) and dissolved inorganic nitrogen (DIN) concentrations respectively. Cyanobacteria were predominant in waters with high SRP, while other organisms were distributed in relation to DIN by their size, with small organisms predominating with low DIN and large ones with high DIN. Spatial effects reflect the importance of location with respect to the water source in this marshland, where flooding areas are very much dominated by autotrophs, while confined areas, which are a long way from nutrient sources, have a more balanced abundance of autotrophs and heterotrophs.

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1. Introduction

The microbial food web includes autotrophic and heterotrophic organisms in the first level of the trophic chain, which are diverse functionally (Sherr and Sherr, 1988). It is this property of the microbial food web that makes it a key element in the trophic web and determines the other components above it. Some authors, for instance, have observed a differential composition (fatty acids, proteins, etc.) of microbial food web organisms according to their trophic strategy (Elser et al., 2000; Müller-Navarra, 2008; Picard and Lair, 2000). In this regard, autotrophic organisms take advantage of nutrient rich situations which lead to shorter, simpler and more productive trophic chains (Pomeroy, 2001; Ryther, 1969).

Heterotrophic (eukaryote and prokaryote) and mixotrophic organisms were common in confined environments (Lopez-Flores et al., 2006; Quintana et al., 1998) and were found to be more nutritive for their predators and therefore more desirable preys than autotrophic organisms (Elser et al., 2000; Müller-Navarra, 2008; Picard and Lair, 2000). Mixotrophic protozoans, for example, are a means by which to obtain additional carbon, which also includes complex organic molecules (PUFAs; de Carvalho and Caramujo, 2014). Confinement is characterized by an accumulation of organic materials and the increase of internal recycling processes and therefore heterotrophic ways are more efficient in these situations (Duarte and Prairie, 2005; Waiser and Robarts, 2004). Moreover, in a scenario of climatic change, Petchey et al. (1999) observed that warming increased decomposition, probably because of both direct effects on decomposer physiology and increased primary production. Temperature and hydrology therefore constitute an essential trade-off for microbial food web functional structure.

In coastal lagoons and wetlands with a Mediterranean climate,

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the hydrological pattern is one of the most important factors determining resource availability and, consequently, the planktonic community composition (Alvarez-Cobelas et al., 2005; Beklioglu et al., 2007; López-Flores et al., 2014; López-flores et al., 2009; Noble et al., 2003). The frequency and intensity of flooding (flooding-confinement gradient), the water input origin (salinity gradient) and the nutrient concentration (eutrophic gradient) can produce several and assorted situations with regard to the functional and taxonomical composition of the microbial community. Nonetheless, variations in microbial food web composition and in its size and trophic structure are described as a result of the trade-off between top-down and bottom-up forces (Allende, 2009; Buskey, 2008). Several authors have shown the supremacy of top-down forces, which can even be responsible for the light: nutrient stoichiometry (Hall et al., 2007). Some works, on the other hand, have also shown how, in some circumstances, bottom-up control, defined as nutrient availability, is the most important driving force (Davis et al., 2014; Lewis, 1978; McQueen et al., 1986; Pitois and Fox, 2006; Zheng and Stevenson, 2006). Lastly, some authors have concluded that top-down control becomes a main factor only when nutrient availability is guaranteed; a scarcity of resources meanwhile puts zooplankton predation control in second place (Allende, 2009; Lopez-Flores et al., 2006; Sala et al., 2008).

The aquatic systems of Doñana National Park are considered to be the most important wetland area in the Iberian Peninsula and one of the largest in Europe with a high significance for wildfowl. Doñana contains an extraordinary variety of aquatic systems, including an extensive marshland on a central floodplain and numerous dune ponds on Aeolian sands. Although the marshland area occupies most of the flooding surface in Doñana, limnological research has produced few quantitative descriptions, probably because of the vast extension of this shallow seasonal ecosystem (Espinar and Serrano, 2009; Serrano et al., 2006). This marshland is the result of extremely complex hydrological patterns (Díaz-Delgado, 2010; Reyes et al., 2008). Its chemical composition is, in fact, influenced by a combination of rainfall, evaporative concentration, groundwater discharge, biogeochemical interactions at the sediment-water interface, and the quality composition of its watershed (Serrano et al., 2006). That complexity is expected to increase in the short to medium term on account of human management of water resources, such as the planned deep-dredging of the estuary and the increase in the water demand for irrigation (Berbel et al., 2011) and, in the long term, because of steady global changes that are predicted to be severe in the south of the Iberian peninsula (Alvarez-Cobelas et al., 2005; Senerpont Domis et al., 2013). As a result, even if the use of groundwater for public supply and irrigation is halted, most future climate simulations predict that the supply of groundwater to the Doñana wetlands is likely to diminish (Guardiola-Albert and Jackson, 2011).

Understanding the behaviour of this complex environment would require long-time series of high-resolution data on time, space and microbial food web compartment analysis. Faced with the same handicap, other authors have used models to predict how the community would respond to certain perturbations, and substitute time series for spatial (sometime latitudinal) nets that include high variability of driving factors (Adrian et al., 2012; Senerpont Domis et al., 2013).

This is the first attempt to understand how the microbial food web in the Doñana marshland works and, more precisely, to analyse the relative contribution of the functional groups (autotrophic, heterotrophic and mixotrophic organisms) to the microbial food web across a wide hydrological gradient based on a spatial-analysis approach. This involved the study of 16 water bodies with different hydrological and trophic conditions distributed within the Doñana marshland. It is hypothesized that in situations of confinement the

microbial food web will be generally predominated by groups of high nutritional value: heterotrophic and mixotrophic. However, in situations involving energy and water exchange and therefore inorganic nutrient inputs, fewer nutritional autotrophic organisms will dominate the microbial community.

2. Study area

Doñana National Park is located on the Atlantic coast of southwest Spain. It covers over 120,000 ha and includes several areas with different degrees of environmental protection, which can have either National Park or Natural Park status (García Novo and Marín Cabrera, 2007). The area is characterized by a Mediterranean climate with Atlantic influence, generally classified as dry sub-humid, with very hot, dry summers and short, mild winters. Rainfall is quite variable, both throughout the year and over the years, with an annual average of 580 mm, 80% of which falls in a wet period from the end of September to the beginning of April. Water balance is generally deficient as rainfall exceeds evapotranspiration for only 3–4 months of the year (Castroviejo, 1993; Siljeström and Clemente, 1990).

Doñana has an extraordinary variety of aquatic systems that are classified according to their location on either Aeolian sands or on the marshland area, the latter of which has a more complex hydrology and has been studied less (Serrano et al., 2003). This study focuses on the marshland area, a great alluvial plain that covers an area of approximately 34,000 ha, has an average height of 1.35 m above sea level and is formed by impermeable clay soils. The alteration of this alluvial plain at a topographic scale of a few centimetres creates depressions that form temporary water bodies (García Novo and Marín Cabrera, 2007; García Viñas et al., 2005; Serrano et al., 2006).

The hydrological pattern of the Doñana marshland exhibits a typical Mediterranean climate dynamic, with sudden and irregular flooding followed by long periods of confinement. Although some points are influenced by the Atlantic tides of the Guadalquivir River estuary, most of the Doñana inland marshland has no tidal influence (Clemente et al., 2004; Reyes et al., 2007). It receives surface waters through different networks of both natural and artificial watercourses, from different origins and of varying characteristics depending on the season: these include the Guadiamar River basin, the Guadalquivir River estuary and the outlet of the rice paddies (Reyes et al., 2007; Serrano et al., 2006). The water entering these rivers and streams that maintains the marshland originates both from rainfall and from the underlying aquifer (hydrological unit 05.51 Almonte-Marismas) on its north and western sides, even though this water input is currently affected by the rate of groundwater abstractions for irrigation. Extractions have a clear impact on water availability and therefore alter the local hydrological cycle significantly both in quality and quantity (García Novo and Marín Cabrera, 2007; Manzano et al., 2002).

3. Methods

The sampling campaign was carried out from 15 to 20 July 2010, a period in which the marshes were still flooded, albeit with a high degree of confinement. Sixteen sampling sites were located within the study area (Fig. 1). These selected sites have a broad environmental gradient, with different renewal rates, salinity and nutrient concentrations and over 40 cm deep in all cases.

3.1. Physical and chemical analysis

Water temperature (°C), electrical conductivity (EC_{25}), pH and dissolved oxygen (mg l^{-1}) were measured in situ. Filtered samples

SITE	NAME
1	Caño Travieso/Punta Negra
2	Lucio Mari López
3	Caño Buen Tiro
4	Lucio de la casa del Cherri
5	Lucio de los Ánsares (NE)
6	Lucio de las Nuevas/C. Buen Tiro
7	Lucio Pelón
8	Lucio del Molinillo
9	Lucio margen derecho/ Vetas altas
10	Lucio del Cangrejo Chico
11	Lucio de Vetalalengua
12	Lucio del Membrillo
13	Salinas de Sant Rafael
14	Marisma del Rocío
15	Caño Martinazo
16	Lucio del Caballero

Fig. 1. Map showing the location of Doñana National Park in southwest Spain and the location and name of the basins included in the study area.

(Whatman GF/F) were frozen for subsequent analysis of dissolved inorganic nutrients (DIN: $NH_4^+ + NO_3^- + NO_2^-$) and soluble reactive phosphate (SRP). Unfiltered samples were either frozen for later analysis of total nitrogen (TN) and total phosphorus (TP) or refrigerated for total organic carbon (TOC) analysis. Nutrient analyses were carried out as described in Grasshoff et al. (1983) and total organic carbon was measured using a TOC analyser (TOC500 SHIMADZU, Shimadzu Scientific Instruments, Columbia, USA). Gilvin (g440) was also measured according to Kirk (1994) to estimate the presence of humic acids by measuring the absorbance at 440 nm of samples. In addition, filtered samples (Nylon 0.45 μm pore) were analysed by ionic chromatography (761 Compact IC, Metrohm, Switzerland) to establish the concentration of anions (SO_4^{2-}, Cl^-) and cations (K^+, Na^+, Mg^{2+}, Ca^{2+}).

3.2. Biological parameters

Water samples were collected at each sampling point to determine the abundance and biomass of zooplankton and of different groups in the microbial food web: microphytoplankton, picophytoplankton and nanophytoplankton (autotrophic organisms), as well as bacterioplankton and ciliates (heterotrophic organisms).

Water samples of 150 ml were fixed with Lugol's iodine (1% final concentration), according to Utermöhl (1958), in order to determine microphytoplankton and ciliate abundance. The method described in Andersen and Throndsen (2003) using an inverted microscope (Zeiss Axiovert 135, Göttingen, Germany) was followed. Biovolume was calculated on the basis of measurements of linear dimensions of cells taken under the microscope, using the appropriate geometric formulae (Sun, 2003) and subsequently biomass for the entire sample was calculated as stated in (Gaedke et al., 2004).

Picophytoplankton, nanophytoplankton and bacterioplankton abundance and biovolume were measured with a flow cytometer (FACScalibur from Becton & Dickinson, USA) equipped with a laser

emitting at 488 nm. Samples were filtered through 20 μm mesh, fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration), deep-frozen in liquid nitrogen and stored frozen at −20 °C. Sample processing and data analyses involved methods detailed in (López-Flores et al., 2006a). Bacterial biomass estimations were made as described in (Troussellier et al., 1999). Data were acquired in list mode and processed with CellQuest™ software (BD Biosciences, USA).

For zooplankton samples, 5 L of water were filtered through a 50-μm size mesh and preserved in situ in 4% formalin. An inverted microscope was used to count and identify the zooplankton taxa. Biomass dry weight estimations were obtained, for most invertebrate species, from the allometric correlation between weight and body length (Malley et al., 1989; McCauley, 1984). For rotifers and ciliates, biomass was calculated by converting volume into dry weight (Bottrell et al., 1976; JOHNSTONE, 1995; Malley et al., 1989; Putt and Stoecker, 1989; Ruttner-Kolisko, 1977; Schönborn, 1992).

The abundance of mixotrophic organisms and their ingestion rate of bacteria were determined by means of grazing experiments, which were performed by in situ incubation of collected water from the surface mixed layer, previously filtered through 50 μm mesh. Triplicate sterile falcons (30 ml) containing water from all the waterbodies were inoculated with 0.5 ml of fluorescence-labelled bacteria (FLB; *Escherichia coli* - K-12 strain- Bioparticles®, Alexa Fluor®488 conjugate, Molecular Probes, Invitrogen). FLB were prepared in accordance with the manufacturer's instructions. Incubation lasted 50 min, which was time enough to allow the ingestion of bacteria by phagotrophic organisms (Christaki et al., 1999). Samples were fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration) and were filtered (Whatman GTTP 3 μm pore) in the laboratory with a manual vacuum pump. Filters, when dry, were mounted on slides with a drop of low-fluorescence immersion oil. One drop of oil was then added above the filter before covering it. Filters were stored frozen at −20 °C until their examination by epifluorescence microscopy.

Trophic strategies (autotrophy and heterotrophy) were distinguished by means of red fluorescence (chlorophyll) and the absence of fluorescence. In addition, all the organisms were examined in order to determine their ingestion rate of FLB. Autotrophic organisms that ingested FLB were classified as mixotrophs.

3.3. Statistical analysis

To determine the dominance of autotrophic (A) or heterotrophic (H) organisms, the A/H balance was calculated and used as a single dependent variable. This balance was computed using techniques of compositional data analysis (Aitchison, 1986), which are suitable when dealing with components of a composition. According to this method, the A/H balance is:

$$A/H = \frac{1}{\sqrt{2}} \ln \frac{A}{H} \qquad (1)$$

where A is the sum of the biomass (pgC/ml) of autotrophic organisms, including picophytoplankton, nanophytoplankton, microphytoplankton and colonial cyanobacteria, and H is the sum of the biomass of heterotrophic organisms, including bacteria and ciliates. This balance takes the form of a log ratio, where $1/\sqrt{2}$ is added for normalization (Pawlowsky-Glahn and Buccianti, 2011). This A/H balance is used in statistical analyses, thus avoiding incorrect correlations that appear when using non-log transformed ratios (Aitchison and Egozcue, 2005; Pawlowsky-Glahn and Buccianti, 2011). For the purposes of representation, however, the non-log transformed ratio may be more illustrative. Hereafter, the term "A/H" will be used to refer to the log ratio defined in Equation (1) and the term "A:H biomass ratio" to refer generally to the non-log relationship between A and H biomasses. Other ratios, such as those found in Table 1, were transformed using the same Equation

(1).

Three sets of predictors were considered in the analysis: spatial data (UTMx and UTMy), environmental variables (conductivity, gilvin (g_{440}), TOC, DOC, DIN, TN, TP, SRP, SO_4^{2-}, Cl^-, K^+, Na^+, Mg^{2+}, and Ca^{2+}) and the biomass of the different zooplankton taxa (ciliates, *Brachionus* rotifers, other rotifers, cladocerans, calanoid copepods and cyclopoid copepods). The zooplankton and environmental data were log-transformed and centered (centered log-ratio). The spatial data were extracted using Moran's eigenvector maps (MEMs), as detailed in Dray et al. (2006). First, Universal Transverse Mercator (UTM) coordinates were used to construct a topology-based map of water body position (using the Delaunay triangulation function as a measure of neighbourhood), which was binary-transformed and weighted. The resulting eigenvector matrix is a model of the spatial relationships of water bodies within each area. Only positive and significant eigenvectors were retained, as they represent positive spatial associations of water bodies (Griffith and Peres-Neto, 2006). 999 permutations were made and 4 spatial eigenvectors for the area were retained for further analyses (MEM1, MEM2, MEM3 and MEM4). All spatial analyses were performed using the spacemakeR package (Dray et al., 2006).

The impact of the sets of predictor variables (biotic, abiotic and spatial) on A/H was determined by means of variation partitioning (Borcard et al., 1992; Legendre and Legendre, 1998) (using the 'vegan' package (Oksanen et al., 2009) of the R language (R Development Core Team, 2011). Variation partitioning decomposes the variation of dependent variables in unique (or pure) and shared (or joint) effects of a set of predictors. Variation partitioning is achieved by partial regression and redundancy analysis for single (community parameters) and multiple dependent variables (species or functional matrices), respectively. The vegan package primarily uses adjusted R squares to assess the partitions

Table 1
Mean and SD values of several physical and chemical variables in the different groups of basins (1, 2 and 3). Results of the same row followed by the same letter are not significantly different (p < 0.05). In bold, the variables with significantly different. From A/H on, ratios were log transformed as described in Equation (1) (see methods).

Variable	Group of basins		
	1	2	3
A:H biomass ratio	**1.71 (1,72) a**	**4.49 (2.15) b**	**12.98 (7.81) c**
A/H	**0.076 (0.65) a**	**0.98 (0.38) b**	**1,64 (0.58) c**
Temperature (°C)	27,12 (2,47) a	27,40 (4,27) a	27,47 (1,24) a
Conductivity (mS cm⁻¹)	6,16 (5,87) a	7,87 (5,36) a	3,63 (1,53) a
% Oxygen saturation	158,49 (57,62) a	147,93 (50,17) a	126,00 (20,16) a
pH	8,93 (0,75) a	8,59 (0,42) a	8,68 (0,07) a
Gilvin (g_{440})	0,07 (0,08) a	0,05 (0,42) a	0,03 (0,01) a
TOC (µM)	33.38 (13.63) a	24.68 (8.87) a	31.53 (6.30) a
Chla (µg/l)	39,31 (66,31) a	10,58 (10,10) a	6,82 (2,28) a
Nt (µM)	**256,98 (194,85) a**	**218,66 (130,56) a**	**115,44 (13,38) b**
Pt (µM)	11,56 (12,19) a	9,98 (6,88) a	10,26 (2,17) a
DIN (µM)	3,65 (2,87) a	27,60 (50,25) a	3,15 (0,92) a
N organic (µM)	**253,33 (195,28) a**	**191,06 (87,88) a**	**112,29 (13,24) b**
[PO_4^{3+}] (µM)	0,54 (0,62) a	0,30 (0,51) a	0,64 (0,54) a
P orgànic (µM)	11,03 (12,16) a	9,67 (7,00) a	9,63 (2,40) a
Na+ (ppm)	956,90 (1006,57) a	1026,31 (557,25) a	586,15 (267,89) a
K+ (ppm)	69,78 (87,87) a	52,12 (37,02) a	38,88 (13,95) a
Ca²⁺ (ppm)	94,94 (87,26) a	172,13 (174,58) a	59,02 (9,86) a
Mg ²⁺(ppm)	129,24 (184,95) a	138,08 (97,17) a	71,61 (35,75) a
Cl⁻ (ppm)	2757,11 (3096) a	2782,91 (1573) a	1527,97 (720) a
SO4²⁻ (ppm)	322,95 (620,87) a	545,17 (589,05) a	51,62 (9,53) a
Nt/Pt	**2,57 (0,80) a**	**3,51 (1,19) b**	**2,86 (1,50) ab**
DIN/N organic	−3,17 (1,17) a	−2,79 (1,68) a	−2,56 (0,26) a
Pinorg/Porg	−2,18 (0,81) a	−3,39 (1,84) a	−3,10 (2,25) a
DIN/SRP	**1,32 (1,13) a**	**2,79 (1,47) b**	**2,30 (2,24) ab**
TOC/Nt	3,86 (0,61) a	3,58 (1,13) a	4,04 (0,23) a
SRP/PT	−2,24 (0,71) a	−3,43 (1,79) a	−3,15 (2,21) a
Na+/Ca²⁺	1,59 (0,98) a	1,47 (0,51) a	1,57 (0,28) a
Cl⁻/SO4²⁻	1,82 (0,71) a	1,69 (0,76) a	2,34 (0,19) a

explained by the predictors and their combinations, as this is the only unbiased method (Peres-Neto et al., 2006). Collinear variables were used in the explanatory tables and hence the variables do not have to be removed prior to partitioning. Multiple regression analysis was performed in order to model the response of *A/H* to main variable sets (partition variation results). The regression model was determined using an analysis with forward selection to choose spatial and environmental variables significant at a level of 5%. Basins were clustered into 3 groups according to their *A/H* value (from the regression model). The Pearson chi-square statistic was used to compare phytoplankton taxonomic composition in different A/H categories clustered. All statistical analyses were performed with R v. 2.13.0 (R Development Core Team, 2011).

In order to quantify the influence of the different sets of variables on the microbial food web, a redundancy detrended analysis (RDA; Rao, 1964) was performed. The groups in the microbial food web considered were separated according to their trophic strategy and their size (see methods: Analysis of biological parameters). All canonical axes were used to evaluate the significant variables under analysis by means of a Monte Carlo test (1000 permutations). RDA tests were run using version 4.5 of CANOCO (Ter Braak and Smilauer, 2002). Three data matrices were used. The first included the biomass of microbial food web groups; the second included the environmental variables and zooplankton groups that may affect plankton variability among lagoons. Spatial impact, moreover, was removed to prevent interference with environmental variables and zooplankton, the spatial matrix was introduced as a covariable. Biological data (plankton and zooplankton) were log- ($x+1$) transformed and centered (centered log-ratio) and the environmental matrix was transformed with log ($x+1$) transformation (Pawlowsky-Glahn and Buccianti, 2011).

4. Results

The microbial food web of the water bodies analysed in the Doñana marshland was predominated by autotrophic organisms (values of A:H biomass ratio ranged between 0.3 and 25.3). A:H biomass ratio was different invariable for all the sampled sites, although we found values > 1 in most of the water bodies, indicating that autotrophic organisms were usually more abundant than heterotrophic organisms. The autotrophic community was principally composed of nanophytoplankton and colonial cyanobacteria. The biomass of heterotrophic organisms was mainly represented by bacteria, while heterotrophic microplankton densities were low. No mixotrophic organisms were found: potential mixotrophic organisms were very scarce and no particle ingestion by autotrophic organisms was detected in the incubations performed.

The three data sets (spatial distribution, environmental variables and zooplankton) explained 77% of total *A/H* variance. Spatial distribution accounted for the largest independent portion of total *A/H* variance (16%), followed by environmental variables that accounted for the 13% of total variance (Fig. 2). Zooplankton had no significant influence on the *A/H* ratio (3%). The variation shared between the environmental variables and spatial distribution was high (24%), while the variation shared between the environmental variables and the zooplankton matrix was lower (8%).

A significant relationship was found ($r^2 = 0.5$; $p < 0.001$) in the multiple regression analysis between *A/H* (response variable) and the MEM values of the spatial distribution data set (explanatory variables). According to this regression analysis, basins can be clustered into 3 groups according to their estimated *A/H* ratio (Fig. 3; Table 1). Group 1 basins (low *A/H* values, A:H biomass ratio <2; autotrophic biomass was less than twice heterotrophic biomass) were isolated and located mainly in the western
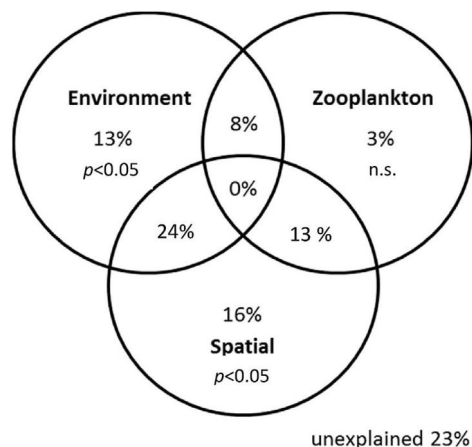
Fig. 2. Variation partitioning of *A/H* into independent and shared variation (%) from three set of variables: environmental variables, zooplankton biomass and spatial distribution of waterbodies.

periphery of the marshland and have the lowest *Nt/Pt* and *DIN/SRP* ratios. Group 2 (intermediate *A/H* values, A:H biomass ratio between 2 and 10) were isolated basins located in the eastern part of the marshland, with the highest *Nt/Pt* and *DIN/SRP* ratios. Lastly, group 3 (high *A/H* values, A:H biomass ratio >10; autotrophic biomass is one order of magnitude higher than heterotrophic) included the basins located closest to the effluent of the aquaculture farm that discharges into a tidal dead-arm of the Guadalquivir River (Brazo de la Torre; Fig. 3; Table 2). This group showed intermediate *DIN/SRP* and *Nt/Pt* ratios, but the lowest Nt and organic-N values. No significant differences among groups were found for other environmental variables. Insofar as the taxonomical composition of phytoplankton is concerned, filamentous cyanobacteria *Anabaena* predominated in low A/H basins (group 1); small green algae (chlorophytes and prasinophytes) in intermediate A/H basins (group 2) and the three high A/H basins (group 3) were predominated by euglenoids (chi-square = 81.0; df = 60; $p = 0.037$; Fig. 4).

In the RDA analysis different trophic groups of the community were included and the results did not discriminate among the different A/H groups described. Only four environmental variables were significant in this analysis: DIN, SRP, conductivity and gilvin (g440). The model did not select any variable related to zooplankton composition. The RDA performed, after the removal of spatial effects, accounted for 43.2% of the total variance of trophic groups and environmental and zooplankton data in the two first dimensions. The first axis of RDA analysis explained the 35.4% and the second axis explained the 7.8% variance (Fig. 5). SRP and DIN appeared as orthogonal in RDA on the two first axes, while SRP and DIN correlated positively to the first and second RDA axes respectively. Gilvin (g440) was positively correlated with the first axis and conductivity with the second. The first axis was also related positively with colonial cyanobacteria and negatively with autotrophic microplankton. The other microbial groups manifested a negative relationship with this axis albeit not so intensely. Organisms were distributed along the second axis according to their size: with bacteria, autotrophic picoplankton and autotrophic nanoplankton related to negative coordinates and heterotrophic microplankton to positive.

5. Discussion

The importance of including spatial relationships in the
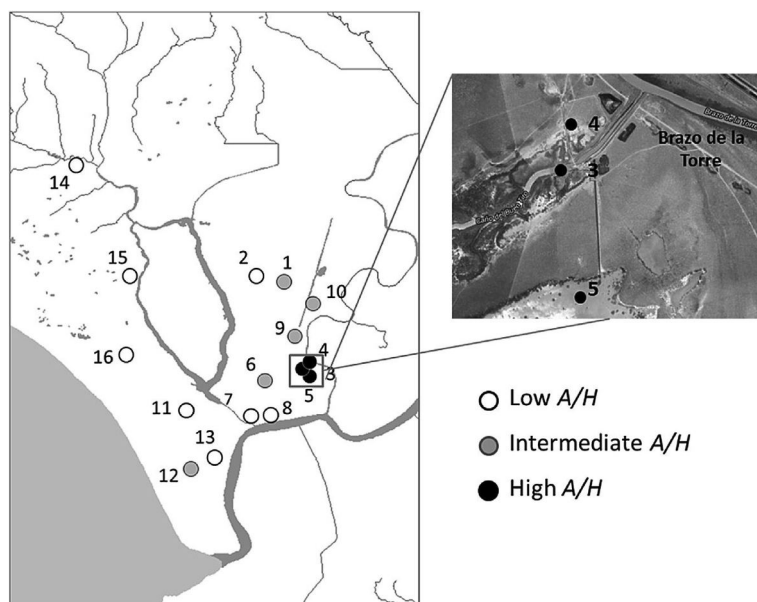
Fig. 3. Map with groups of basins based on spatial model of *A/H* (white circles = group 1, grey circles = group 2 and black circles = group 3). Detail of sites in group 3 hydrological distribution.

Table 2
Ratio autotrophic/heterotrophic biomass in the 16 sites related with main hydrological characteristics and distance from the effluent of the aquaculture farm-wetland complex of Veta la Palma (Google Earth).

Site	Freshwater/tidal	Shortest distance travelled by tidal water (m)	Distance to effluent (km)	*A/H*
1	F/T	1640	11	Intermediate
2	F	Almost isolated	Isolated	Low
3	T	700	3,7	High
4	T	600	3,6	High
5	F/T	1200	4,2	High
6	F/T	4000	7	Intermediate
7	F	Almost isolated	Isolated	Low
8	F	Almost isolated	Isolated	Low
9	T	700	6,9	Intermediate
10	T	360	5,4	Intermediate
11	F	Almost isolated	Isolated	Low
12	F	Almost isolated	Isolated	Intermediate
13	F	Almost isolated	Isolated	Low
14	F	Almost isolated	Isolated	Low
15	F	Almost isolated	Isolated	Low
16	F	Almost isolated	Isolated	Low

interpretation of ecological data has been widely acknowledged (Fortin and Dale, 2005; Legendre and Legendre, 1998). The Doñana wetlands have traditionally been classified using spatial-related variables (Espinar and Serrano, 2009). In the Doñana marshland, hydrogeochemical features are related to the frequency and persistence of the flood in each particular basin which, in turn, depend on its location with respect to the water source (freshwater *vs.* tidal water), the net flow of surface water, and the degree of groundwater discharge (Custodio et al., 2008; Manzano et al., 2005; Serrano et al., 2006). This complexity has created a patchy distribution of nutrient and planktonic chlorophyll concentrations that has prevented any further differentiation of wetlands, except on a very fine spatial scale (Espinar et al., 2002). Species composition rather than biomass has been used to differentiate vegetation units (Espinar et al., 2002). Hydrology and basin location are also expected to play an important role in the composition of

phytoplankton assemblages, as different water inputs would allow the colonization and the survival of organisms from different origins (Comín et al., 1999; López-Flores et al., 2006b). Both temporal and spatial factors produced a pattern in the phytoplankton assemblages of the NE Doñana marshland predominated by freshwater inputs during winter flooding and tidal inputs in summer, and where the spatial segregation of phytoplankton was likely the result of differences in retention time and distance to each water input (Reyes et al., 2008).

In our study, spatial distribution of waterbodies was the most important source of variability of *A/H*. Most factors concerning the catchment area are directly related to hydrology. Moreover, spatial distribution in the Doñana marshland very much differentiates water bodies with unlike hydrologies. The results therefore, suggest the importance of hydrology in these marshlands. Environmental variables and their combination with spatial distribution were the
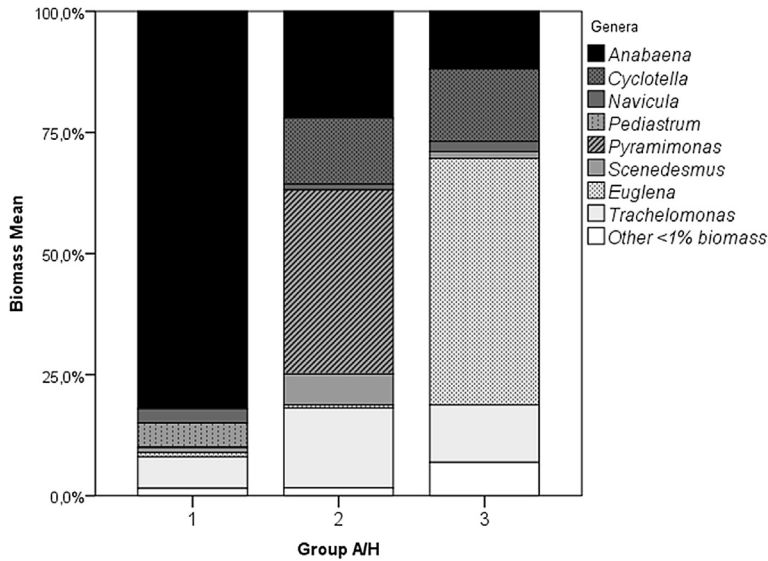
Fig. 4. Biomass mean of major phytoplankton genera clustered by A/H category. Colors were defined following higher taxonomic classification (cyanobacteria, diatoms, chlorophytes and euglena). Taxa representing less of 1% of biomass were clustered together.
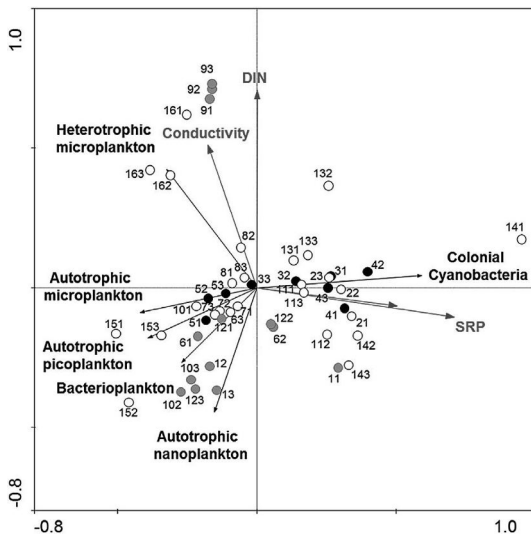
Fig. 5. Representation of the first two axes of the RDA analysis of main microbial loop trophic groups (bacterioplankton, heterotrophic microplankton, colonial cyanobacteria and autotrophic microplankton, picoplankton and nanoplankton) with environmental variables and zooplankton. Only significant variables are represented (p < 0.05, Monte Carlo test). RDA showing groups of basins based on spatial model of A/H (white circles = group 1, grey circles = group 2 and black circles = group 3).

next most significant factors.

The spatially-explicit regression model allowed for the segregation of waterbodies into three groups according to their A/H balance. Although all the basins exhibited a predominance of autotrophic organisms, the first group contained the waterbodies with a relatively higher load of heterotrophic organisms. Groups 2 and 3 were formed by waterbodies completely predominated by autotrophic organisms (autotrophic biomass was more than twice

that of the heterotrophs, or 10 times more, respectively). Our data confirmed previous studies with regard to a greater abundance of heterotrophic organisms in confined environments (López-flores et al., 2009; López-Flores et al., 2006a). Group 1 (low A/H) included the basins that are isolated from tidal inputs and located either close to the dune front or further inland and are only fed by freshwater (mainly rainfall and groundwater discharges). These basins presented the lowest values of Nt/Pt and DIN/SRP, but the highest values of organic nitrogen, probably because of their confinement. In contrast, group 3 (high A/H) comprised those basins supplied entirely by tidal waters or by a combination of fresh and tidal water, and located at less than 4.5 km from the effluent of the aquaculture farm-wetland complex of Veta la Palma. Basins in this group presented the lowest values of Nt and organic nitrogen and intermediate values of Nt/Pt and DIN/SRP. Group 2 (intermediate A/H) occupied an intermediate position as regards A/H balance, with basins supplied by a combination of water sources, albeit located more than 5 km from the aquaculture effluent. They obtained the highest values of Nt/Pt and DIN/SRP. The only exception was site 12 (Lucio del Membrillo), which is located by the dune front and isolated from the tidal waters.

Neither zooplankton group was determinant of the prevailing microbial food web trophic strategy (A or H). Zooplankton data were not significant when testing the variance of A/H nor in the RDA of trophic microbial groups. These results therefore pointed to the existence of a bottom-up control in the microbial food web of this ecosystem. This control was also reflected in the importance of N/P ratios. The low values of DIN/SRP ratios found in most of the study basins suggested that N acts as a limiting nutrient. The N/SRP ratio was higher than 16 in only one basin (site 9), where nitrate concentration was very high. This particular area of the marshland receives the overflow of some streams that are chronically polluted with nitrate (Serrano et al., 2006). Internal processes may explain the general lack of N, as strong N limitation has been described in Mediterranean saltmarshes that are highly confined because of denitrification (Comín and Valiela, 1993; Nixon, 1981; Quintana et al., 1999). In the Doñana wetlands particularly, López-Flores

et al. (2014) have described a generalized low balance between potential nitrification and denitrification rates that may lead to a global N loss.

The high inter- and intra-annual hydrologic variability is produced by irregular energy pulses, whereby communities face situations with a high variability in the availability of resources, which arrive in abundance during flood pulses but become limited during the confinement period. In confinement conditions, the concentration of dissolved inorganic nitrogen (DIN) is very low (Bañeras et al., 2012). Scarcity of nitrogen combined with a relatively easy P release from sediment induces nitrogen-fixation and hence favours the development of species capable of fixing molecular nitrogen (Blomqvist et al., 1994) such as cyanobacteria (Stal et al., 2000). Cyanobacteria are widely described as having a lesser dependence on inorganic nitrogen on account of their ability to fix atmospheric nitrogen (Havens et al., 1998; Reynolds, 2006; Scheffer, 1997; Shapiro, 1990).

Indeed, the strong correlation we found between SRP and cyanobacteria biomass suggested that cyanobacteria were more influenced by the availability of P than by the lack of N. The distribution of the other organisms throughout the RDA analysis also pointed to the importance of N in the productive processes of the Doñana marshland. Along the RDA axis associated with DIN, organisms were distributed according to their size. In situations of high productivity with available inorganic nitrogen, large body sized organisms predominate (microplankton), while in low nitrogen availability plankton is predominated by small organisms (bacterioplankton, picoplankton and nanoplankton). Under less productive conditions, smaller organisms take advantage of their high ratio surface area: volume, which makes them more efficient in nutrient uptake (Capblancq, 1990; Litchman and Klausmeier, 2008).

We found a higher proportion of organic than inorganic fractions of P in the water column. Some particulated inorganic P was also likely present in those sites with some re-suspended sediment because of the high capacity of the marshland silty substrate to adsorb P (Reina et al., 2006). Results found in other Doñana wetlands (Díaz-Espejo et al., 1999; Serrano et al., 2003, 1999) have also shown that the bioavailability of P is very high due to the release of phosphate from the sediment. For this reason, the sediment does not act as a sink for P in these shallow ecosystems, as P availability depends on the chemical balance between the water and the sediment (Golterman, 2004, 1995, 1975). Considering that nutrient input sources usually supply both nutrients together (N and P), their orthogonality in the RDA suggests that internal processes are more important than external inputs in the nutrient dynamics of this marshland.

Efficient energy transfer in a trophic web depends on basal level quality. Heterotrophic (eukaryote and prokaryote) and mixotrophic organisms have a higher nutritive quality (Picard and Lair, 2000; Elser et al., 2000; Müller-Navarra, 2008; de Carvalho and Caramujo, 2014) and therefore exhibit a higher efficiency energy transfer to higher levels than autotrophic organisms. Picard and Lair (2000) concluded that by feeding essentially on bacteria, Daphnia growth and reproduction were guaranteed under conditions of scarcity. Müller-Navarra (2008) also asserted that although bacteria are rich in protein, they do not generally contain HUFA or they have a very limited HUFA content. Our data revealed a heterotrophic compartment of mainly bacteria and ciliates. In the Doñana marshland, heterotrophic resources may therefore be of a lower quality on account of a possible scarcity of some HUFA compounds. The predominance of autotrophic organisms in all the basins studied would however complement a heterotrophic diet with complex lipids. Some authors have determined that autotrophic organisms dominate the total plankton biomass in eutrophic systems, while heterotrophic organisms —mainly bacterioplankton— predominate in oligotrophic waters. The existence of this tendency towards a decreasing A/H ratio from eutrophic to oligotrophic environments has been observed in other studies of plankton communities, both in freshwater and in the ocean (Cotner and Biddanda, 2002; Del Giorgio and Gasol, 1995). In this study, we have noticed that A/H ratio variation is primarily driven by spatial distribution of waterbodies and, in consequence, by hydrology. Secondary, trophic variables modulated the final composition of the community. In the Doñana marshland, nitrogen is naturally scarce (Golterman, 1995) and it limits the production of the microbial food web. The trophic web is nevertheless sustained mainly by a basal level predominated by autotrophic organisms, a situation more typical of systems with short trophic webs where nutrient supplies are guaranteed (Sommer et al., 2002). Higher efficiency of the prevailing low-sized cells (*Navicula* and *Cyclotella*; Capblancq, 1990; Legendre and Rassoulzadegan, 1995), combined with a capacity to fix cyanobacteria nitrogen and an intermediate level in the trophic web predominated by protozoan before the predators (Sommer and Stibor, 2002; Sommer et al., 2002) could shed light on this initially unforeseen situation. Both the tidal dynamic of the estuary and the distance of each site to the outflowing water of the aquaculture-wetland complex of Veta la Palma were crucial in this spatial pattern. This aquaculture-wetland complex is located adjacent to the Doñana National Park and is managed to sustain large microalgae productivity (Fernández-Rodríguez et al., 2015). The arrival of microalgae blooms in the Doñana marshland with incoming tidal water has also been reported by Reyes et al. (2008).

In warm temperate and arid climates, the predicted reduction in precipitation and higher evaporation on account of Global Change will lead to a severe drop in run-off, whereas diffuse may decrease as a result and concentrations of nutrients in inflows may increase (Jeppesen et al., 2011, 2009). Furthermore, in drier climates, lakes might also completely disappear (Schindler, 2009), a possibility exacerbated by likely compensatory increases in water extraction for irrigation (Jeppesen et al., 2011). Our study reflects the importance of hydrology and trophic condition variations in configuring microbial food web structure and, consequently, the higher levels of the associated trophic web. The non-natural increase of freshwater inflow into the marshland and the consequent increase in the eutrophication level can lead to a simplification of the aquatic community, which now is already mainly sustained by autotrophic biomass. Results also suggest that confinement could favour an increase in energy transfer between trophic levels and a more complex trophic web as observed previously by (Amils et al., 2007; Sommer and Stibor, 2002; Sommer et al., 2002) in scarce-resource environments. An accurate management of local and regional hydrology, which takes basal trophic level dynamics into account, will be essential to prevent the predicted consequences of non-natural and global changes.

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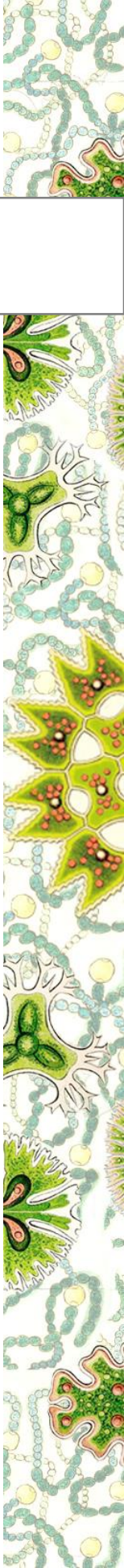López-Flores, R., Garcés, E., Boix, D., Badosa, A., Brucet, S., Masó, M., Quintana, X.D.,

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Microbial food web composition in Mediterranean coastal aquatic ecosystems under extreme drought conditions

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Microbial food web composition in Mediterranean coastal aquatic ecosystems under extreme drought conditions

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ABSTRACT

Mediterranean coastal aquatic ecosystems are heterogeneous systems covering a wide range of hydrological characteristics. Differences in the hydrological pattern determine the nutrient dynamics and microbial food web composition of these habitats. We analysed the composition of the microbial food web and the factors affecting the predominance of autotrophic (A) or heterotrophic (H) microorganisms in 17 coastal water bodies of the Empordà and Baix Ter wetlands (NE Iberian Peninsula) during mid-summer under severe drought conditions. We sampled three types of water bodies with differences in their water turnover: 6 confined euhaline basins, 8 oligohaline basins and the estuaries of the three rivers that flood into the area. Our aim was to determine if differences exist in the microbial food web structure in Mediterranean coastal water bodies with different water turnover under conditions of severe drought or, on the other hand, if the lack of flooding during the summer makes confined, choked and leaky ecosystems more similar. Confined basins showed higher values than estuaries for temperature, conductivity and the proportion of nutrients in organic forms. Regarding microbial food web composition, microphytoplankton (mainly dinoflagellates) dominated in confined lagoons, while picoplankton dominated in estuaries. We found no significant differences between oligohaline basins and both estuaries and confined basins for either physical and

chemical variables or microbial food web composition. Only the proportion between autotrophic and heterotrophic organisms was lower in oligohaline basins than in confined ones. We conclude that the estuaries of rivers with measurable flow during the summer differ from confined lagoons long isolated from any surface water input, and similarities that we expected to find between leaky and confined ecosystems under drought conditions arise in only oligohaline habitats with intermediate water turnover rates.

INTRODUCTION

Coastal aquatic ecosystems, defined according to the Water Framework Directive (WFD) as transitional waters (Borja, 2005; Hering et al., 2010; Moss et al., 2003), develop at the interface between coastal terrestrial and marine ecosystems and can be permanently open or intermittently closed off from the adjacent sea by depositional barriers (Gönenç and Wolflin, 2004; Kjerfve, 1994; Lankford, 1977). Transitional waters are heterogeneous systems covering a wide range of hydrological characteristics, from estuaries or leaky coastal systems with free connections to the open sea and thus with a high circulation of marine and river water, to choked lagoons with limited marine or freshwater circulation (Kjerfve and Magill, 1989), or even confined ecosystems with surface water circulation limited to flooding events and isolated from the sea and from any freshwater input for most of the year (Badosa et al., 2006; López-Flores et al., 2006a; Quintana et al., 1998).

Differences in hydrological patterns between more or less connected coastal ecosystems determine the nutrient dynamics and microbial food web composition in these habitats. In open estuaries, ecological processes are mainly driven by external nutrient inputs coming from the river, while in choked and confined ecosystems internal loading processes become more important than external inputs (Dugdale and Goering, 1967; Gamito et al., 2005; Gilbert et al., 2010). Moreover, these ecosystems differ in their microbial food web compositions. According to Gilbert et al., (2010) estuarine phytoplankton communities are dominated by larger-

sized organisms, such as diatoms, responding to runoff nutrient inputs where oxidized forms of nitrogen (nitrate) dominate. On the other hand, lagoons are dominated by picophytoplankton and sustain greater flow through the microbial food web, supported by nutrients regenerated from the benthos mainly in reduced forms in the case of nitrogen (ammonia or urea).

Coupling between autotrophic and heterotrophic processes strongly affects the microbial food web structure and dynamics (Del Giorgio et al., 1999). The relative contribution of autotrophic (A) or heterotrophic (H) organisms in aquatic food webs has been described as being not constant, depending on several factors such as primary productivity or the relative amount of allochthonous carbon supplies (Del Giorgio et al., 1999; Del Giorgio and Gasol, 1995; Gasol et al., 1997). Heterotrophic production has been considered to be important in estuaries where allochthonous organic matter supplies are high (Coffin and Sharp, 1987; Cole et al., 1988; Findlay et al., 1991). However, nutrient supplies in estuarine ecosystems may also increase the *A/H* ratio by increasing autotrophic growth (Wiegner et al., 2003). In confined environments, the lack of allochthonous supplies may reduce the relative importance of heterotrophic processes; on the other hand, the accumulation of organic matter and the increase of internal recycling processes may favour the heterotrophic pathway (Duarte and Prairie, 2005; Waiser and Robarts, 2004), thus reducing the *A/H* ratio (Àvila et al., 2016b; López-Flores et al., 2009, 2006a).

According to Gilbert et al., (2010) the differences between coastal lagoons and riverine estuaries of temperate or warm regions may become minimal in summer due to flow reduction in estuaries. This is the case in Mediterranean coastal ecosystems, where climatic conditions are characterized by a low tidal influence and a strong irregularity in flooding events and water level fluctuations (Alvarez Cobelas et al., 2005; Beklioglu et al., 2007). Moreover, the model of confinement described for Mediterranean coastal ecosystems proposes a zonation based on the time of renewal of the components of marine origin where environments with strong differences in water turnover rates have similarities in organisms' biomass,

abundance and diversity (Gamito et al., 2005; Guelorget and Perthuisot, 1983; Tagliapietra et al., 2009). This suggests that drought conditions would minimize differences in nutrient dynamics and microbial food web structures between estuaries and choked lagoons in the Mediterranean region. The question is whether these similarities between estuaries and choked ecosystems persist under extreme confinement conditions. Actually, it is hard to imagine that under conditions of severe drought, estuaries that still maintain some water flow are similar to confined waters that have not received water inputs for a long time.

We analyse the microbial food web structure and the dominance of A or H organisms in several Mediterranean coastal water bodies with differences in their water turnover, from open estuaries to strongly confined lagoons, under severe drought conditions to ascertain if differences in microbial food web related to differences in water turnover can be observed. We hypothesize that similarities between estuaries and choked ecosystems do not appear in confined ecosystems, where abiotic constrictions caused by the prolonged lack of water inputs during periods of severe drought strongly determine the nutrient dynamics and community structure.

METHODS

Study area

The study was undertaken on a group of 17 coastal water bodies of the Alt Empordà and Baix Ter wetlands (NE Iberian Peninsula), a set of shallow lentic waters which are greatly affected by their proximity to the sea (always less than 4 km from the sea shore), but with no tidal influence (Figure 1). They lie on a plain formed by sedimentary materials from three rivers: the Muga, Fluvià and Ter, which have a Mediterranean hydrology with highly variable and irregular flow rates and with a strong reduction in flow during the summer (Julià et al., 1994; Trobajo et al., 2002). Samples were taken once during midsummer (between July and August 2010) at three different points in each water body. From a geographical and limnological

perspective, the studied water bodies are a good sample of the diversity of water bodies in this area. Water bodies were classified into three groups according to their hydrology: Confined basins (6 water bodies). These are euhaline ecosystems with high and variable salinity that are not connected to the sea and which lack a continuous supply of freshwater. Flooding in these ecosystems is always sudden and mainly sea water, usually coinciding with sea storms, after which the water remains confined without surface water inputs, causing a decrease in water level and an increase in salinity. These water bodies were classified by Trobajo et al., (2002) as

SITE	NAME
1	Fra Ramón
2	PN2
3	Ter Vell TV1
4	Salins
5	Clot de la Llúdriga
6	Illa del Moro
7	La Rogera
8	Riereta
9	Basses d'en Coll
10	Mas Pinell
11	La Llarga
12	Rec Madral
13	Ter
14	Riuet
15	Sirvent
16	Fluvià
17	Muga
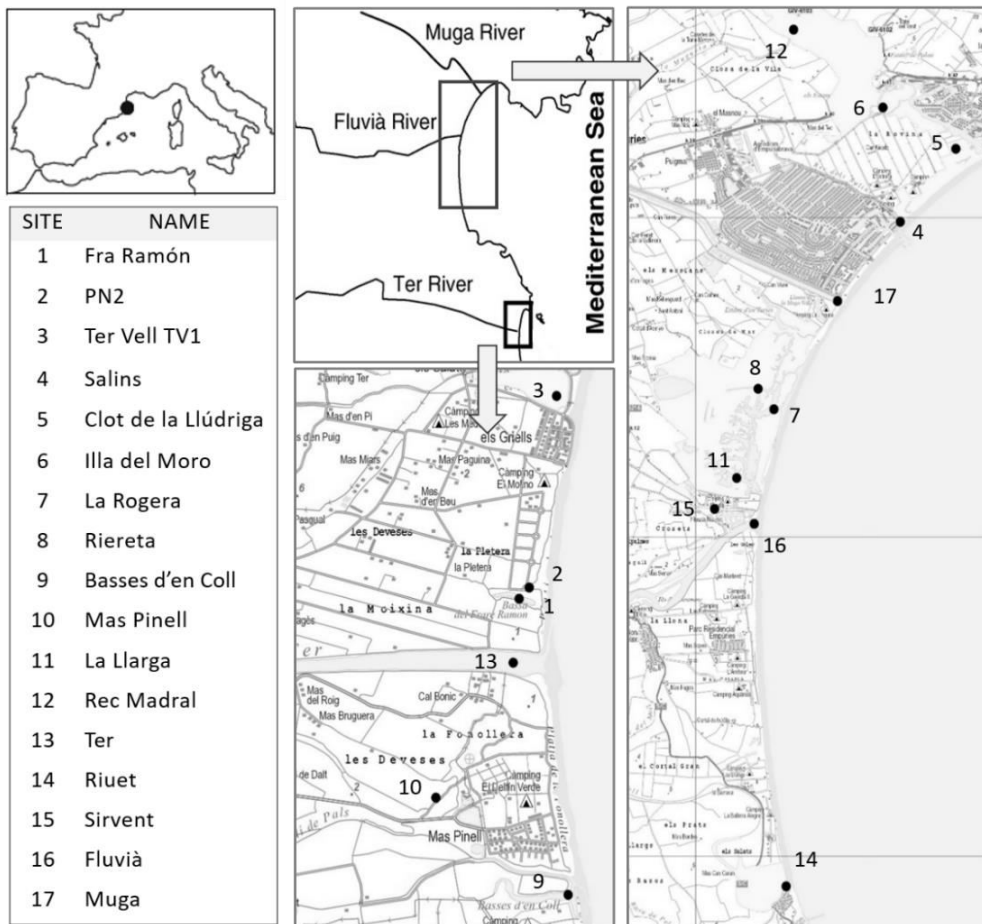
Figure 1. Map showing the location of the Empordà wetlands in the NE of the Iberian Peninsula and the location of the different water bodies in the study area (black circles = confined basins, grey circles = oligohaline basins and white circles = estuaries).

confined and semi-confined brackish coastal lagoons and ponds. Oligohaline basins (8 water bodies). These are oligohaline and unconfined ecosystems, defined by Trobajo et al., (2002) as fluctuating freshwater systems where a certain flux of freshwater occurs more or less continuously. This group includes ecosystems with a wide gradient of freshwater circulation, from water bodies with an estuarine behaviour with more or less continuous freshwater flooding (even in midsummer) to water bodies without a surface water supply channel, where freshwater enters mainly via groundwater. Estuaries (3 water bodies), including the estuaries of the three rivers that flow into the zone (the Ter, Fluvià and Muga rivers). Some limnological characteristics of the selected water bodies are listed in Table 1.

Physical and chemical analyses and biomass estimations of organisms

Water temperature (°C), electrical conductivity (EC_{25}), pH and dissolved oxygen (mg/L) were measured in situ. Filtered samples (Whatman GF/F) were frozen for the later analysis of dissolved inorganic nutrients (DIN: $NH_4^+ + NO_2^- + NO_3^-$) and soluble reactive phosphate (SRP). Unfiltered samples were either frozen for later analysis of total nitrogen (Nt) and total phosphorus (Pt) or refrigerated for total organic carbon analysis (TOC). Nutrient analyses were carried out as described in Grasshoff et al., (1983) and total organic carbon was measured using a TOC analyser (TOC500 SHIMADZU, Shimadzu Scientific Instruments, Columbia, USA). Gilvin (g440) was also measured according to Kirk (1994), which determines the presence of humic acids by measuring the absorbance of samples at 440 nm.

The following different groups of organisms that make up the microbial food web were analysed: colonial cyanobacteria, picophytoplankton, nanophytoplankton and microphytoplankton (autotrophic organisms); heterotrophic microplankton (ciliates and *Oxyrrhis marina*) and bacterioplankton (heterotrophic organisms). Samples of zooplankton (> 50 μm) were also collected. The abundance and biomass of bacterioplankton, picoplankton and nanoplankton were measured with a flow cytometer (FACScalibur by BD Biosciences). Microphytoplankton, colonial

Table 1. Environmental characteristics measured in the different groups of basins (1, Confined basins; 2, Oligohaline basins; and 3, Estuaries). NAD not available data.

Group	Water body name	Water body	Temperature (sampling day) ºC	Conductivity (sampling day) mS/cm	Conductivity annual mean (Std. dev.) mS/cm	Distance to the sea, m	Marine and/or freshwater surface inputs	References
CONFINED	Fra Ramon	1	31.07	57.00	43.75 (16.73)	200	Absence of freshwater inputs.	López-Flores et al. 2006
	PN2	2	29.10	18.52	43.50(14.69)	175	Connection to the sea limited to sea storm events	Moreno-Amich et al. 1998
	Clot de la Llüdriga	5	30.77	79.80	40.17(10.27)	460	Occasional connection to the sea	Unpublished data
	Illa del Moro	6	30.27	62.63	NAD	1650		
	La Rogera	7	27.77	55.03	14.89(7.9)	230	Absence of freshwater inputs.	Brucet et al. 2005
	Riereta	8	32.17	83.80	13.20(7.16)	540	Connection to the sea limited to sea storm events	Moreno-Amich et al. 1998
OLIGOHALINE	Ter Vell TV1	3	30.40	10.20	5.50 (8.70)	170	A freshwater channel provides water inputs several days after a rainfall	Badosa et al., 2008
	Salins	4	26.70	0.99	0.60 (0.18)	110		Moreno-Amich et al. 1998
	Basses d'en Coll	9	23.07	1,18	1.56 (0.63)	120	Active flow during summer due to rice field surpluses	Badosa et al. 2006b
	Mas Pinell	10	30.97	4.79	2.83 (0.94)	960	A freshwater confined lagoon with no surface water supplies	Ruhí et al. 2009
	La Llarga	11	27.00	29.83	NAD	520	A freshwater channel that provides water inputs during the whole wet season	
	Rec Madral	12	27.63	1.55	2.11 (2.53)	3600	A drainage channel of a freshwater wetland	Moreno-Amich et al. 1998
	Riuet	14	23.23	0.77	1.63(0.54)	115	The old estuary of the river Fluvià	Unpublished data
	Sirvent	15	26.63	41.27	1.28 (0.94)	710	A drainage channel of a freshwater wetland	Moreno-Amich et al. 1998
ESTUARIES	Ter	13	24.60	0.79	4.15(5.97)	150	Estuary of the river Ter	Unpublished data
	Fluvià	16	26.60	44.23	1.08(0.32)	90	Estuary of the river Fluvià	Moreno-Amich et al. 1998
	Muga	17	26.97	17.17	0.60(0.14)	150	Estuary of the river Muga	

cyanobacteria, ciliates and zooplankton were counted and measured using an inverted microscope (Zeiss Axiovert 135, Göttingen, Germany). More details on sampling, counting and biomass estimations are described in López-Flores et al., (2006a) and Àvila et al., (2016a).

Statistical analysis

A principal component analysis (PCA) was carried out to explore whether the three different water body groups differ in their physical and chemical compositions. The variables included in the PCA were temperature (Temp), conductivity (Cond), dissolved oxygen percentage (O_2%), pH, gilvin, inorganic nitrogen forms (NH_4^+, NO_2^-, NO_3^-), phosphate (SRP), organic phosphorus (Porg), organic nitrogen (Norg), total organic carbon (TOC) and planktonic chlorophyll a (Chl-a). PCA was carried out using PRIMER v6+ software (Clarke and Gorley, 2006). All variables were normalized prior to application of the PCA.

As a proxy of the dominance of autotrophic (A) or heterotrophic (H) organisms, we calculated the *A/H* balance using techniques based on compositional data analysis (Aitchison, 1986; Pawlowsky-Glahn and Buccianti, 2011), which are suitable when dealing with components of a composition and avoid spurious correlations. According to this, the *A/H* balance is

$$A/H = \frac{1}{\sqrt{2}} \ln \frac{A}{H},$$

where A is the sum of the biomass (pg C/ml) of the autotrophic organisms including picophytoplankton, nanophytoplankton, microphytoplankton and colonial cyanobacteria, and H is the total biomass (pg C/ml) of heterotrophic organisms including bacteria, ciliates and *Oxyrrhis marina*. This balance takes the form of a log ratio, where $1/\sqrt{2}$ has been added for normalization (Pawlowsky-Glahn and Buccianti, 2011). Other ratios, such as those found in table 2, were transformed using the same equation.

To quantify the influence of environmental variables and to see if the three water body types differ in microbial food web composition, we carried out a redundancy detrended analysis (RDA; Rao, 1964). Two data matrices were used. The first included the biomass of the microbial food web groups separated according to their trophic strategies and their size (previous section); the second included the environmental variables and zooplankton biomasses (Wzoo). Only significant variables were included in the analysis. The biological data (plankton and zooplankton) were log (x+1) transformed and centred (centred log ratio), and the environmental matrix was log (x+1) transformed (Pawlowsky-Glahn and Buccianti, 2011). All canonical axes were used to evaluate the significant variables under analysis by means of a Monte Carlo test (1000 permutations). RDA tests were performed using version 4.5 of CANOCO (Ter Braak and Smilauer, 2002).

RESULTS

Environmental variables and *A/H* ratio

Confined basins showed higher values of conductivity, temperature and TOC and lower values of inorganic nitrogen forms compared with estuaries. In oligohaline basins, intermediate values were found (Table 2). Although no significant differences were observed in phosphate and total nutrient concentrations, higher values of the ratios between nutrients and organic matter were found in estuaries than in confined basins (Table 3), indicating higher relative amounts of inorganic nutrients and higher proportions of reduced forms of nitrogen in estuaries. Oligohaline basins again showed intermediate values. The first two axes of the PCA analysis including environmental variables (explaining 62.3% of the variance) discriminated confined basins (generally with low values in both the PC1 and PC2 axes) from the other two groups, although a certain overlap was observed (Figure 2). Temperature and conductivity were positively related to confined basins, while inorganic nutrients were negatively related. No difference was found between estuaries and oligohaline basins.

A/H median values were slightly positive in confined basins, but negative in estuaries and oligohaline basins (Figure 3). Although the differences found in physical and chemical composition were mainly between confined basins and estuaries, the *A/H* differences were significant between confined and oligohaline basins; estuaries were extremely variable in *A/H* values and were not significantly different from any other basin group. Consequently, confined basins were dominated by autotrophs, while heterotrophs dominated in oligohaline systems.

Table 2. Mean and SD values of several physical and chemical variables in the different groups of basins (1, Confined basins; 2, Oligohaline basins; and 3, Estuaries). The results of the same row followed by the same letter do not differ significantly (post-hoc test; *p <0.05*). Bold indicates the variables with significant differences.

VARIABLE	TYPE OF BASIN		
	Confined	**Oligohaline**	**Estuaries**
Temperature (°C)	**30.19 (1.64) a**	**26.96 (2.78) b**	**26.06 (1.12) b**
Conductivity (µS/cm)	**59.46 (21.9) a**	**11.32 (14.89) b**	**20.73 (19.01) b**
% Oxygen saturation	113.57 (45.09) a	118.43 (59.79) a	108.76 (16.25) a
pH	8.31 (0.31) a	8.14 (0.31) a	8.33 (0.32) a
Gilvin (g_{440})	0.05 (0.05) a	0.06 (0.07) a	0.04 (0.04) a
TOC (µM)	**1497.36 (941.1) a**	**937.22 (704.4) a**	**332.80 (143.2) b**
Chlorophyll *a* (µg/L)	22.38 (23.14) a	41.67 (44.45) a	67.58 (86.5) a
Nt (µM)	188.28 (128.14) a	144.70 (84.22) a	156.71 (107.59) a
Pt (µM)	10.44 (13.27) a	17.01 (20.44) a	12.65 (14.27) a
DIN (µM)	**0.67 (0.92) a**	**21.20 (39.45) b**	**53.22 (43.69) c**
[NH_4^+] µM	**0.61 (0.92) a**	**4.45 (7.97) b**	**10.80 (13.66) b**
[NO_2^-] µM	**0,04 (0.02) a**	**0,41 (0,697) b**	**3.46 (4.01) b**
[NO_3^-] µM	**0.02 (0.02) a**	**16.34 (31.59) b**	**38.97 (46.56) c**
Norg (µM)	187.61 (128.05) a	123.50 (93.19) a	103.49 (88.04) a
[SRP] (µM)	2.20 (3.72) a	6.75 (9.6) a	7.24 (8.61) a
Porg (µM)	8.25 (10.34) a	10.26 (13.24) a	5.41 (5.72) a

Table 3. Mean and SD values of different balances in the different groups of basins (1, Confined basins; 2, Oligohaline basins; and 3, Estuaries). The results of the same row followed by the same letter do not differ significantly (post-hoc test; p < 0.05). Bold indicates significantly different variables. These balances were log transformed as described in the Methods equation.

VARIABLE	TYPE OF BASIN		
	Confined	Oligohaline	Estuaries
Nt/Pt	1.40 (1.43) a	1.82 (1.98) a	2.92 (0.94) a
DIN/Norg	**-4.46 (1.01) a**	**-2.39 (2.06) b**	**-0.53 (0.87) c**
SRP/Porg	-1.37 (1.09) a	-1.03 (1.54) a	-0.28 (0.74) a
DIN/SRP	**-0.56 (1.46) a**	**0.61 (2.42) ab**	**1.93 (0.98) b**
Nt/TOC	**-4.25 (0.73) a**	**-3.04 (1.17) b**	**-1.53 (0.69) c**
Norg/TOC	-1.45 (0.31) a	-1.43 (0.21) a	-1.06 (0.54) a
Pt/TOC	**-5.39 (1.19) a**	**-4.53 (1.22) ab**	**-3.91 (1.08) b**
Porg/TOC	**-3.99 (0.77) a**	**-3.40 (0.53) b**	**-3.25 (0,59) b**
NH_4^+/NO_3^-	**1.77 (1.83) a**	**1.24 (2.33) a**	**-1.17 (1.45) b**
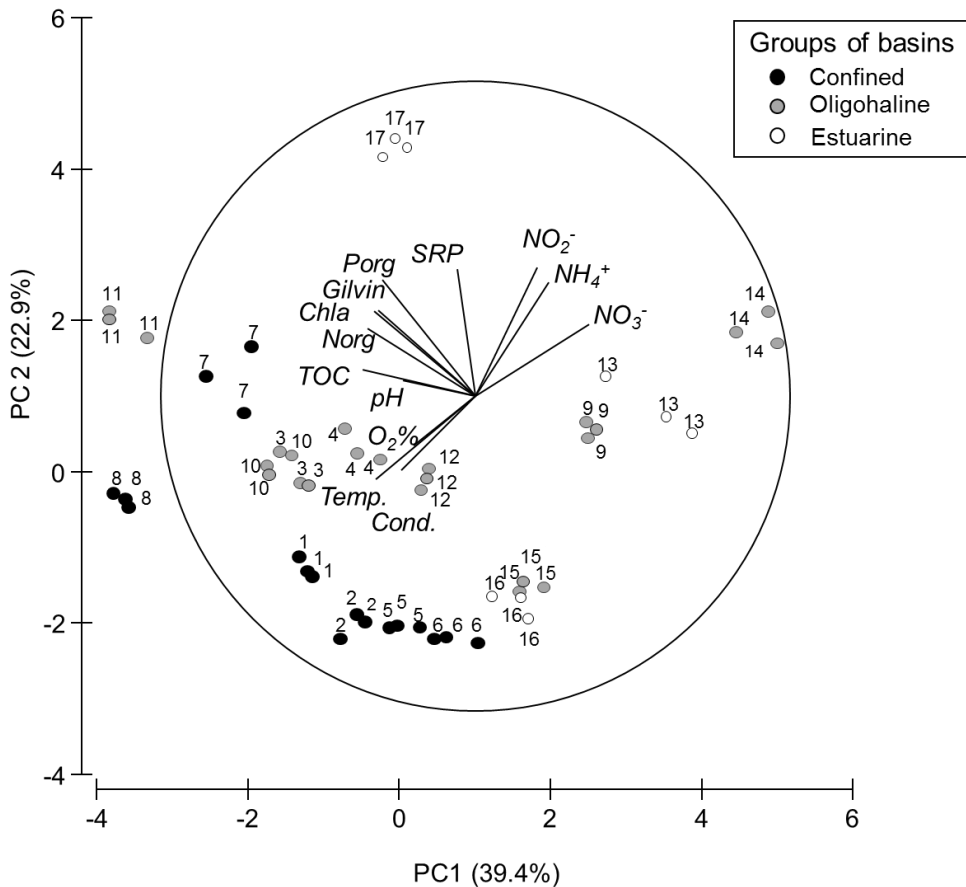
Figure 2. The first two axes of the PCA analysis using the environmental variables of the 17 sampled water bodies. The numbers correspond to the water body numbers listed in Table 1 (black circles = confined basins, grey circles = oligohaline basins, and white circles = estuaries).

Microbial food web composition in summer conditions

The biomass of heterotrophic organisms was mainly represented by bacteria in the three basin groups, with some heterotrophic microplankton composed mainly of ciliates appearing in confined basins (Figure 4). Autotrophic microphytoplankton in confined basins and estuaries were dominated by dinoflagellates (100% and 75%, respectively). Oligohaline basins were dominated by more varied groups: Euglenophyta (52%), Dinoflagellata (19%), Chlorophyta (12%) and Cyanobacteria (11%) (Figure 5).

The two first axes of the RDA analysis including environmental variables and microbial food web composition (Figure 6) explained 39.5% of the total variance (29.8% and 9.7%, respectively). Five environmental variables (conductivity, NO_3^-, Norg, TOC and Wzoo) contributed significantly (p <0.05) to the distribution of microbial food web groups. The first axis related to conductivity and discriminated assemblages dominated by autotrophic nano- and picoplankton (positive values) from those with colonial cyanobacteria. The second axis is related to inorganic forms of nitrogen and discriminated assemblages dominated by autotrophic microplankton (low inorganic nitrogen content) from those dominated by bacteria (high inorganic nitrogen content). RDA analysis did not discriminate between basin types since a large overlap was observed in the coordinates of the different basin types (Figure 6).
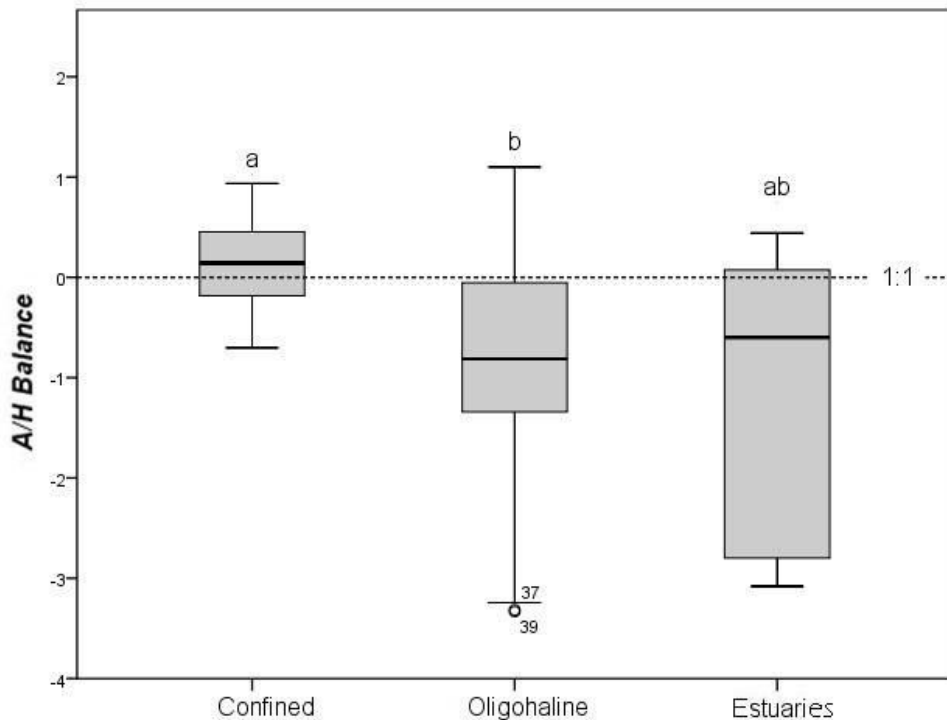
 Figure 3. Box plot showing the *A/H* values in the different groups of basins (1, 2 and 3). The ratios were log transformed as described in Methods, and the *A/H* values marked with the same letter do not differ significantly (post-hoc test; $p < 0.05$). The 1:1 line corresponds to the biomass of A and H being equivalent.
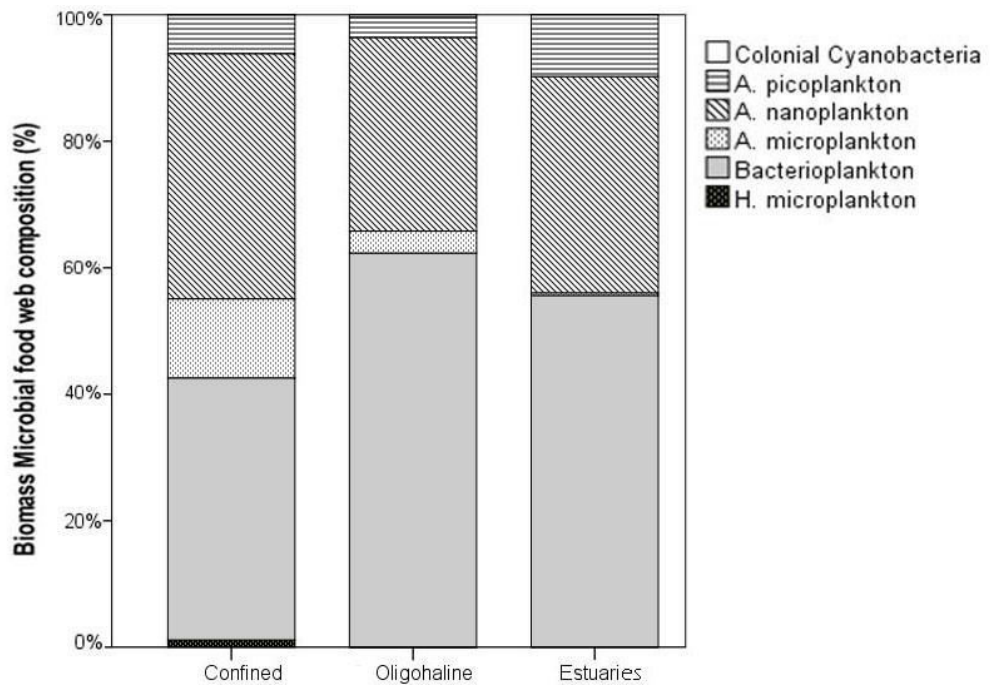
Figure 4. Percentage of the total biomass (µg/L) of the different groups composing the microbial food web in each group of basins.
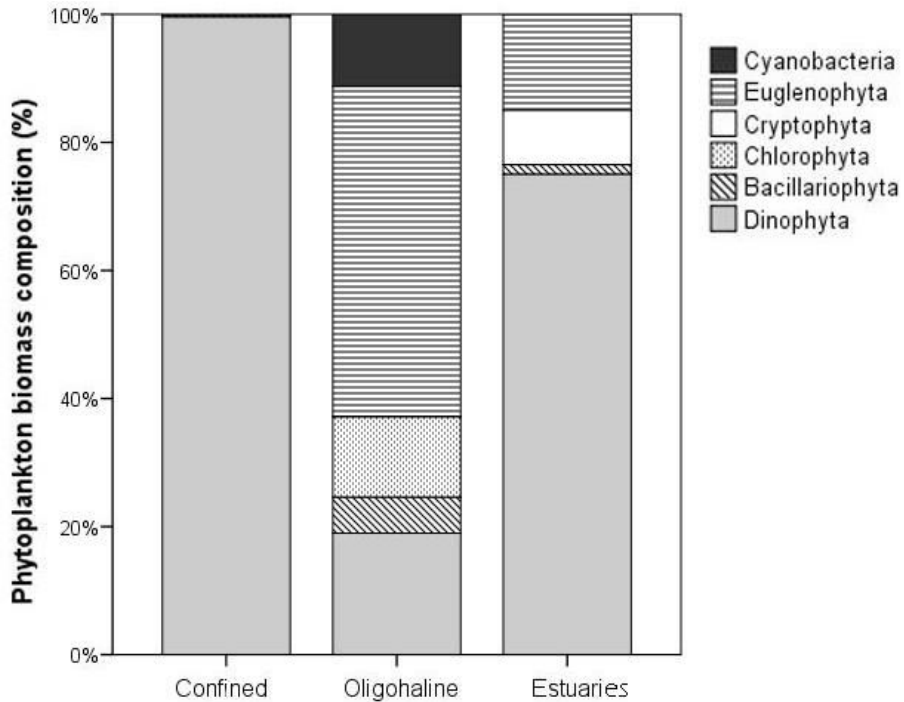
Figure 5. Biomass percentage of the different autotrophic phytoplankton groups in each group of basins.
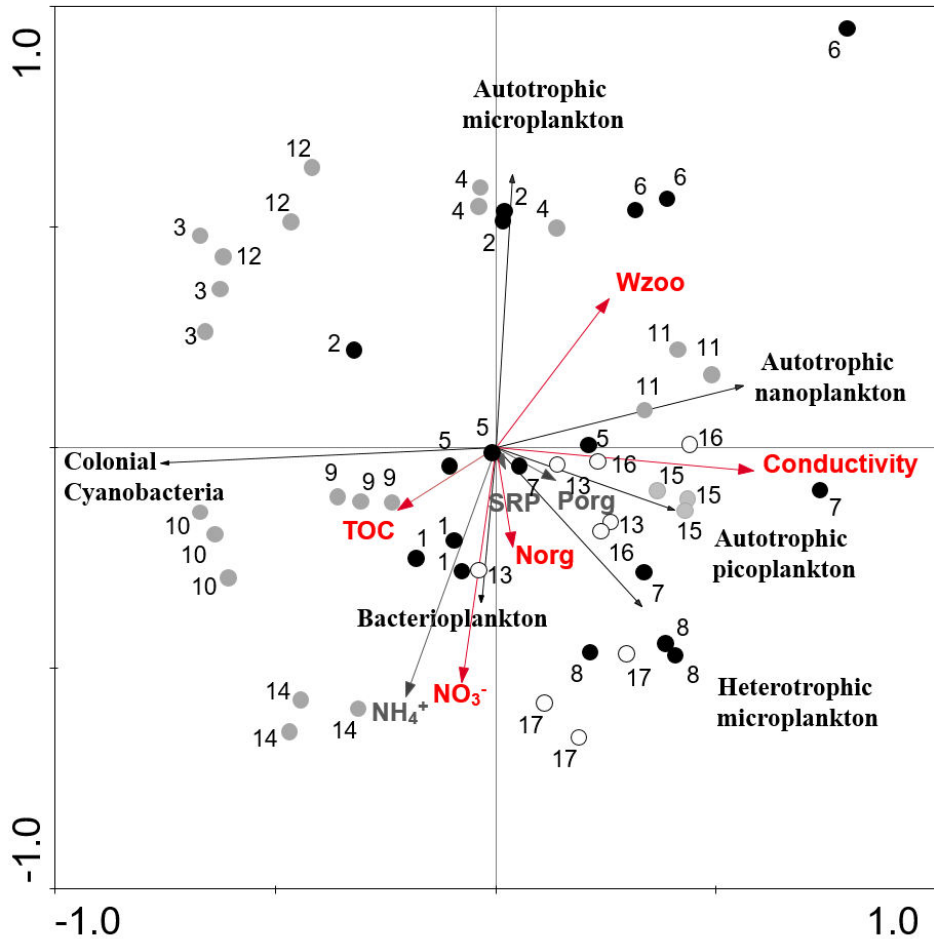
Figure 6. Plot of the two first axes of the RDA analysis including environmental variables and the different microbial food web groups (bacterioplankton, heterotrophic microplankton, colonial cyanobacteria and autotrophic microplankton, picoplankton and nanoplankton). Significant variables ($p < 0.05$, Monte Carlo test) are shown in red, and supplementary variables are shown in grey. The numbers correspond to the water body numbers listed in table 1. Black circles = confined basins, grey circles = oligohaline basins, and white circles = estuaries.

Conductivity values in Mediterranean coastal wetlands may increase during summer due to excess evaporation under maximum confinement conditions, but also because of marine water inputs coinciding with low freshwater supplies, as is the case in a salt-wedge estuary. Thus, an increase in conductivity may appear under both flooding conditions and a maximum degree of water stagnation. The same possible double effect can be applied to nutrient contents since total nutrients can

increase with water inputs and also with an increase in concentration during evaporation. To avoid these possible double effects and to see if differences exist in the relative amounts of the different nutrients, we conducted a second RDA removing conductivity from the analysis and substituting nutrient concentrations by the nutrient ratios listed in Table 3.

In this second RDA (Figure 7), only one environmental ratio, the balance between inorganic and total nitrogen (*DIN/Norg*), contributed significantly (*p <0.05*) to the analysis. Actually, *DIN/Norg* correlated with most of the other ratios listed in table 3 (data not shown). The first two axes explained 49% and 5.4% of the total variance, respectively (Figure 7). The first axis of this second RDA clearly discriminated between confined basins and estuaries, the former with low *DIN/Norg* values and dominated by autotrophic microplankton and the latter with high *DIN/Norg* values and dominated by bacterioplankton. The second RDA axis discriminated between samples dominated by colonial cyanobacteria from those dominated by autotrophic nano- and picoplankton. Oligohaline basins were distributed throughout the RDA plot, with a high overlap of both estuaries and confined basins.

DISCUSSION

Our results show differences in environmental variables and microbial food web compositions between estuaries and confined coastal lagoons in the Mediterranean under the strong drought situations typical of the summer period. While confined ecosystems present higher temperature and conductivity values and a higher proportion of nutrients in organic forms, estuaries are characterized by higher proportions of inorganic nutrients, especially of the three inorganic forms of nitrogen (ammonia, nitrite and nitrate). Regarding organisms, bacterio- and picoplankton appear to be more abundant in estuaries since microplankton, composed mainly of autotrophic dinoflagellates, are more abundant in confined ecosystems.
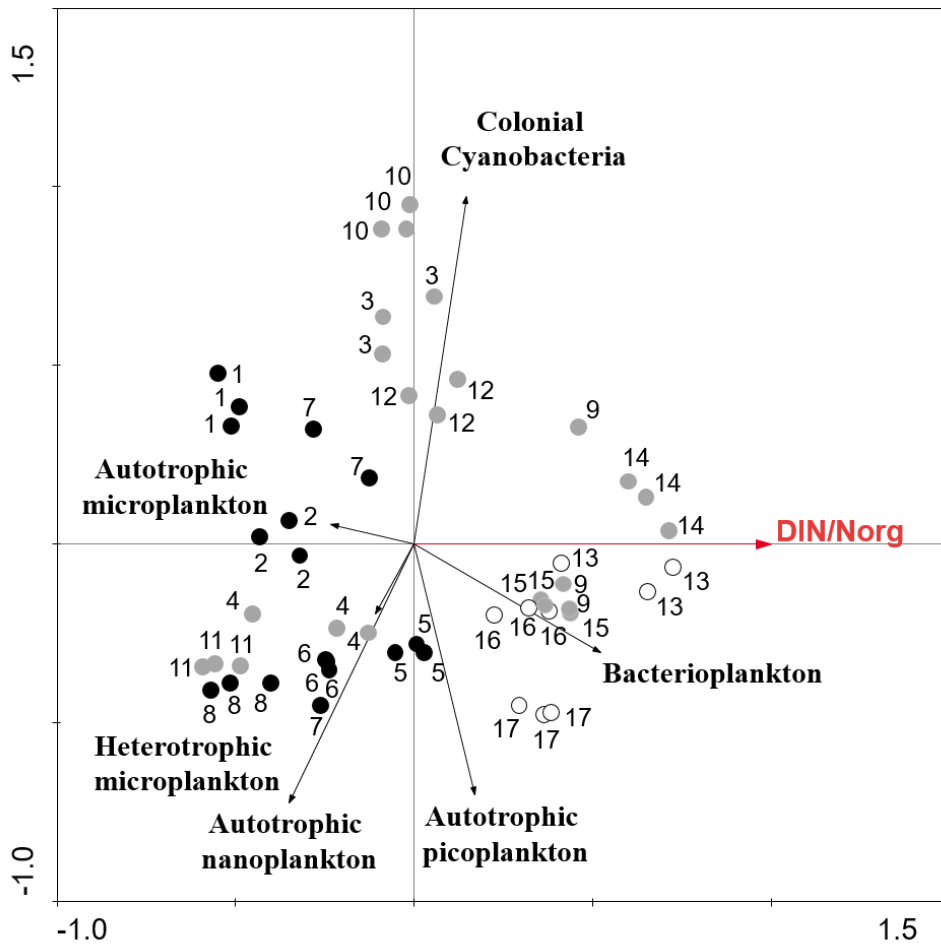
Figure 7. Results of RDA analysis with the same variables as in figure 5, but using the ratios of the environmental variables (listed in table 4) instead of the raw data of environmental variables. Only significant variables are represented (p <0.05, Monte Carlo test). The numbers correspond to the water body numbers listed in table 1. Black circles = confined basins, grey circles = oligohaline basins, and white circles = estuaries.

Neither the PCA analysis using the physical and chemical composition nor the RDAs including organisms discriminated oligohaline basins from estuaries and confined basins. In fact, oligohaline waters along the Mediterranean coast include a wide range of ecosystems with strong differences in their hydrologies, from choked ecosystems receiving a relatively high amount of freshwater during summer, mostly coming from artificial supplies of irrigation surplus (e.g., Comin and Valiela, 1993;

Romo et al., 2005; Badosa et al., 2008) to small estuaries that remain isolated during the summer period. Thus, the similarities that we expected to find between leaky and confined ecosystems arise in only oligohaline habitats, where the hydrology is highly variable at both the temporal and spatial scales. On the other hand, estuaries of rivers with measurable flows even during summer differ markedly from confined lagoons, which remain isolated from surface water inputs for most of the year.

Differences between estuaries and confined waters in their microbial food web compositions did not appear in the RDA analysis using the absolute values of nutrients (Figure 6), but did appear in the RDA using nutrient ratios (Figure 7). The increase in the total amount of nutrients may be the result of two opposite patterns: the increase of external loadings with increasing flooding or the accumulation due to evaporation during extreme confinement conditions (Gamito et al., 2005; Guelorget and Perthuisot, 1983). However, our results show that these two patterns differ in nutrient speciation. External loadings promote waters with nutrients in inorganic forms, while internal loading processes dominating during confinement conditions lead to the predominance of nutrients in organic forms. Denitrification has been described as the main cause of nitrogen losses in confined waters, affecting all inorganic nitrogen forms (Golterman, 1995; López-Flores et al., 2014) and causing a strong nitrogen limitation in these habitats (Àvila et al., 2016b; Badosa et al., 2006; Comin and Valiela, 1993; López-Flores et al., 2014, 2009, 2006b; Quintana et al., 1998). Regarding phosphorus, the relative amount of inorganic phosphate in the water depends on the chemical equilibrium with the phosphate precipitated in the sediment, mainly bound to Fe or Ca (Golterman 1995, 2004). A higher amount of soluble phosphate in water has been associated with allochthonous inputs (Golterman, 2004; Reina et al., 2006).

Gilbert et al., (2010) discriminate between coastal lagoons and riverine estuaries in temperate regions. According to these authors, coastal lagoons sustain greater energy flow through the microbial loop dominated by small picophytoplankters supported by nutrients regenerated from the benthic community, which provide

nitrogen mainly in reduced forms (ammonia or urea); estuaries are dominated by larger planktonic organisms, mainly diatoms, responding to runoff nutrient inputs where the dominant nitrogen form is nitrate. In agreement with the model described by Gilbert et al., (2010), we found higher values of the NH_4^+/NO_3^- ratio in confined lagoons than in estuaries. Furthermore, RDA analyses selected the inorganic/organic nitrogen ratio as the main environmental factor discriminating between the microbial food web compositions of estuaries and confined water bodies. Regarding organisms, we did not find an increase of phytoplankton body size with increasing water turnover as described by Gilbert et al., (2010). On the other hand, picoplankton dominated in estuaries, while microplankton, mainly composed of dinoflagellates, was more abundant in confined waters. Probably the abiotic stress under strong confinement conditions and their effects on the microphytoplankton species composition may explain these differences: flooding conditions would provide high inorganic nutrient inputs favouring the development of r organisms such as diatoms, whereas strong confinement would select only K species adapted to extreme environmental conditions of inorganic nutrient scarcity, such as dinoflagellates. Several studies show similar patterns in Mediterranean coastal waters (e.g., Giacobbe et al., 1996; López-Flores et al., 2006a, 2009; Bosak et al., 2012).

We found a higher dominance of autotrophic organisms' biomass in confined than in oligohaline waters, suggesting that autotrophic processes become more important with increasing confinement. These differences are only applicable to confined and oligohaline waterbodies since the *A/H* balance in estuaries was extremely variable and did not differ significantly from other water body types. Something similar happens in lotic ecosystems; the increased water flow and the consequent increase in allochthonous supplies have been described as promoting heterotrophic growth (Findlay et al., 1991; Gasith and Resh, 1999; Wiegner et al., 2003). Allochthonous organic matter supplies also increase the relative amount of heterotrophs in marine systems (Del Giorgio et al., 1999, 1997). However, this pattern appears not to coincide with that observed in lentic coastal floodplains,

where a positive relationship between autotrophic dominance and water circulation has been observed (Àvila et al., 2016b). Other factors besides water circulation may affect the dominance between autotrophic and heterotrophic organisms in lentic shallow waters, such as the degree of eutrophy (Àvila et al., 2016a) or the existence of anthropogenic impacts in the drainage basin (Àvila et al., 2016b). Moreover, a unimodal relationship between water turnover and A or H dominance cannot be discarded, with maximum heterotrophic dominance at intermediate degrees of confinement. Flooding conditions provide inorganic nutrients, whereas excessive confinement limits growth to the use of recycled nutrients, both favouring opposite life strategies (r and K) among the autotrophic pathways. The existence of unimodal relationships with water turnover are common in the Mediterranean, where abiotic restrictions prevail at both extremes, maximum flooding and severe drought, while biotic control predominates at intermediate turnover rates (Boix et al., 2004; Gascón et al., 2016; Gasith and Resh, 1999; Trobajo et al., 2002). Diatoms and dinoflagellates would be the organisms dominating autotrophic species compositions in the two extreme conditions.

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DISCUSIÓN GENERAL

1. El papel de la cuenca hidrológica en los aportes a las lagunas.

2. Dinámica de nutrientes y materia orgánica en lagunas someras.

3. Dominancia de autótrofos (A) versus heterótrofos (H).
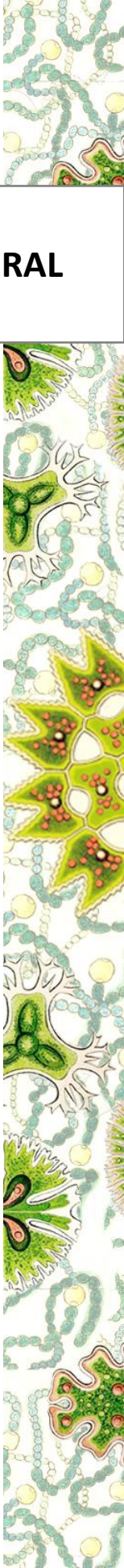
1. El papel de la cuenca hidrológica en los aportes a las lagunas

Conocer el funcionamiento hidrológico de las lagunas someras es importante para poder determinar la estructura de la red trófica microbiana, dado que la hidrología es uno de los principales factores que determina la composición de la comunidad planctónica (Alvarez Cobelas et al., 2005; López-Flores et al., 2006a; Beklioglu et al., 2007). Todos los ecosistemas incluidos en esta tesis presentan algún tipo de protección legal, sin embargo, son especialmente relevantes las diferentes presiones antrópicas que se ejercen sobre ellos, causando la alteración de su patrón hidrológico y funcionamiento natural. De forma concreta, los humedales costeros como son las marismas de Doñana (capítulo 2) y las lagunas y marismas del Empordà (capítulo 3), son sistemas que se ven afectados por las actividades que se realizan en el conjunto de la cuenca hidrológica que drena en estas. Estas cuencas están sometidas a fuertes presiones antropogénicas (agricultura, pastoreo, modificación de cauces, urbanización, actividades urbanas, industriales y turísticas) que en conjunto modifican el uso de los recursos hídricos de la zona causando elevadas variaciones en el nivel del agua, salinidad, temperatura y composición de nutrientes (Quintana et al., 1998; Trobajo et al., 2002; Alvarez Cobelas et al., 2005; García-Novo & Marín Cabrera, 2007). Los efectos de las actividades humanas se hacen más evidentes en los tramos finales de la cuenca hidrológica debido a sus efectos acumulativos (Manfrin et al., 2013; Bruno et al., 2014), por tanto, son más evidentes en los sistemas de humedales costeros (capítulo 2 y 3) que en los que se encuentran en tramos más iniciales o intermedios de la cuenca como es el caso de las lagunas someras de interior estudiadas en el capítulo 1 de esta tesis. Otra de las diferencias entre las lagunas interiores y costeras es que las primeras se encuentran en cuencas bien conservadas y forestadas lejos de grandes urbanizaciones y con actividad humana extensiva (Ballón et al., 2016). Este bajo nivel de presión antrópica y de buena conservación de la cuenca en la que se encuentran, se ve reflejada en las elevadas concentraciones de DOC que presentan. Se sabe que la materia orgánica en los ecosistemas acuáticos someros tiene mayoritariamente un origen alóctono,

procedente de la materia orgánica presente en los residuos vegetales y en los suelos de las vertientes de las cuencas de captación (Aitkenhead-Peterson et al., 2003; Thurman, 2012). Diversos autores han observado que las entradas alóctonas en lagunas localizadas en zonas perturbadas o deforestadas o que reciben agua que ha tenido un largo recorrido, como es el caso de las llanuras aluviales (Tockner et al., 1999; Amoros & Roux, 1988; Amoros & Bornette, 2002) o lagunas costeras (Glibert et al., 2010) muestran concentraciones más importantes de nutrientes inorgánicos que de DOC. Esto, como se ha comentado, estaría relacionado con los efectos de la actividad humana en los sistemas más perturbados, donde se observa un aumento en la carga de nutrientes a causa de la alteración de flujos, la entrada de aguas residuales y el flujo difuso de nutrientes procedentes de los campos agrícolas (Nixon, 1995; López-Flores et al., 2003; Howarth & Marino, 2006; Salvado et al., 2006).

2. Dinámica de nutrientes y materia orgánica en lagunas someras

La composición y la dinámica de los nutrientes y de la materia orgánica en las lagunas someras se ve fuertemente influenciada por la hidrología (Comín et al., 1987; Quintana et al., 1998; López-Flores et al., 2006a, 2006b; Beklioglu et al., 2007). Todos los sistemas estudiados a lo largo de esta tesis presentan una situación común de baja entrada de energía y nutrientes en la laguna puesto que todos los muestreos se realizaron en condiciones de estabilidad e incluso de máximo confinamiento. En el contexto de esta tesis, por tanto, no se ha tratado cómo sería el funcionamiento de estos sistemas en condiciones inestables de inundación, fluctuación o entradas de energía y nutrientes.

De forma general se ha observado un gradiente de nutrientes y materia orgánica relacionado con el grado de confinamiento (Figura 1). En las lagunas someras que presentan una situación de máximo confinamiento, la falta de entradas de agua y la elevada evaporación durante este período causa una acumulación progresiva de materia orgánica y de nutrientes totales (sobre todo en forma orgánica), como también una disminución de los nutrientes inorgánicos dando lugar a un bajo cociente entre

nutrientes inorgánicos: orgánicos típico de estos sistemas (Quintana et al., 1998; Quintana & Moreno-Amich, 2002; López-Flores et al., 2006a). En cambio, los estuarios de río o aquellas lagunas cercanas a las entradas de agua, se caracterizan por mayores proporciones de nutrientes inorgánicos, especialmente de las formas inorgánicas del nitrógeno. Se ha visto que elevadas entradas de nitrógeno están usualmente relacionadas con entradas de agua dulce (Chapelle et al., 2000; Lucena et al., 2002; Perez-Ruzafa et al., 2005). Esta entrada de nutrientes inorgánicos se observa sobre todo en los humedales costeros (capitulo 2 y 3) dado que se pone de manifiesto la influencia de la cuenca, tal como ya se ha comentado.
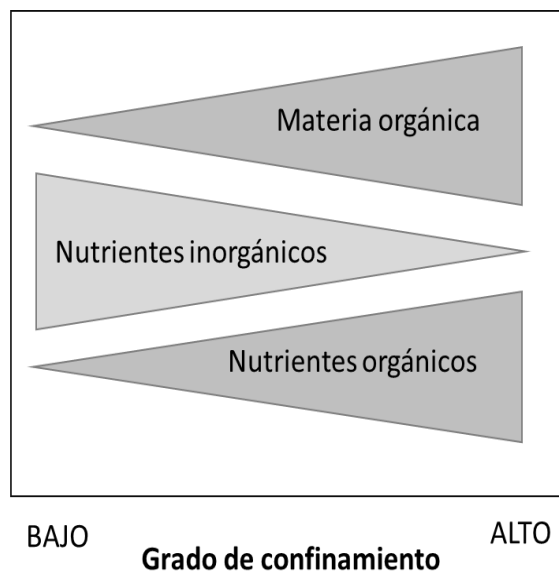
Figura 1. Relación entre el grado de confinamiento y la carga de nutrientes y materia orgánica en las lagunas someras.

Nuestros resultados también muestran que la disponibilidad del nitrógeno es limitada en todos los sistemas dado que la relación entre el nitrógeno inorgánico disuelto (DIN) y fósforo total (PT) presenta valores inferiores a 2 indicando la limitación de nitrógeno (Ptacnik et al., 2010). El DIN, principalmente en formas inorgánicas oxidadas, disminuye debido a la producción primaria y a la elevada tasa de desnitrificación, muy común en

estos sistemas cuando las entradas de agua disminuyen (Comin & Valiela, 1993; Quintana et al., 1998; Sylaios & Theocharis, 2002; López-Flores et al., 2014). En estas situaciones, el nitrógeno inorgánico se vuelve un factor limitante para la producción primaria (Quintana et al., 1998; Frascari et al., 2002; Mendoza-Salgado et al., 2005; López-Flores et al., 2009). En relación al contenido de fósforo inorgánico, la falta de diferencias significativas para los diferentes sistemas estaría de acuerdo con el hecho de que la dinámica del fósforo no depende tanto de las entradas de agua como lo hace la dinámica del nitrógeno, sino más bien de los procesos de acumulación y del reciclado interno (Gomez et al., 1998; Frascari et al., 2002). Esta es una característica común en la mayoría de las lagunas costeras (Souchu et al., 1997; Victor et al., 1997; Kormas et al., 2001; Lucena et al., 2002; Pérez-Ruzafa et al., 2002; Serrano et al., 2004).

En contraste con la homogeneidad observada para el fósforo inorgánico, el contenido de fósforo total en las lagunas confinadas es siempre mayor que en las que reciben directamente las entradas de agua dulce (capítulos 2 y 3). A diferencia del nitrógeno que se agota rápidamente y solo queda disponible en forma de nitrógeno atmosférico para aquellos organismos capaces de fijarlo, el fósforo tiende a acumularse progresivamente en el sedimento (Gomez et al., 1998; Golterman, 1999; Valiela et al., 2000; Teal & Howes, 2002; Reina et al., 2006). Este fósforo puede estar biodisponible si se mineraliza a través de la descomposición de los organismos o si se redisuelve del sedimento, en función del potencial redox y el pH de la laguna (Mortimer, 1941; Andersen, 1975; Søndergaard et al., 1992). En el caso concreto de Doñana, los resultados de estudios previos (Díaz-Espejo et al., 1999; Serrano et al., 1999, 2003) han demostrado que la biodisponibilidad de fósforo es muy elevada debido a la liberación de fosfato a partir de los sedimentos. Por esta razón, el sedimento no actúa como un sumidero de fósforo en estos ecosistemas poco profundos donde la disponibilidad de fósforo depende del equilibrio químico entre el agua y el sedimento (Golterman, 1975, 1995, 2004).

3. Dominancia de autótrofos (A) *versus* heterótrofos (H)

En conjunto, nuestros resultados ponen de manifiesto la importancia de la hidrología en la dominancia de A *versus* H. El hecho de que un sistema esté dominado por una estrategia trófica u otra depende principalmente del régimen hidrológico del sistema y de los factores relacionados con este, como son sobre todo, la distribución espacial, el gradiente renovación de agua-confinamiento, la permanencia del agua y la concentración de nutrientes. El efecto *top-down* del zooplancton sobre el balance A:H es poco significativo.

De forma más concreta, nuestros resultados nos permiten crear un modelo conceptual en el que se muestra como el balance A:H presenta un patrón unimodal con el gradiente de renovación del agua en lagunas someras. La importancia de la renovación del agua y de los aportes alóctonos sería la principal diferencia entre lagunas someras y los ecosistemas acuáticos profundos, donde la relación A:H aumenta a lo largo de un gradiente de productividad (Del Giorgio & Gasol, 1995; Gasol et al., 1997). En este modelo conceptual se englobarían las lagunas confinadas y oligohalinas del Empordà (capítulo 3) y las marismas de Doñana (capítulo 2; Figura 2).

Una primera observación que podemos hacer en este modelo conceptual, es el hecho de que el balance A:H se relaciona de forma directa y lineal con el gradiente de renovación del agua si no hay confinamiento (Figura 2; nº1). Por tanto, puede decirse que la proporción de autótrofos en la comunidad microbiana crece a medida que la renovación del agua es mayor, así como el aporte de nutrientes inorgánicos alóctonos.

Las lagunas oligohalinas (capítulo 3; Figura 2; nº 2), durante la época estival, presentan una red trófica microbiana dominada por una estrategia heterótrofa (bacterioplancton). Estas lagunas incluyen una amplia gama de ecosistemas con fuertes diferencias en su hidrología, desde los ecosistemas con baja renovación del agua, pero con aportes en verano procedentes principalmente de excedentes de riego (Comín y Valiela, 1993, Romos et al., 2005, Badosa et al., 2008) hasta pequeños estuarios que permanecen aislados durante el período estival.

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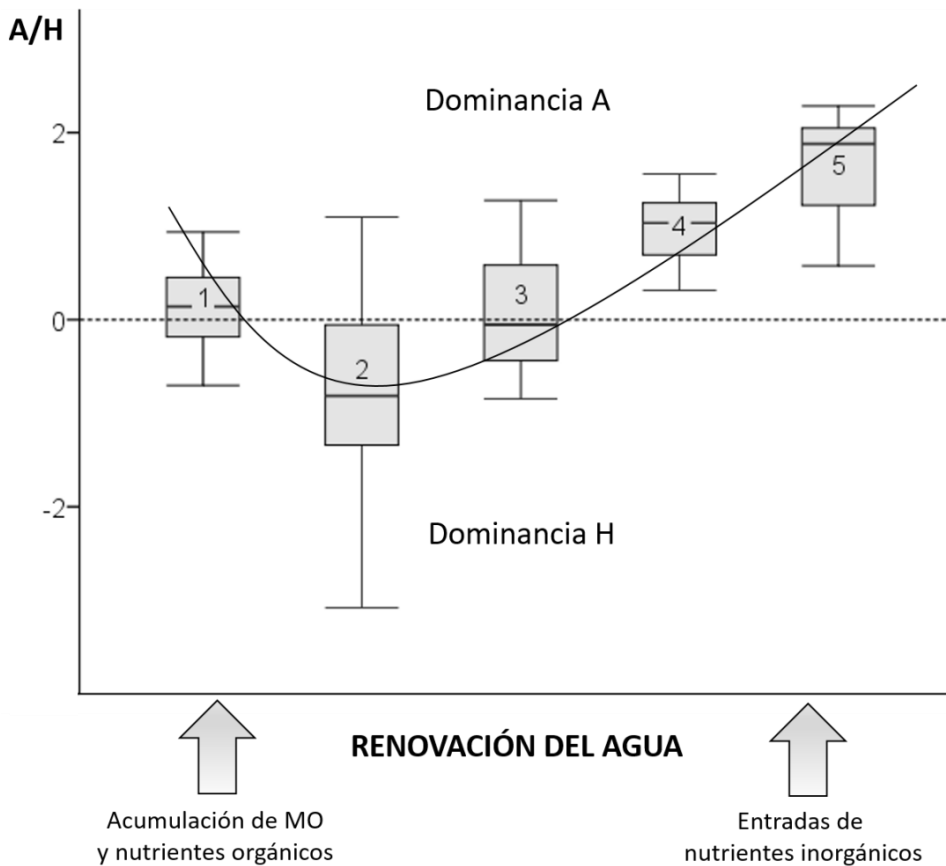
Figura 2. Gráfico que muestra la relación unimodal entre el balance A/H y la renovación del agua (1: lagunas confinadas (Cap. 3), 2: lagunas oligohalinas (Cap. 3); 3: lagunas aisladas de las entradas de agua (Cap. 2; grupo 1), 4: lagunas con flujo intermedio (Cap. 2; grupo 2), 5: lagunas cercanas a las entradas de agua (Cap. 2; grupo 3)).

Las marismas de Doñana (capítulo 2; Figura 2; nº 3, 4, 5) presentan una red trófica microbiana dominada por organismos autótrofos, tanto en aquellas lagunas con una alta renovación como en las que presentan una situación intermedia o de poca renovación. Aun y así, se observan pequeñas variaciones en el balance A:H debidas principalmente a la distribución espacial de las masas de agua. Concretamente, aquellas lagunas que se encuentran más aisladas de las entradas de agua (Figura 2; nº3) son las que presentan una proporción relativamente mayor de organismos heterótrofos. Así,

en condiciones de baja renovación, la materia orgánica alóctona se acumula y los nutrientes inorgánicos se agotan y sólo el reciclaje interno puede proporcionar disponibilidad de recursos (Charpy & Charpy-Roubaud, 1990; Quintana et al., 1998; Quintana & Moreno-Amich, 2002) favoreciendo de este modo al bacterioplancton. En relación a los grupos funcionales del fitoplancton, se observa que el grupo que domina el microfitoplancton autótrofo son las cianobacterias coloniales (*Anabaena*), esto estaría relacionado con el hecho de que, en condiciones de baja renovación, la concentración de nitrógeno inorgánico disuelto (DIN) es muy baja (Bañeras et al., 2012). En estos sistemas, la escasez de nitrógeno combinado con una liberación de fósforo desde el sedimento favorece el desarrollo de organismos capaces de fijar el nitrógeno molecular (Blomqvist et al., 1994), tales como cianobacterias (Stal et al., 2000) descritas ampliamente por tener una menor dependencia del nitrógeno inorgánico debido, precisamente, a su capacidad de fijar el nitrógeno atmosférico (Shapiro, 1990; Scheffer et al, 1997; Havens et al., 1998; Reynolds, 2006).

Finalmente, cuando se incluyen las lagunas más confinadas en el modelo conceptual (capitulo 3; Figura 2; nº1), la relación entre el balance A:H y la renovación del agua pasa a describir una curva unimodal. En las lagunas confinadas, en contra de lo esperable, los autótrofos vuelven a dominar la comunidad. En las lagunas y marismas del Empordà el confinamiento va asociado a una elevada concentración de materia orgánica y nutrientes en forma orgánica. Aquí, el balance A:H es similar al visto en las lagunas más aisladas de Doñana (Figura 2; nº3). Sin embargo, en este caso, el confinamiento excesivo que se da en estas lagunas limita el crecimiento de los organismos al uso de los nutrientes reciclados, favoreciendo las estrategias de vida K. El microfitoplancton autótrofo se encuentra dominado por dinoflagelados, autótrofos de crecimiento lento y con posibilidad de usar estrategias alternativas como la mixotrofia para suplir las carencias de energía y nutrientes (Isaksson, 1998; Jones, 2000). Modelos unimodales relacionados con la tasa de renovación en lagunas someras también se han descrito para otros parámetros como el grado de eutrófia o la dominancia de macrófitos o fitoplancton (Trobajo et al., 2002).

Tanto las lagunas temporales de interior (capítulo 1) como los estuarios de ríos (capítulo 3) debido a su gran diferencia hidrológica con el resto de sistemas, quedarían fuera de la explicación del modelo conceptual visto. Ambos sistemas, presentan una red trófica dominada por una estrategia heterótrofa, concretamente por bacterioplancton. Estos ecosistemas tienen un comportamiento más parecido al que se da en los ecosistemas lóticos, donde el aumento del flujo de agua y el consiguiente aumento de los suministros alóctonos se ha descrito como promotor del crecimiento heterotrófico (Findlay et al., 1991, Wiegner et al., 2003). Esto está de acuerdo con lo que proponen los modelos conceptuales en sistemas lóticos, el *river continuum concept* (Vannote et al., 1980) y el *river wave concept* (Humphries et al., 2014). En las lagunas temporales de interior, que se encuentran en una situación de estabilidad sin entradas de nutrientes inorgánicos y con acumulación de materia y nutrientes inorgánicos, el DOC es la única variable ambiental relacionada significativamente con el balance A:H. Cuando las concentraciones de DOC aumentan el balance disminuye, sugiriendo que el DOC favorece un aumento de la producción bacteriana (Hessen, 1992; Salonen et al., 1992; Del Giorgio & Peters, 1993). Como se ha comentado en el apartado anterior de esta discusión la proporción de DOC con respecto a los nutrientes inorgánicos sería mayor en aquellas lagunas que presentan cuencas forestadas como es el caso de estos sistemas de interior, y más bajas en zonas perturbadas o deforestadas como los sistemas del capítulo 2 y 3, donde el suministro alóctono puede proporcionar, de forma relativa, más nutrientes inorgánicos que DOC. Aun y así, estudios realizados por López-Flores et al., (2009) determinaron que había una fuerte correlación entre el DOC y el fitoplancton. Esa relación del DOC tanto con el bacterioplancton como con el fitoplancton estaría de acuerdo con la respuesta unimodal encontrada por Seekell et al., (2015) entre la producción primaria y el DOC en ecosistemas profundos. A bajas concentraciones el incremento de DOC estimularía la producción (actividad autótrofa) pero por encima de una concentración umbral este incremento afectaría de forma negativa a la producción primaria. Los valores de DOC observados en las lagunas del capítulo 1 están muy por encima del umbral definido por Seekell et al., (2015).

CONCLUSIONES
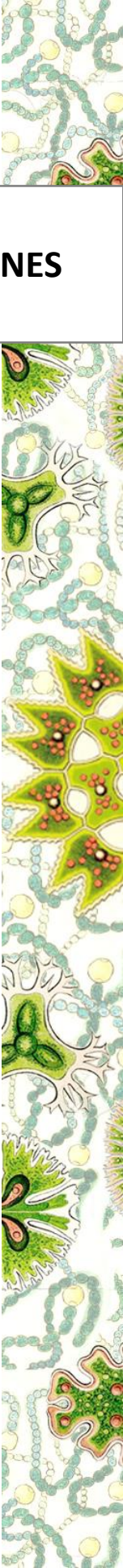
1. La red trófica microbiana de lagunas someras de interior se encuentra dominada por organismos heterótrofos sobre todo por bacterioplancton.

2. La única variable significativamente relacionada con el balance A/H en las lagunas someras de interior es el DOC. Cuando la proporción de DOC aumenta el balance A/H disminuye sugiriendo así que el DOC favorece un aumento de la producción bacteriana.

3. Esta correlación entre el DOC y los organismos heterótrofos se relaciona con el hecho de que estas lagunas someras de interior se encuentran localizadas en cuencas forestadas y bien conservadas, donde las entradas alóctonas de carbono, procedente de la escorrentía de la cuenca que rodea la laguna, son más importantes que las entradas de nutrientes inorgánicos.

4. No se han encontrado otras relaciones significativas entre el balance A/H y las variables ambientales analizadas; nutrientes inorgánicos, ácido fúlvico, Chl-a, biomasa total de macrófitos y zooplancton.

5. En las lagunas someras de interior al considerarse el zooplancton por separado el balance A/H se relaciona de forma positiva con la biomasa de harpacticoides y negativa con la biomasa de los cladóceros. Sin embargo, el efecto de la dinámica del DOC y los nutrientes es siempre más importante que un posible control top-down.

6. La elevada variabilidad espacial encontrada entre las lagunas y también entre los sistemas sugiere que en un mismo momento de muestreo las diferentes lagunas pueden presentar diferentes estados del hidroperiodo y de la sucesión de organismos después de la inundación.

7. La red trófica microbiana en las marismas de Doñana se encuentra dominada por organismos autótrofos, principalmente nanoplancton.

8. La distribución espacial de las lagunas de Doñana es la principal causa de variabilidad en el balance A/H, combinado con el efecto de las variables ambientales consideradas. La biomasa total de zooplancton no influye de forma significativa en este balance.

9. La distribución espacial de las lagunas de Doñana refleja la importancia de la localización con respecto a las entradas de agua en la marisma y por tanto está muy relacionada con la hidrología del sistema que, a su vez, influye en la composición y la dinámica de los nutrientes y de la materia orgánica en las lagunas.

10. Las lagunas de Doñana que se encuentran más aisladas de la entrada de agua, aunque se encuentran dominadas por autótrofos, son las que presentan una proporción relativamente mayor de organismos heterótrofos. En cambio, en las lagunas cercanas a las entradas de agua y nutrientes, los organismos autótrofos dominan el sistema.

11. Las lagunas de Doñana más aisladas de las entradas de agua presentan un microfitoplancton dominado por cianobacterias coloniales. Estas lagunas presentan un bajo cociente nutrientes inorgánicos: orgánicos y el nitrógeno inorgánico es especialmente escaso debido a la desnitrificación. Esta situación combinada con la liberación de fósforo procedente del sedimento que se da en estos sistemas favorece el desarrollo de organismos capaces de fijar el nitrógeno molecular como son las cianobacterias.

12. En condiciones de sequía, se siguen observando diferencias significativas entre las lagunas confinadas y los estuarios del Empordà, en la dinámica de nutrientes y en la estructura de la red trófica microbiana, a pesar de que la reducción del caudal en estuarios hace suponer que estas diferencias se reduzcan.

13. Las lagunas oligohalinas del Empordà, dado que incluyen una amplia gama de ecosistemas con fuertes diferencias hidrológicas, no presentan diferencias significativas ni en la dinámica de nutrientes ni en la estructura de la red microbiana, con las lagunas confinadas ni con los estuarios.

14. El balance A/H en las lagunas del Empordà presenta diferencias significativas entre las lagunas confinadas donde dominan los organismos autótrofos (nanoplancton), y las lagunas oligohalinas donde dominan los organismos heterótrofos (bacterioplancton). Los estuarios, dominados por heterótrofos (bacterioplancton), son sistemas muy variables y no se diferencian significativamente del resto.

15. El factor ambiental que discrimina la diferente composición taxonómica microbiana encontrada entre estuarios y lagunas confinadas del Empordà es el balance DIN/Norg.

16. En estas lagunas, el tamaño del fitoplancton dominante varía en función de la renovación del agua. El picoplancton domina en los estuarios (alta renovación del agua), mientras que en situaciones de confinamiento (baja renovación del agua) dominan los dinoflagelados.

17. A lo largo de esta tesis se ha observado la relación entre el balance A/H y la tasa de renovación del agua sigue un patrón unimodal. En general, cuanto menor es la renovación del agua, menor es la proporción de autótrofos. Sin embargo, en condiciones de máximo confinamiento la proporción de autótrofos vuelve a aumentar. En estas condiciones de máximo confinamiento, los dinoflagelados mixótrofos dominan el fitoplancton.

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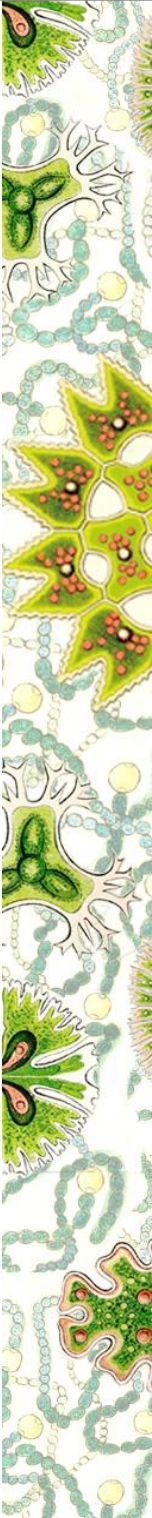
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