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Zooplankton grazing on natural algae and bacteria under hypertrophic conditions

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ABSTRACT

Zooplankton grazing on natural algae and bacteria under hypertrophic conditions

The grazing characteristics of the natural zooplankton community in the shallow hypertrophic lagoon Albufera de Valencia (Spain) were compared in two periods of the annual cycle: (1) at the onset of spring, after a period of increased water flow through the lagoon that reduces phytoplankton density and cyanobacterial prevalence, and (2) in mid-spring, after the spring phytoplankton bloom and under conditions of cyanobacterial dominance. Clearance, ingestion and assimilation rates were measured by labelling natural seston with ^{14}C-bicarbonate and ^{3}H-thymidine/leucine. The clearance rates (CRs) of *Daphnia magna*, the dominant species at the onset of spring, were low, very likely as a consequence of the high abundance of filamentous cyanobacteria in the lagoon. The CRs were nearly equal for phytoplankton and bacteria, corroborating the unselective feeding of *Daphnia*. Bacteria supplied $\approx 40\%$ of the planktonic carbon ingested by *D. magna*, and this carbon was assimilated with the same efficiency as that provided by phytoplankton. This suggests a strong coupling between the microbial loop and the classic grazer trophic web at the onset of spring in the lagoon and, consequently, an important contribution of the microbial loop to energy transfer to higher trophic levels (fish). The CRs of *Bosmina longirostris*, the dominant species in mid-spring, were also low probably because the food concentrations were well above the incipient limiting level. The CRs were higher for 1-15 μm algae than for < 1 μm bacteria, indicating positive selective feeding towards larger cells. Algae sized 3-15 μm provided most of the planktonic carbon ingested by *B. longirostris*. Similarly, regarding the total mid-spring zooplankton community, phytoplankton contributed 90 % of the planktonic carbon ingested. This suggests that during the spring phytoplankton bloom when cyanobacteria dominate, the energy transferred to zooplankton via bacteria constitutes a smaller fraction than during periods of lower phytoplankton density. Finally, our results indicate that zooplankton consumed a very small proportion of phytoplankton and bacterioplankton production during the two studied periods, which highlights the reduced ability of zooplankton to control phytoplankton growth in hypertrophic systems.

Key words: *Daphnia magna*, *Bosmina longirostris*, clearance rates, shallow lake, carbon flux.

RESUMEN

Alimentación del zooplancton sobre comunidades naturales de algas y bacterias en condiciones hipertróficas

Las características filtradoras de la comunidad natural de zooplancton en el lago somero hipertrófico Albufera de Valencia (España) fue comparada en dos periodos del ciclo anual: (1) al inicio de la primavera, tras un periodo de intenso flujo en el lago que reduce la densidad fitoplantónica y la prevalencia de cianobacterias y (2) a mediados de primavera, tras el pico de crecimiento de fitoplancton y en condiciones de dominancia de cianobacterias. Las tasas de filtración, ingestión y asimilación fueron medidas marcando el seston natural con ^{14}C-bicarbonato y ^{3}H-Timidina/Leucina. Las tasas de filtración (CR's) de Daphnia magna, *la especie dominante al inicio de la primavera, fueron bajas, probablemente como consecuencia de la elevada abundancia de cianobacterias filamentosas en el lago. Las CR's fueron prácticamente iguales para fitoplancton y bacterias, corroborando la alimentación no selectiva de* Daphnia. *Las bacterias proporcionaron el $\approx 40\%$ del carbono planctónico ingerido por* D. magna, *y este carbono fue asimilado con la misma eficiencia que el suministrado por el fitoplancton. Esto sugiere un estrecho acoplamiento entre el bucle microbiano y la red trófica clásica a principios de primavera en el lago y en consecuencia una contribución importante del bucle microbiano a la transferencia de energía a niveles tróficos superiores*

(peces). Las CR's de Bosmina longirostris, *la especie dominante a mediados de primavera, fueron bajas, posiblemente debido a que las concentraciones de alimento superaban el "nivel saturante de ingestión". Las CR's fueron más altas para algas de 1-15 μ m que para bacterias < 1 μ m, indicando una selección positiva de las células de mayor tamaño. Las algas de 3-15 μ m proporcionaron la mayor parte del carbono planctónico ingerido por* B. longirostris. *Asimismo, si se considera toda la comunidad zooplanctónica de mediados de primavera, el fitoplancton aportó el 90 % del carbono planctónico ingerido. Esto sugiere que durante picos de crecimiento fitoplanctónico en los que dominan las cianobacterias, la energía transferida al zooplancton a través de las bacterias constituye una fracción más pequeña en comparación con periodos de menor densidad fitoplanctónica. Finalmente, nuestros resultados indican que durante los periodos estudiados, el zooplancton consumió una mínima proporción de la producción fitoplanctónica y bacteriana, lo que evidencia la reducida capacidad del zooplancton para controlar el crecimiento de fitoplancton en sistemas hipertróficos.*

Palabras clave: Daphnia magna, Bosmina longirostris, *tasas de filtración, lago somero, flujo de carbono.*

INTRODUCTION

Hypertrophic lakes are characterized by sustaining an exceptionally high phytoplankton biomass. However, the majority of this biomass generally consists of filamentous and colonial cyanobacteria, inedible to most zooplankton and responsible for additional distress to the feeding mechanism of large cladocerans (DeMott *et al*., 2001). Moreover, eutrophication typically involves an increase in the proportion of planktivorous and omnivorous fish at the expense of piscivorous species, which results in increased predation control over zooplankton (Jeppesen *et al*., 2007). Both feeding inhibition by filamentous cyanobacteria and fish predation have been found to favour small-bodied species in eutrophic lakes (Gliwicz, 1990). Consequently, hypertrophic lakes are often dominated by cyclopoid copepods and rotifers and present a low zooplankton:phytoplankton biomass ratio (Havens *et al*., 2007; Fermani *et al*., 2013).

In addition to phytoplankton, heterotrophic bacteria might provide an alternative food source for zooplankton. The bacterivory of zooplankton has been widely described in the literature (Tóth & Kato, 1997; Work & Havens 2003; Miracle *et al*., 2014), which leads to the question of the importance of bacteria in channelling energy to zooplankton through the microbial food web in hypertrophic lakes.

Numerous studies have investigated the food selectivity and grazing characteristics of zooplankton. However, the majority of them involved the use of cultured cell suspensions that were often monospecific (Lair, 1991; Tóth & Kato, 1997; He & Wang, 2006). There are few data on grazing estimations based on natural plankton suspensions, especially containing both algae and bacteria from the natural assemblage. Further, the available information in this regard is especially scarce for hypertrophic lakes.

The shallow hypertrophic lagoon Albufera de Valencia provides a representative case study for this type of systems. This lagoon shifted from a clear to a turbid state as a consequence of an eutrophication process that began in the 1960s (Vicente & Miracle, 1992; Romo *et al*., 2005). Today, the lagoon completely lacks macrophytes and is in a turbid state dominated by phytoplankton, namely cyanobacteria (Onandia *et al*., 2014a). Nonetheless, the lagoon experiences short "clear-water" events in late winter (January-March). These events are the result of the management of the lagoon, which is "flushed" in winter when the water contained in the neighbouring rice paddies flows through the lagoon into the adjacent Mediterranean Sea. During this period, there is a decrease in algal biomass mainly caused by the decline of cyanobacteria. After the flushing period, the water flow through the lagoon is strongly reduced, and cyanobacterial dominance is re-established.

The main objective of this work was to study the grazing characteristics of the natural zooplankton community in the Albufera de Valencia

lagoon during periods of contrasting cyanobacterial dominance, *i.e.*, the onset of spring *vs* mid-spring, characterized by non-cyanobacterial *vs* cyanobacterial dominance. More specifically, we aimed to assess the importance of different algal and bacterioplankton size fractions to zooplankton feeding.

MATERIALS AND METHODS

Study site and general methods

Albufera de Valencia is a shallow, oligohaline (salinity ≈ 1‰) lagoon on the Mediterranean coast, 15 km south of the town of Valencia (Spain). With an extension of roughly 24 km^2 and a mean depth of approximately 1 m, the Albufera de Valencia is currently a hypertrophic, cyanobacteria-dominated lagoon with chlorophyll-*a* (Chl-*a*) concentrations over 100 μg/L during most of the year and over 300 μg/L in spring (Onandia *et al.*, 2015). More detailed information on the history and limnological characteristics of the lagoon can be found in Vicente & Miracle (1992) and Romo *et al.* (2008).

The study consisted of two sets of experiments aimed to assess the grazing aspects at the onset of spring and mid-spring, namely on March 26, 2011 and May 16, 2012, respectively. Additionally, a number of selected variables were measured at the experimental site on these dates as described in Onandia *et al.* (2014b). Water conductivity (Cond), pH, salinity and temperature (T) were measured in situ with a multiparameter probe (WTW Multi 350i). The Secchi disk depth was recorded, and vertical profiles of photosynthetically active radiation were obtained with a flat-faced quantum sensor (LiCor Li-1000) to calculate the light attenuation coefficient K_d. Water samples for chemical and biological analyses were taken with a Ruttner bottle (50 cm length) by placing the top of the bottle at approximately 30-40 cm depth. Total nitrogen (TN), ammonia, nitrate, nitrite, total phosphorus (TP) and alkalinity (Alk) were estimated following the methodology described in APHA (1992). Dissolved inorganic nitrogen (DIN),

calculated as the sum of ammonia, nitrate and nitrite, was subsequently substracted from TN (TN-DIN). Chl-*a* concentrations were determined spectrophotometrically as described by Shoaf & Lium (1976). Samples from lagoon water and feeding suspensions were preserved in Lugol's solution until the enumeration of algal cells and protists (ciliates and heterotrophic flagellates) in 3 mL sedimentation chambers with an inverted microscope at 1000×. Given the low abundance of heterotrophic flagellates, we opted to enumerate them with an inverted microscope in the algal samples because the water volume explored was notably larger than that of the bacterial DAPI-stained samples. Cell biovolume was calculated as described by Hillebrand *et al.* (1999), and phytoplankton biovolume was transformed into biomass using a conversion factor of 0.225 pg C/μm^3 for mixed phytoplankton populations (Reynolds, 2006).

Samples of lagoon water and feeding suspensions for the enumeration of bacteria were preserved in a mixture of paraformaldehyde and glutaraldehyde (100 g/L wv and 0.5 % final concentrations, respectively). Subsamples of 200/250 μL were filtered on black polycarbonate filters (Millipore, 0.2 μm) and stained with DAPI (Porter & Feig, 1980). Bacteria were then counted on epifluorescence microscope microphotographs (Zeiss III RS). Cell volume was estimated based on the dimensions measured in these microphotographs using a general formula (Sommaruga, 1995) and transformed to carbon by applying a conversion factor of 0.2 pg C/μm^3 (Simon & Azam, 1989). Autotrophic picocyanobacteria were also evaluated in the same microscope slides under green light excitation.

Zooplankton samples were taken from lagoon water by filtering two Ruttner bottles (2.7 L capacity each) through a 30 μm nytal mesh and preserved with formalin 4 %. Zooplankton specimens from these samples and from the experimental vials were counted and identified using an inverted microscope. The rotifer biomass was calculated using species-specific carbon conversion factors (Latja & Salonen, 1978; Telesh *et al.*, 1998). Microcrustacean length, once measured, was converted into biomass according to Dumont

et al. (1975) and Bottrell *et al*. (1976) and into C by assuming that the C content was 0.48 of the dry weight (Andersen & Hessen, 1991).

Grazing experiments at the onset of spring

Our experimental design targeted the estimation of clearance rates (CRs), ingestion rates (IRs), mass-specific ingestion rates (MSIRs), assimilation rates (ARs) and assimilation efficiency (AE) of natural algae and bacteria by *D. magna,* the dominant zooplankton species at the end of March 2011, under natural-resembling conditions.

Twenty-four hours prior to the grazing experiments, a suspension of radiolabelled phytoplankton and bacteria was prepared. Freshly collected water from the Albufera de Valencia lagoon was filtered through a 25 µm nytal mesh and labelled with both [methyl-^{3}H]-thymidine (Perkin Elmer; specific activity = 2.59 to 3.33 TBq/mmol; final concentration = 5 nM) and NaH^{14}CO$_3$ (DHI; specific activity = 39035 kBq/mL; final concentration = 4 µCi/mL). The suspension was incubated for 24 hours at 15 °C and 110 µEinsteins m^{-2} s^{-1} (16 h light:8 h dark cycle). The goal of the incubation period was to label bacteria with [methyl-^{3}H]-thymidine and phytoplankton with ^{14}C. Nonetheless, a small proportion of both radiotracers might have been transferred to other components of the food web (namely to heterotrophic flagellates and ciliates, as well as to heterotrophic bacteria in the case of ^{14}C). After the incubation period, the suspension was centrifuged (12 m at 3500 r.p.m) and aspirated, and the remaining pellets were rinsed with lagoon water that had been filtered through glass-fibre filters (GF/F, Whatman, hereafter referred to as pure lagoon water), centrifuged, aspirated again, and resuspended in pure lagoon water.

One day before the grazing experiments, 500 mL of freshly collected water from the lagoon was filtered through a 25 µm sieve and distributed into 10 plastic vessels: 8 replicates and 2 controls (containing 50 mL each). Five adult *Daphnia magna* individuals (collected from the lagoon at the same time) were added to each vessel and allowed to acclimate for 24 h under the same conditions as in the radiolabelled suspension. To estimate the CRs, 5 mL of water were extracted from 5 zooplankton vessels and replaced by 5 mL of radiolabelled solution. *Daphnia* were allowed to feed for 8 minutes, then anaesthetized with carbonized water and subsequently fixed with sucrose-formalin (40 %).

To determine the ARs, 5 mL of water was extracted from the 5 remaining zooplankton vessels and replaced by 5 mL of radiolabelled food solution. *Daphnia* were allowed to feed for 30 minutes, then filtered through a 50 µm sieve and immediately transferred to a vessel containing 50 mL of unlabelled lagoon water that had been filtered through a 25 µm sieve. The animals were allowed to evacuate their guts for a period of 45 min, then anaesthetized with carbonized water and subsequently fixed with sucrose-formalin (40 %).

In all experiments, after the animals were fixed, they were collected on a 90 µm sieve and fully rinsed with a 4 % sucrose-water solution to eliminate any radiolabelled cells potentially attached to them. The same procedure was followed for the blanks, although the animals were killed with sucrose-formalin (40 %) before the start of the CRs or ARs experiments. The animals contained in each microcosm were then placed in glass vessels containing a 4 % sucrose-water solution and stored at 4 °C. Within 1 day, these animals were transferred with a minimum amount of liquid to corresponding scintillation vials.

Mid-spring grazing experiments

Our experimental design targeted the estimation of the CRs, IRs, MSIRs, ARs and AE of *Bosmina longirostris,* the dominant zooplankton species in mid-May 2012. Additionally, we aimed to estimate the CRs of the 50-100 µm zooplankton size fraction, together with the CRs, IRs and MSIRs of the entire zooplankton community within this period.

Twenty-four hours prior to the grazing experiments, two food suspensions were prepared. The preparation of the first suspension aimed to radiolabel the < 15 µm phytoplankton cells present in the natural assemblage. To this end, freshly collected water from the Albufera de Valencia lagoon was filtered through a 15 µm nytal mesh

and labelled with NaH^{14}CO$_3$ (same activity as in the early spring experiment). The preparation of the second suspension aimed to radiolabel the < 3 μm heterotrophic bacteria naturally present in the lagoon water. To do so, freshly collected water was filtered through a 3 μm filter and labelled with both [methyl-^{3}H]-thymidine (Perkin Elmer; same activity as in the early spring experiment) and L-[3,4,5-^{3}H (N)]-leucine (Perkin Elmer; specific activity = 3.7 to 5.56 TBq/mmol; final concentration = 5 nM). Both suspensions were incubated for 20 hours at 20 °C and 110 μEinsteins m^{-2} s^{-1} (16 h light: 8 h dark cycle). Thereafter, the suspension containing the radiolabelled < 15 μm phytoplankton cells was filtered through a 3 μm filter (Whatman Nuclepore Track-Etched Membrane PC MB, 47 mm) to obtain the 3-15 μm and < 3 μm algal size fractions. Likewise, the suspension containing the radiolabelled < 3 μm heterotrophic bacteria was serially fractioned to obtain the 1-3 μm (Whatman Nuclepore Track-Etched Membrane Nuclpo, 47 mm, 1 μm) and 0.2-1 μm (Whatman Cyclopore Track-Etched membrane Cyclpo PE, 47 mm, 0.2 μm) bacterial size fractions. To maximize the similarity between the food suspensions and the natural plankton community (< 15 μm), the size fractions of the labelled algae/bacteria were resuspended in unlabelled lagoon water containing the complementary plankton size fractions, *i.e.*, the 3-15 μm algal fraction was resuspended in unlabelled < 3 μm filtrate.

The next step involved the preparation of 7 sets of 4 microcosms (3 replicates plus a control) consisting of 60 mL of freshly collected unfiltered lagoon water containing the natural zooplankton community. To estimate the CRs of the zooplankton community on food suspensions containing combinations of different natural plankton size fractions, every microcosm set was subjected to one of the following treatments, which involved the addition of the following suspensions:

a. 5 mL 3-15 μm ^{14}C + 5 mL < 3 ^{14}C

b. 5 mL 3-15 μm ^{14}C + 5 mL 1-3 ^{3}H

c. 5 mL 3-15 μm ^{14}C + 5 mL < 1 ^{3}H

d. 5 mL < 3 ^{14}C + 5 mL 1-3 ^{3}H

e. 5 mL < 3 ^{14}C + 5 mL < 1 ^{3}H

f. 5 mL 1-3 ^{3}H

g. 5 mL < 1 ^{3}H

A volume equivalent to that to be added to the suspensions was removed from the microcosms prior to the suspension addition. The volume was removed by aspiration through a 20 μm filter. All treatments had the same total concentrations of phytoplankton and bacteria found in the < 15 μm natural water filtrate. After the radiolabelled suspensions were added, the animals were allowed to feed for 15 minutes, anaesthetized with carbonized water, filtered through a 50 μm sieve and fully rinsed with water (pH = 7.8) to eliminate any radiolabelled cells potentially attached to them.

For the assimilation experiments, two sets of 4 microcosms (3 replicates + 1 control) were prepared with 60 mL of unfiltered lagoon water. Similar to the CR experiments, a volume equivalent to that to be added to the suspensions was removed from the microcosms prior to the suspension addition by aspiration through a 20 μm filter. Each set of microcosms was either subjected to treatment b or e. After the radiolabelled suspensions were added, the zooplankton were allowed to feed for 50 minutes, filtered through a 50 μm sieve, rinsed carefully with water and immediately transferred to a vessel containing 50 mL of unlabelled lagoon water that had been filtered through a 15 μm sieve. The animals were allowed to evacuate their guts for a period of 45 min, anaesthetized with carbonized water, filtered through a 50 μm sieve and fully rinsed as explained above.

In all experiments, once the animals were filtered and rinsed, they were immediately transferred to a Petri dish containing a 4 % sucrose-water solution, fixed with formalin (4 %) and stored at 4 °C. The same procedure was followed for the blanks, although the animals were killed with sucrose-formalin (4 %) before the start of the CR or AR experiments. Within 1 day, the animals from each microcosm were separated as fol-

lows: 20 individuals of *B. longirostris* were first removed from the sucrose-water solution and the rest of the animals were counted and then serially filtered, first through a 100 μm and then through a 50 μm nytal sieve. The 20 individuals of *B. longirostris* and the nytal sieves (15 mm diameter) with the collected animals were transferred with a minimum amount of liquid to corresponding separate scintillation vials.

Radioactivity measurements and calculations

In all experiments (involving *D. magna* or *B. longirostris* and others), after the animals were transferred to scintillation vials and the remaining liquid was evaporated, 250 mL of Soluene-350 (Perkin Elmer) was added to them. After 24 hours, 10 mL of scintillation cocktail (Sigma-Fluor TM High Performance LSC Cocktail, for aqueous samples, S4023) was added. The radioactivity accumulated by the animals and the radioactivity in the grazing suspensions of each microcosm were measured with a PerkinElmer LSA Tri-Carb 2810TR liquid scintillation counter.

The CRs were calculated as follows:

$$CR = (dpm_{individual} - dpm_{blank}) / dpm_{grazing\ suspension} \times t,$$

where CR = μL individual^{-1} h^{-1} and t = time in hours. The IRs (μm^3 ind^{-1} h^{-1}) were calculated by multiplying the CR by the corresponding biovolume of algae or bacteria in the feeding suspension. To estimate the IRs on algae, only the biovolume corresponding to the edible organisms present in the radiolabelled solution was taken into account; chlorophytes, diatoms and a minor part of cyanophyceae (excluding filaments of the genus *Planktolyngbya* and *Pseudanabaena*, as well as colonies of the genus *Merismopedia*, *Aphanothece* and *Aphanocapsa*) were considered edible. In the case of the IR of heterotrophic bacteria, all present morphotypes were considered edible. To estimate the MSIRs (pg C pg C^{-1} h^{-1}), the carbon content of ingested biovolume per hour was calculated and divided by the zooplankton carbon content in the corresponding microcosms. Assimilation rates (AR) were calculated as described for the CR, and the AE was subsequently calculated as, AE = CR/AR × 100.

Although the algal and bacterial abundance in the food solutions was lower than in the lake, the IRs estimated in the microcosms could be extrapolated to those expected in the lake because the food concentrations in the feeding solutions were above the incipient level. Thus, we could estimate the relative grazing rate of lake's phytoplankton and bacterioplankton biomass as well as the relative grazing rate of primary production and bacterial production. These relative grazing rates were calculated as the percentage of the daily IRs with respect to the *i*) total phytoplankton and bacterioplankton biomass and *ii*) daily primary (PP$_{day}$) and bacterial production (PB$_{day}$), respectively. The values for PP$_{day}$ (g C m^{-3} d^{-1}) and BP$_{day}$ (mg C m^{-3} d^{-1}) were obtained in previous studies carried out in the same locality and seasonal period (Onandia *et al.*, 2014a; b).

RESULTS

The physicochemical conditions at the time of the zooplankton grazing experiments in the lagoon are shown in Table 1. The hypertrophic state of the lagoon is illustrated by the high TN, TP and Chl-*a* concentrations, together with the water turbidity (Wetzel, 2001). The water temperature was 5 °C higher in the mid-spring period. The elevated phytoplankton spring growth is indicated by the markedly high values of Chl-*a* in May, which were nearly double those found in March. Correspondingly, the water turbidity (il-

Table 1. Main limnological characteristics on the experimental dates (March 2011: 03/26/2011 and May 2012: 05/16/2012). See text for abbreviations. *Principales características limnológicas en las fe*chas experimentales (Marzo 2011: 26/03/2011 y Mayo 2012: 16/05/2012). Consultar en el texto las abreviaturas.

	March 2011	May 2012
T (°C)	16.2	21.2
pH	8.9	9.6
Cond (μS)	1426	1656
Chl-*a* (μg/L)	177	301
Secchi (m)	0.4	0.35
K$_d$	4.7	7.1
TP (μg/L)	174	262
TN (mg/L)	4.7	5
TN-DIN (mg/L)	2.8	4.3
Alk (mg/L)	24.4	15.8

lustrated by K_d and the Secchi depth), TN and TP were also higher. Similarly, variables associated with photosynthetic activity reflected this spring upsurge (high pH, low alkalinity).

Grazing experiments at the onset of spring

On 26 March 2011, cladocera represented more than 50 % of the total zooplankton biomass (Fig. 1). *Daphnia magna* was the dominant species (31 %), although *Alona rectangula* (14 %) and *Chydorus sphaericus* (6 %) were also well represented. The cyclopoid copepod *Acanthocyclops americanus* and rotifera each contributed roughly 25 % of the zooplankton biomass. The main rotifer species were *Brachionus variabilis*

(*D. magna* epibiont) and *Keratella cochlearis*, with a moderate presence of *Polyarthra* sp. and a minor presence of *Synchaeta* sp. and *Keratella tropica*. However, these proportions differed in terms of individuals (Table 2).

The phytoplankton community (biomass 38 mm³/L) was noticeably dominated by diatoms (mostly *Nitzschia* sp. and *Fragilaria* sp.), which contributed 42 % of the total phytoplankton biomass (Fig. 1). The conjugatophycea *Cosmarium abbreviatum* also represented a significant biomass fraction (20 %), slightly higher than the fraction composed by a wide variety of pooled chlorophytes species (*e.g., Scenedesmus* sp., *Desmodesmus* sp., *Monoraphidium* sp., *Tetraedron* sp.). Cyanophyceae amounted to 11 % of the
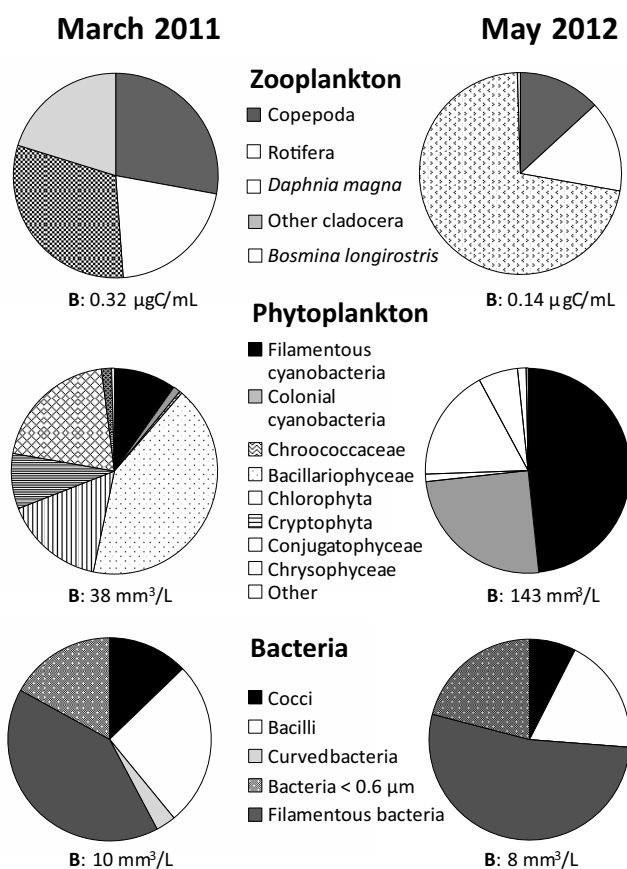
Figure 1. Relative contribution of the different groups of zooplankton, phytoplankton and bacterioplankton to the natural plankton biomass in the lake on the experimental dates (March 2011: 03/26/2011 and May 2012: 05/16/2012). B: biomass (μg C/mL or mm³/L). *Contribución relativa de los diferentes grupos de zooplancton, fitoplancton y bacteria a la biomasa planctónica natural del lago en durante la realización de los experimentos en Marzo de 2011 (26/03/2011) y Mayo de 2012 (16/05/2012). B: biomasa (μgC/mL o mm³/L).*

total pool, with the main contributors being oscillatoriales such as *Pseudanabaena galeata*. Minor but still well represented groups were cryptophytes, namely *Cryptomonas* sp., and chrysophytes. In terms of relative abundance, the proportions changed: cyanobacterial filaments (mainly *Pseudanabaena galeata*) and colonies represented 10 % and 1 %, respectively. Regarding the bacterial community, the most significant biomass contribution was from filamentous bacteria (40 %, Fig. 1), although its relative abundance with respect to total bacterial individuals was small (Table 2).

In the suspension containing labelled organisms < 25 μm, the proportion of the different phytoplankton groups was very similar to that observed in the natural assemblage. However, total phytoplankton biomass was 17.2 mm³/L, approximately half of that found in the unfiltered lagoon sample. *Fragilaria* sp. and *Nitzschia* spp. were the dominant species (42 %) in terms of biomass, but *Cosmarium abbreviatum* also accounted for an important fraction (25 %). Chlorophytes and cyanobacteria contributed 17 % and

12 % to the total biomass, whereas cryptophytes and chrysophytes represented 2.6 % and 0.7 %, respectively. Similarly, bacteria in the labelled suspension were distributed as described in the water sample (Fig. 1, Table 2). In terms of individuals, flagellates and ciliates presented very low relative abundances (lower than that in the whole water sample, Table 2). Flagellates and ciliates amounted to approximately 1 % and below 0.1 %, respectively, of the abundance of phytoplankton individuals and were an order of magnitude lower than the bacterial numbers. When compared to phytoplankton biomass, flagellates and ciliates contributed at somewhat higher percentages, but still below 2 % and 1 %, respectively; conversely, these percentages were 4 times higher than the bacterial biomass.

Daphnia magna CRs were nearly equal for both groups of radiolabelled organisms. During the period in which the food suspension was incubated with ^{14}C, this carbon was incorporated into the phytoplankton biomass, but part of this carbon was presumably released as dissolved organic matter and subsequently incorporated by

Table 2. Abundance (ind/mL) of the main zooplankton, phytoplankton and bacterial groups. *Abundancia (ind/mL) de los principales grupos de zooplancton, fitoplancton y bacterias.*

	Abundance (ind/mL)	
	March 2011	May 2012
Zooplankton		
Daphnia magna + other cladocera	0.04 + 0.02	—
Bosmina longirostris + other cladocera	—	1.4 + 0.04
Copepoda	0.5	0.5
Rotifera	0.9	0.6
Ciliates	300	643
Heterotrophic flagellates	3600	19166
Phytoplankton ($\times 10^3$)		
Filamentous and colonial cyanobacteria (inedible)	69	764
Edible cyanobacteria	7	200
Diatoms	43	107
Chlorophytes	31	44
Other	51	6
Heterotrophic bacteria ($\times 10^6$)		
Bacteria < 0.6 μm	18.2	17
Bacteria 0.7-3 μm	12.8	8.4
Filamentous bacteria	1.3	1.9

heterotrophic bacteria. However, previous results in the lagoon showed that, in spring, less than 15 % of the extracellular dissolved organic carbon released by phytoplankton is consumed by heterotrophic bacterial gross production (see bacterial carbon demand in Table 2 in Onandia *et al.*, 2014a); therefore, we assumed that the labelled carbon was mainly incorporated into phytoplankton biomass. Likewise, during the incubation period, a fraction of the radiolabelled compounds was very likely transferred to different protists. Given the low relative abundance of ciliates and heterotrophic flagellates with respect to phytoplankton and bacteria, we considered that the labelled thymidine was largely incorporated into the heterotrophic bacterial biomass, although labelled flagellates were very likely present.

Taking into account these considerations, the measure of CR as the suspension volume filtered by *Daphnia* can be considered a good estimate and therefore used to calculate the IR for the algae and bacteria in the suspension by knowing their corresponding concentrations, even though we had an unevenly labelled pool of organisms. Moreover, because no significant differences were found between CRs using the two radiotracers, our results indicate that there were no differences between the CRs of phytoplankton and heterotrophic bacteria. However, the IRs were significantly higher for algae than for bacteria (Fig. 4). Regarding the bacterial carbon ingested by *D. magna*, bacteria in the size range of 1-3 μm amounted to 83 % of the total bacterial biomass, and therefore, assuming even CRs for all bacteria, this size range would probably provide most of the bacterial carbon ingested by *D. magna*.

The mean MSIRs of *D. magna* for algae and bacteria are shown in Table 3. These results indicate that bacteria contributed ≈ 40 % of the total (algae + bacteria) ingested carbon. The AEs for phytoplankton, as well as for bacteria, were approximately 50 % and did not differ significantly ($p > 0.05$, $n = 8$, ANOVA test). According to these results, at the end of March, *D. magna* grazing removed 5 %/d and 15 %/d of the standing stock of total phytoplankton and bacteria, respectively, in the Albufera de Valencia lagoon. The relative grazing rates of PP_{day} and BP_{day} by *D. magna* were 9 %/d and 75 %/d, respectively.
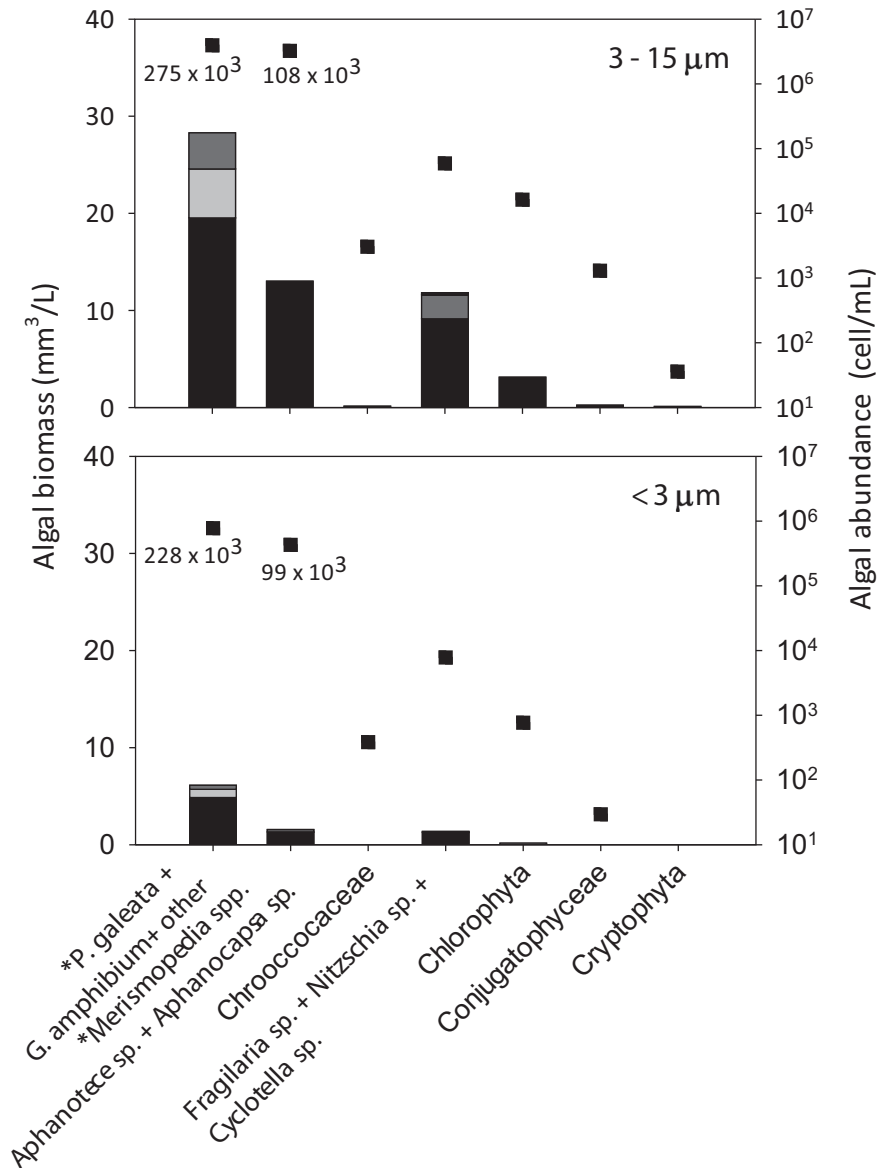
Mid-spring grazing experiments

In mid-spring 2012, the zooplankton community was clearly dominated by the cladocera *Bosmina longirostris,* which amounted to 72 % of the zooplankton biomass (Fig. 1). Other cladocera species such as *Chydorus sphaericus*, *Alona rectangula* and *Ceriodaphnia laticaudata* were found, but they represented a negligible biomass fraction. The rest of the zooplankton biomass consisted of *Acanthocyclops americanus* and rotifers in similar proportions. The main rotifer species were *Brachionus calyciflorus* and *Keratella cochlearis,* although *Keratella tropica*, *Lecane bulla*, *Polyarthra* sp. and *Hexarthra fennica* were also present in low numbers (Fig. 1). The proportions varied in terms of their abun-

Table 3. Mass-specific ingestion rates (MSIRs, pg C pg C^{-1} h^{-1}) and assimilation efficiency (AE, %) of *D. magna* and *B. longirostris* for different labelled organisms. *Tasas de ingestión por unidad de biomasa (MSIRs, pgC pgC^{-1} h^{-1}) y eficiencia de asimilación (AE, %) de* D. magna *y* B. longirostris *para los distintos organismos marcados.*

	Labelled size in food suspensions (μm)				
	Algae			Bacteria	
Daphnia magna	< 25			< 3	
Mass specific ingestion rate (pgC pgC^{-1} h^{-1})	0.054 ± 0.005			0.036 ± 0.004	
Assimilation efficiency (%)	56 ± 2			$51 \pm$	
Bosmina longirostris	1-15	3-15	1-3	1-3	< 1
Mass specific ingestion rate (pgC pgC^{-1} h^{-1})	32.5	21.4 ± 5.9	3 ± 0.3	4.6 ± 1.4	0.7 ± 0.2
Assimilation efficiency (%)		44 ± 20	52 ± 8	53 ± 20	73 ± 11

dances (Table 2). Cyanobacteria were the main algal group, contributing 75 % of total phytoplankton biomass. We assumed that only a small proportion of the cyanobacterial biomass (24 %) was ingested by zooplankton because the species with larger biomass contributions were considered inedible, such as the filamentous *Pseudanabaena galeata*, (37 % abundance, 37 % biomass)


Figure 2. Phytoplankton biomass (bars) and abundance (squares) in the radiolabelled food suspensions containing (a) 3-15 μm and (b) < 3 μm algae used in the mid-spring experiments (May 2012). Bar fractions are ordered corresponding to taxon names on the x-axis. *Filamentous and colonial cyanobacteria were also counted as individuals: numbers under the corresponding squares indicate abundance (ind/mL). *Biomasa (barras) y abundancia (cuadrados) de fitoplancton en las suspensiones de alimentación radioactivas con algas de (a) 3-15 μm y (b) < 3 μm en los experimentos de final de primavera (mayo de 2012). Las fracciones de las barras están ordenadas siguiendo los nombres de los taxones del eje horizontal. *Las cianobacterias filamentosas y coloniales fueron también contados como individuos: la abundancia (ind/mL) se indica bajo los cuadrados correspondientes.*

and the colonial *Merismopedia* sp. (38 % abundance, 25 % biomass). Next in importance were bacillariophytes (18 %), mainly represented by species of the genus *Fragilaria* sp. and *Nitzschia* sp., in addition to *Cyclotella* sp. and chlorophytes (6 %), with species such as *Oocystis* sp., *Scenedesmu*s sp., *Tetraedron* sp., or *Lagerheimia* sp. (Fig. 1). Filamentous bacteria also dominated the heterotrophic bacterial community in terms of biomass (50 %), but their relative abundance was low (7 %). Bacteria of the smallest size ($< 0.6 \, \mu m$) were always more numerous (62 % of the relative abundance), but the other bacteria (mostly 1-3 μm) were also abundant (Fig. 1).

The phytoplankton community composition of the two radiolabelled food suspensions (15-3 μm and $< 3 \, \mu m$ seston fractions) resembled that of the natural phytoplankton assemblage (Figs. 1-2). *P. galeata* and *Merismopedia* sp. dominated in terms of biomass, but *Fragilaria* sp. also made an important contribution. However, the relative abundance of filaments or colonies was low with respect to the total phytoplankton entities. In the 3-15 μm fraction, the phytoplankton abundance and biomass were markedly higher than in the $< 3 \, \mu m$ fraction. The former phytoplankton biomass amounted to 56.6 mm^3/L, and the latter was 9.2 mm^3/L. Likewise, the phytoplankton abundance decreased from 7.3×10^6 cell/mL in the 3-15 μm fraction to 1.2×10^6 cell/mL in the $< 3 \, \mu m$ fraction (Fig. 2). The bacterial abundance was negligible in the 3-15 μm fraction (although a small proportion of filamentous bacteria were present), and the relative proportion of the 1-3 μm and $< 1 \, \mu m$ bacterial sizes was very similar to that of the lake water. The relative contribution of flagellates and ciliates in these fractions was very low; flagellates amounted to approximately 0.03 % of the number of phytoplankton individuals in both fractions (and less than 2 % of phytoplankton biomass), while ciliates amounted to 0.003 % of the number of phytoplankton individuals in the 3-15 μm (0.3 % of phytoplankton biomass) and to a negligible proportion both in numbers and biomass in the $< 3 \, \mu m$ fraction.

B. longirostris was found to clear all radiolabelled suspensions (Fig. 3). However, the CRs differed significantly across the suspensions containing organisms with different size ranges. The CRs on algae in the size range of 1-15 μm were significantly higher than the CRs on $< 1 \, \mu m$ bacteria ($p < 0.05$, $n = 31$, ANOVA test; Tukey-b test). No significant differences were found be-
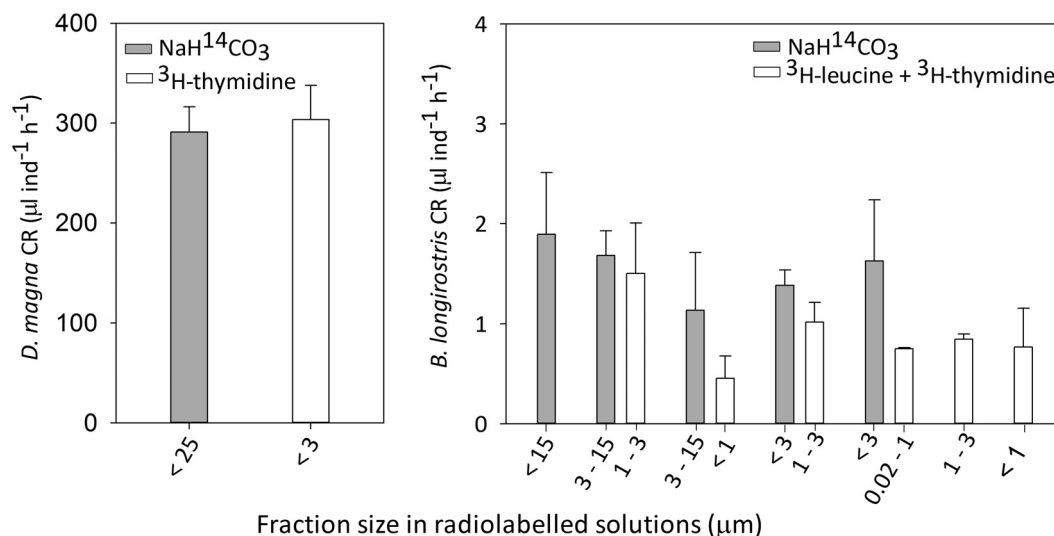
Figure 3. Mean clearance rates (CR, μL ind^{-1} h^{-1}) of *D. magna* and *B. longirostris* in March 2011 and May 2012 for the different labelled organisms. Error bars indicate the standard deviation. *Tasas de filtración medias (CR, μL ind^{-1} h^{-1}) de* D. magna *y* B. longirostris *en marzo de 2011 y mayo de 2012 para los diferentes organismos marcados.*
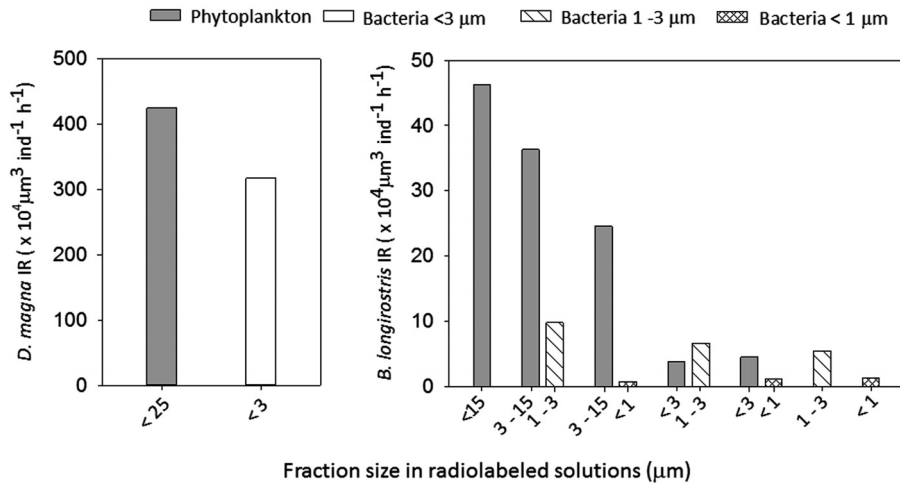
Figure 4. Mean ingestion rates (IR, μm^3 ind^{-1} h^{-1}) of *D. magna* and *B. longirostris* in March 2011 and May 2012 for the different labelled organisms. *Tasas de ingestión media (IR, μm^3 ind^{-1} h^{-1}) de* D. magna *y* B. longirostris *en marzo de 2011 y mayo de 2012 para los diferentes organismos marcados.*

tween the CRs on the two algal size fractions. Similarly, no significant differences were found between the CRs on the two bacterial size fractions, but the minimum CR was found for bacteria of the 0.2-1 μm size class (Fig. 3). The IRs of *B. longirostris* are shown in Figure 4. Given the important contribution of *Fragilaria* sp. to the edible phytoplankton biomass in the radiolabelled suspensions, this species probably proportionated the largest amount of carbon ingested by *B. longirostris*.

The mean MSIRs for *B. longirostris* decreased with the size of food particles in the tested food suspensions (Table 3). Bacteria contributed ≈ 10 % of the total (algae + bacteria) ingested carbon. *B. longirostris* displayed an AE of approximately 50 % for the different plankton size fractions contained in the food suspensions, with the exception of higher values for <1 μm bacteria (Table 3).

The CRs of the 50-100 μm zooplankton size fraction, consisting of *Keratella cochlearis* and a small proportion of *A. americanus* nauplii (Fig. 5), significantly differed across the different food suspensions. The CRs on algae in the size range of 3-15 μm were significantly higher than those on algae in the size range of 1-3 μm and on bacteria ($p < 0.05$, $n = 28$, Kruskal-Wallis test; pairwise comparison). No signal was found
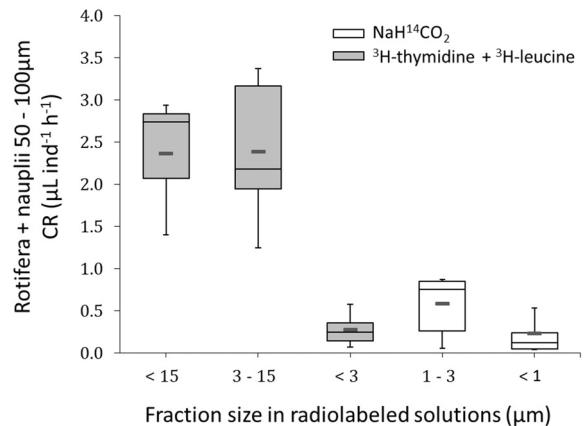
Figure 5. Clearance rates of 50-100 μm zooplankton (CR, μL ind^{-1} h^{-1}) for the different labelled organisms in May 2012. Zooplankton consisted mainly of *Keratella cochlearis* and some *Acanthocyclops americanus* nauplii. The line within the box represents the median, the black rectangles indicate the means, and the whiskers indicate the 95 % confidence intervals. *Tasas de filtración del zooplancton de 50-100 μm (CR, μL ind^{-1} h^{-1}) para los diferentes organismos marcados en mayo de 2012. El zooplancton estaba constituido por* K. cochlearis *y algunos naplius de* A. americanus. *La línea dentro de las cajas indica la mediana, los rectángulos negros la media y las barras de error los intervalos de confianza del 95 % .*

in the separately analysed adult copepods or the last copepodite stages.

The CRs computed globally, pooling together cladocerans, cyclopoid nauplii and rotifers (mainly *Bosmina*, cyclopoid nauplii and *Keratella*),
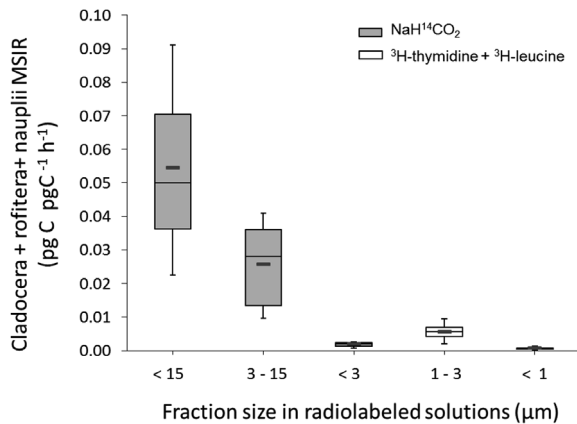
Figure 6. Total zooplankton community mass-specific ingestion rates (MSIR, pg C pg C^{-1} h^{-1}) in May 2012. The line within the box represents the median, the black rectangles indicate the means, and the whiskers indicate the 95 % confidence intervals. *Tasa de ingestión por unidad de biomasa de la comunidad total de zooplancton en (MSIR, pgC pg C^{-1} h^{-1}) mayo de 2012. La línea dentro de las cajas indica la mediana, los rectángulos negros la media y las barras de error los intervalos de confianza del 95 %.*

were relatively higher for algae than for bacteria. There were significant differences across the tested food suspensions. Namely, CRs were significantly higher in the treatment with algae in the size range 1-15 μm than in the other treatments. Likewise, CRs on algae in the size range of 3-15 μm were significantly higher than the CRs for 1-3 μm algae and for the two bacterial size ranges ($p < 0.05$, $n = 31$, Kruskal-Wallis test; pairwise comparisons). The Cladocera + rotifer + nauplii IR was estimated to be 98×10^3 μm^3/h and 37×10^3 μm^3/h for edible algae and bacteria, respectively. The mean estimates of MSIRs are shown in Figure 6 and amounted to a total of 0.08 pg C pg C^{-1} h^{-1} for algae and approximately 0.006 pg C pg C^{-1} h^{-1} for bacteria. These results indicate that bacteria contributed $\approx$ 10 % of the total carbon ingested (algae + bacteria). Subsequently, we could estimate that the total zooplankton community consumed 1 %/d and 4 %/d of the total algal and bacterial standing stock in Albufera de Valencia in mid-spring. The relative grazing rates of PP_{day} and BP_{day} by the total zooplankton community were 2 %/d and 0.6 %/d, respectively.

DISCUSSION

Grazing experiments at the onset of spring

D. magna is markedly seasonal in Albufera de Valencia, occurring mainly during winter months (Miracle *et al*., 2002) and peaking at the end of this season, namely at the time of winter flushing, thus aiding in water clearing. Its occurrence is usually extended to early spring. *Daphnia* is known to be an efficient filter feeder. However, the CRs of *D. magna* that we measured were quite low (0.3 mL ind^{-1} h^{-1}). Our rates were at the bottom of the 0.05 – 2.1 mL ind^{-1} h^{-1} range found at 15 °C in Dutch lakes and were well below the CR of $\approx$ 2 mL ind^{-1} h^{-1} on *Scenedesmus* sp. reported for 1.8 mm length individuals of 4 *Daphnia* species (Gulati, 1978; DeMott *et al*., 2001).

These low rates were very likely caused by the high abundance of filamentous cyanobacteria (30.6×10^3 filaments/mL). Previous experiments in Albufera de Valencia with seston dilutions showed a marked decrease in *D. magna* CRs with increasing filament concentrations (Sahuquillo *et al*., 2007). By using a different methodology (beads), these authors reported a CR ≈ 0.3 mL ind^{-1} h^{-1} for a filament concentration very close to that tested in our experiments, thus completely agreeing with our results. Similarly, a study comprising different food suspensions with different proportions of *Cylindrospermopsis raciborskii* that resembled eutrophic conditions indicated that the presence of *C. raciborskii* relative to the suitable food resulted in reduced *D. magna* CRs (Panosso & Lürling, 2010). The extremely low CR of $\approx$ 0.02 mL ind^{-1} h^{-1} on filtered seston from hypertrophic Lake Breukeleveen (with $\approx$ 2×10^5 filaments/mL) obtained for individuals 1.8 mm in length further supports our hypothesis (DeMott *et al*., 2001). With regard to daphnids grazing on bacteria, the CRs of *D. pulex* (1.8 mm in length) estimated in the laboratory with natural bacterioplankton from Toolik Lake in Alaska were approximately 0.75 mL ind^{-1} h^{-1} (Peterson *et al*., 1978), a value that is approximately double our estimations. However, the CRs on bacteria were practically equal to those reported for *D*.

magna feeding on the yeast *Rhodotorula glutinis* at 15 °C (Burns, 1968). Given that the CRs on algae and bacteria were simultaneously estimated from a common food suspension, the mechanical interference caused by the high abundance of filamentous cyanobacteria very likely indirectly reduced the ability of *D. magna* to ingest bacteria.

The *D. magna* CRs were nearly identical for the organisms labelled with the two radiotracers. Despite the fact that the radiolabelling food suspension procedure targeted the incorporation of ^{14}C and ^3H-thymidine by phytoplankton and bacteria, respectively, a fraction of the radiotracers might have been channelled to different planktonic groups. However, as we indicated in the results section, because the amount of dissolved organic carbon excreted by algae was low with respect to the total dissolved organic carbon and the relative abundance of flagellates and ciliates was low with respect to bacteria in the grazing suspensions, phytoplankton and bacteria should be the main labelled groups of organisms. Therefore, while acknowledging a certain roughness in our methodology, our results corroborate the unselective feeding of *Daphnia* (Lampert, 1987). Few data are available on *D. magna* feeding under natural-resembling conditions, but, in accordance with our results, individual *D. galeata* CRs estimated from the natural planktonic community of the eutrophic Bautzen reservoir were practically equal for phytoplankton and bacteria (Kamjunke *et al.*, 1999). Likewise, on a study involving cultures of *Chlamydomonas reinhardtii* (5-7 μm) and *Aerobacter aerogenes* (0.85 μm) as food suspensions, the *D. magna* CRs were found to be similar for both food types (DeMott, 1982).

The *D. magna* IRs were higher on phytoplankton than on bacteria as a consequence of the higher contribution of phytoplankton to the total biomass of the plankton community on the experimental date. In our study, the largest fraction of ingested carbon (measured as MSIRs) was proportionated by phytoplankton, but the share of bacteria in the total ingested pool was important (≈ 40 %). A high bacterial contribution (42 %) to the total ingested carbon by *D. galeata* was reported during the clear water phase in the

Bautzen reservoir, associated with a low algal biomass (Kamjunke *et al.*, 1999). In Albufera de Valencia, the algal biomass typically decreases at the end of winter (Onandia *et al.*, 2014a; Romo *et al.*, 2005), which might explain the important contribution of bacteria to the *D. magna* carbon ingestion during this period. Additionally, we would like to highlight that detritus as well as protozoa probably provided a part of the ingested carbon but was not considered in the experiment.

According to our experiments, *D. magna* assimilated approximately 50 % of the ingested carbon when feeding on an algal concentration of 3.3 mg C/L. Our values are in good agreement with those found for *D. magna* in Dutch lakes with a seston concentration of 3.5 mg C/L and were close to the range of 34-49 % estimated for *D. magna* feeding on algal suspensions (containing *Chlamydomonas reinhardtii* and *Scenedesmus obliquus*) with a concentration of 3 mg C/L (Gulati, 1978; He & Wang, 2006). There are few studies combining assimilation data on algae and bacteria, but it has been reported that zooplankton assimilates algae (15-90 %) more efficiently than bacteria (3-50 %) (Wetzel, 2001). Although our values lay within the mentioned range, the fact that the MSIRs as well as the AEs were comparable for algae and bacteria highlights the role of bacteria as an important food source for zooplankton at the onset of spring in the lagoon. Moreover, some algae such as *C. abbreviatum,* which contributed a significant fraction of the algal biomass and therefore very likely to the phytoplankton carbon ingested, might not have been assimilated because of the mucilage cover (Coesel, 1997). These results might have broader implications for the annual succession of zooplankton because it has been suggested that bacteria could allow the continuance of a high *Daphnia* biomass during periods of food limitation (*i.e.*, low algal biomass (Kamjunke *et al.*, 1999).

Mid-spring grazing experiments

We found that *B. longirostris*, the dominant zooplankton species in this period and essentially the only cladoceran species, fed preferably on

algae (size ranges of 1-3, 3-15 and 1-15 μm) over < 1 μm bacteria, indicating positive selective feeding towards larger cells. These rates were low because the food concentrations were very high, *i.e.*, well above the incipient limiting level. Nonetheless, they lay within the compiled CR range for *B. longirostris* from numerous grazing studies on algae and bacteria reviewed in Tóth & Kato (1997). There are few studies in which the CRs were estimated on natural plankton, but the referred ones are in good agreement with our results. For instance, the *B. longirostris* CRs estimated in situ in three oligotrophic Ontario lakes indicated that it filtered *Cosmarium impressulum* ($\approx 28 \times 17 \times 12$ μm) more efficiently than *Chlorella vulgaris* (3-7 μm). Similarly, CRs on nearly natural bacterioplankton were higher for 0.9-8.4 μm bacteria than for ≤ 0.45 μm bacteria (Tóth & Kato, 1997). Moreover, higher *Eubosmina* CRs on natural bacterioplankton were obtained for attached bacteria (< 3 μm) than for free-living bacteria (0.2-1 μm) (Schoenberg, 1989). Regarding the results obtained under laboratory conditions, higher *B. longirostris* CRs were found for *Chlamydomonas reinhardtii* (5-7 μm) than for *Aerobacter aerogenes* (0.85 μm) on separate or combined cultures of both species (DeMott, 1982).

The fact that we did not find significant differences between the CRs on 1-3 μm and < 1 μm sized bacteria but the CRs on 1-3 μm algae were higher than those on <1 μm bacteria is possibly due to the higher mean size of algae compared to bacteria (1.5 *vs* 1.2 μm); it also suggests that *B. longirostris* does not discriminate between particles below a certain threshold size (*i.e.*, < 1.2 μm).

Unfortunately, we cannot infer mechanistic information on the selective feeding pattern from our data. Tóth & Kato (1997) attributed the selective feeding to passive selection through the filter mesh size on the fine gnathobasic filter plates rather than to active particle discrimination. However, based on mechanistic observations, DeMott & Kerfoot (1982) ascribed the differential feeding to a bimodal food collection; the first two pairs of thoracic limbs can be spread out to capture particles, and the 3rd to 5th limbs

can be used to filter fine particles. In view of our results, we cannot conclude that *B. longirostris* is a highly effective bacterial grazer. The outcomes of previous studies are controversial. Tóth & Kato (1997) concluded from a literature review that the feeding efficiency of *B. longirostris* on both natural and cultured bacteria is very often low. However, these authors found that *B. longirostris* fed efficiently on natural bacterioplankton and suggested that this cladoceran could importantly shape the structure and size of the bacterial community.

The IRs of *B. longirostris* were markedly higher on phytoplankton than on bacteria as a consequence of the higher contribution of phytoplankton to the total biomass of the plankton community in May 2012. The mean MSIRs for *B. longirostris* were proportional to the cell size in the tested solutions, with 3-15 μm algae representing the main ingested carbon source for this species.

The AEs for 3-15 μm algae were approximately 50 %, substantially lower than the values of 74-92 % estimated for *B. longirostris* feeding on an algal mixture (containing mostly *Scenedesmus* and *Chlorella*) with concentrations of 0.05-2.50 mg C/L (Urabe, 1991). The high food concentration of our 3-15 μm feeding suspension (5.4 mg C/L) might explain this discrepancy because, in agreement with the "superfluous feeding" theory, a negative relationship has been found between the AE of cladocerans and food concentrations (He & Wang, 2006). The AEs were markedly higher for bacteria sized 1-3 μm, but our values lay within the ranges usually reported for zooplankton (see above). The higher AEs for < 1 μm bacteria suggest a higher digestibility of this bacterial group; however, given their small mean mass and the low CRs on this group, these results should be considered with caution; for the same reasons, the contribution of the smallest bacteria to the total ingested carbon by *B. longirostris* can be regarded as of low relevance.

The 50-100 μm zooplankton size fraction consisting mainly of *Keratella cochlearis* and a small proportion of nauplii, showed the highest CRs on 3-15 μm algae. *Keratella cochlearis* is

able to feed on particles of different sizes (*Aerobacter*, *Rhodotorula* and *Chlamydomonas*) including detritus and killed algal cells (Bogdan *et al*., 1980; Weisse & Frahm, 2002; Starkweather & Bogdan, 1980). However, the IRs of bacteria-sized particles is low (Ooms-Wilms *et al*. 1995). Moreover, it rarely ingests "flavoured beads" and appears to have significant selection capabilities (DeMott, 1986). Furthermore, when cultured in filtered lake water, it did not reproduce and exhibited poor survival in the < 3 or < 1 μm filtrates, but it survived and reproduced well in the < 15 μm filtrates (Ooms-Wilms, 1997). Our results confirm *K. cochlearis* bacterivory but also show their preference for phytoplankton.

Regarding the zooplankton community as a whole, bacteria contributed 25 % of the total ingested carbon by zooplankton as a consequence of its lower contribution to the phyto-bacterioplankton biomass. Furthermore, the bacteria with very high relative abundances are very small (< 0.6 μm, Table 2) and represent only 20 % of total bacterial biomass (Fig. 1) thus, their contribution to the ingested C was only 5 %. These results probably reflect the feeding preferences of the dominant *Bosmina longirostris*. Nevertheless, the consumed bacteria represented a much greater percentage than phytoplankton with respect to their respective biomass concentration in the water because of the dense algal concentrations.

Onset of spring versus mid-spring grazing experiments

The physicochemical and biological parameters differed substantially between the study periods. The composition of the phytoplankton community varied markedly. The variations in the phytoplankton assemblage are closely related to the hydrological management of the lagoon. The "flushing" experienced by the lagoon during winter favours the decrease in cyanobacterial biomass and the appearance of diatoms and chlorophytes, which are edible to daphnids. Thereafter, in mid-spring, the strong reduction of the flow through the lagoon promotes the increase in filamentous and colonial cyanobac-

terial biomass and the reestablishment of these groups' dominance (Onandia *et al*., 2014a). This was accompanied by a shift from a zooplankton community dominated by the large cladocera *D. magna* in early spring to a community dominated by the small *B. longirostris* later in the season.

Early works on the annual zooplankton succession in Albufera de Valencia described the occurrence of *D. magna* in winter-spring, followed by the sporadic appearance of *D. pulex* and a brief peak of *B. longirostris* (Blanco, 1974). More recent studies in this lagoon have linked the appearance of *D. magna* to the mentioned decrease in cyanobacteria in early spring during the "flushing" periods (Oltra & Miracle, 1992; Miracle & Sahuquillo, 2002). However, when filamentous cyanobacteria bloomed again in mid-spring, *D. magna* failed to grow, in accordance with what has been observed in the hypertrophic Loosdrecht Lakes (DeMott *et al*., 2001). Filamentous cyanobacteria have been found to inhibit the feeding of *Daphnia* (Sahuquillo *et al*., 2007; Panosso & Lürling, 2010) and often represent an inadequate food source for several reasons. Cyanobacteria might be toxic, nutritionally deficient or difficult to ingest because of mechanical interference (Lampert, 1987; Brett & Müller-Navarra, 1997; DeMott *et al*., 2001). In Albufera de Valencia, a large part of the *D. magna* population at the onset of spring is composed of ephippial females that disappear from the plankton, leaving ephippia that sink to the bottom of the lake (Miracle & Sahuquillo, 2002). The induction of resting eggs could be both due to poor food conditions and the entrance of juvenile fish early in the year in the lagoon (Slusarczyk *et al*., 2013).

In contrast, the spring bloom of filamentous and colonial cyanobacteria favoured the appearance of *B. longirostris*. Small cladocerans often outcompete larger ones under conditions of cyanobacterial dominance (Lampert, 1982; Abrantes *et al*., 2009), with *Bosmina* sp. being less sensitive than daphnids to cyanobacterial filaments (Gliwicz & Lampert, 1990). *B. longirostris* is typically considered an indicator of eutrophy (Alonso, 1996; Haberman & Haldna, 2014). Therefore, the observed changes in the zooplankton community may reflect changes in

the amount and quality of phytoplankton. Our observations support the idea that cladocerans provide a useful indicator of the trophic status in shallow lakes (Haberman *et al*., 2007).

Our results revealed different feeding strategies of the dominant cladocerans in the two studied periods. *D. magna* showed no differential CRs on phytoplankton and bacteria. On the contrary, *B. longirostris* grazed preferentially on algae (size range of 1-15 μm) than on bacteria (mainly < 1 μm). These findings agree with the results from *in vitro* grazing experiments on natural radiolabelled plankton in the shallow hypertrophic Lake Søbygaard (Denmark), indicating that, whereas *B. longirostris* fed mainly on phytoplankton; daphnids equally cleared bacteria and algae (Jeppesen *et al*., 1996).

Our estimations of the relative grazing rates of bacteria and phytoplankton indicate that only a minor proportion of the standing stock of bacteria and especially of phytoplankton was removed by zooplankton grazing, which is in good agreement with the results from other shallow eutrophic lakes (Agasild & Nõges, 2005). Furthermore, the greater part of the phytoplankton production and secondary bacterial production was not consumed by grazing. The low phytoplankton and bacterial biomass losses driven by grazing illustrate the limited ability of the zooplankton to control the lower trophic levels in the lagoon and support the idea of reduced top-down control in warm-temperate lakes (Jeppesen *et al*., 2007).

We found that bacteria supplied a substantial part of the carbon ingested by *D. magna* ($\approx$ 40 %) at the onset of spring. However, in mid-spring, bacteria only provided a minor fraction ($\approx$ 10 %) of the carbon ingested by the zooplankton community. Our results suggest that during periods in which algal biomass is reduced and *Daphnia* dominates, the trophic coupling between the microbial loop and the classical grazer food web might play a more important role in energy transfer in hypertrophic lakes, not only through the microbial pathway but also via direct bacterial carbon flow to macrozooplankton. However, during peaks in phytoplankton growth and cyanobacterial dominance in which

B. longirostris dominates the zooplankton community, the amount of energy transferred via bacterial secondary production is smaller than in the period of lower phytoplankton density.

ACKNOWLEDGEMENTS

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