1 Ms. submitted to FEMS Microbiology Ecology

2 **Effects of large river dam-regulation on bacterioplankton community structure**

3 Clara Ruiz-González.[1], Lorenzo Proia,[1], Isabel Ferrera[2], Josep M. Gasol[2], and Sergi

4 Sabater[1,3]

5 [1] Institute of Aquatic Ecology, University of Girona, E-17071 Girona, Spain.

6 [2] Institut de Ciències del Mar-Consejo Superior de Investigaciones Científicas (ICM-

7 CSIC), E-08003 Barcelona, Spain.

8 [3]Catalan Institute for Water Research (ICRA). H_2O Building, Scientific and

9 Technological Park of the University of Girona, E-17003 Girona, Spain.

10

11 Corresponding author: clara.ruiz.glez@gmail.com

12 Condensed running title: Effects of reservoirs on river bacterioplankton

13 Key words: river regulation; reservoirs; bacterioplankton community composition;

14 CARD-FISH; aerobic anoxygenic phototrophic bacteria

15

16 **Abstract**

17 Large rivers are commonly regulated by damming, yet the effects of such
18 disruption have seldom been studied on prokaryotic communities. We describe the
19 effects of the three large reservoirs of the Ebro river (NE Iberian Peninsula) on
20 bacterioplankton assemblages by comparing several sites located before and after the
21 impoundments on three occasions. We monitored the abundances of several bacterial
22 phylotypes identified by rRNA probing, and those of two functional groups
23 (picocyanobacteria and aerobic anoxygenic phototrophic bacteria-AAPs). Much greater
24 number of particles colonized by bacteria were found in upstream waters compared to
25 downstream sites. Picocyanobacteria were found in negligible numbers at most sites
26 while AAPs comprised up to 14% of total prokaryotes, yet there was not a clear effect
27 of reservoirs on the spatial dynamics of these two groups. Instead, damming caused a
28 pronounced decline in *Betaproteobacteria, Gammaproteobacteria* and *Bacteroidetes*
29 from upstream to downstream sites, while *Alphaproteobacteria* and *Actinobacteria*
30 significantly increased after the reservoirs. Redundancy analysis (RDA) revealed that
31 conductivity, temperature and dissolved inorganic nitrogen were the environmental
32 predictors best explaining the observed variability in bacterial community composition.
33 Our data show that impoundments exerted significant impacts on bacterial riverine
34 assemblages and call attention to the unforeseen ecological consequences of river
35 regulation.

36 **Introduction**

37 Prokaryotes are essential players in aquatic ecosystems, catalyzing significant
38 biogeochemical reactions and holding central roles in aquatic food webs (Cotner and
39 Biddanda 2002, Pernthaler 2005). Natural assemblages of bacterioplankton are highly
40 diverse and can undergo shifts in composition in response to spatial and temporal
41 environmental gradients across ecosystems (Kirchman et al. 2004, Crump and Hobbie
42 2005, Alonso-Sáez et al. 2007, Comte and del Giorgio 2010), fluctuations that may lead
43 to changes in the functional roles of bacterial communities in the biogeochemical
44 cycles. However, our current knowledge of the dynamics of freshwater bacterioplankton
45 diversity is almost entirely based on lake studies (Zwart et al. 2002, Newton et al.
46 2011), and much less attention has been paid to the structure of bacterial communities in
47 rivers.

48 Recent studies using culture-independent approaches (like fluorescent in situ
49 hybridization - FISH and 16S rDNA sequencing) have revealed that typical riverine
50 bacterioplankton assemblages are dominated by taxa belonging to *Betaproteobacteria,*
51 *Bacteroidetes* and *Actinobacteria* (Glöckner et al. 2000, Crump et al. 2009, Portillo et
52 al. 2012). These major bacterial groups and the phylotypes within them show, however,
53 diverging abundances at different temporal and spatial scales. Recent work on the
54 composition of riverine bacterial communities has shown shifts according to seasonal
55 variations in discharge, temperature, nitrate concentration, dissolved organic matter
56 (DOM) and conductivity, and even on episodic events such as freshets caused by rain,
57 ice or snow melting (Leff et al. 1999, Crump and Hobbie 2005, Crump et al. 2009,
58 Portillo et al. 2012). Further, spatial changes in bacterial assemblages along river
59 systems have also been reported and related to phytoplankton development, changes in
60 land use, variations in nutrient and DOM concentration, and quality and intensity of

61 grazing pressure, among other factors (Winter et al. 2007, Levine and Crump 2002, Leff
62 et al 2000).

63 Given that the composition of bacterioplankton communities often seems to
64 change slowly and gradually along rivers (Sekiguchi et al. 2002, Winter et al. 2007), the
65 magnitude of the seasonal variation may exceed that of spatial changes, as observed
66 elsewhere (Leff et al. 1999). However, many rivers are highly regulated for hydropower
67 or water supply purposes, which generates important hydrological disturbances at the
68 spatial scale. Reservoirs constitute a discontinuity for the river system as they regulate
69 water flow circulation, modify water residence time, and affect riverborne nutrient and
70 matter loads through retention of a great fraction of the suspended material transported
71 by the river (Batalla and Vericat 2011). As a result, the upstream and downstream
72 sections of the reservoirs tend to differ greatly in their physico-chemical properties
73 (Sabater et al. 1989, Pozo et al. 1997) and, consequently, changes in the planktonic
74 communities could also be expected. Seston sedimentation due to damming has been
75 shown to cause shifts in the proportion of free-living vs. particle-attached bacteria (e.g.,
76 Kondratieff and Simons 1985) which might imply changes in the composition and
77 metabolic capabilities of the bacterial assemblages from both reaches (Karner and
78 Herndl 1992, DeLong et al. 1993, Besemer et al. 2005). Moreover, studies performed
79 within reservoirs have reported large longitudinal shifts in bacterioplankton community
80 structure that can ultimately be attributed to the extended residence time of water within
81 the impoundment (Mašín et al. 2003). As such, we would expect a clear differentiation
82 between up- and downstream bacterial assemblages, yet given that the response of
83 bacteria to environmental changes is not only due to replacement of the existing
84 phylotypes, but also to functional adjustments of the existing taxa (Comte and del
85 Giorgio 2011), phylogenetically different communities before and after the reservoirs

86 may not always be encountered. Indeed, the very few available studies so far comparing
87 the communities before and after the reservoirs show contrasting results. While clearly
88 different bacterial assemblages were found before and after the reservoir at the small
89 Sinnamary river (Dumestre et al. 2001), large impoundments in the Danube river caused
90 indiscernible effects on bacterial communities (Winter et al. 2007). Our own objective
91 was thus to determine the influence of damming on the spatial and seasonal patterns of
92 bacterial community composition in the large regulated Ebro river (NW Spain).

93 The Ebro is the third largest river system in the Mediterranean basin in terms of
94 watershed area, and has been strongly regulated since 1940. Its largest reservoirs
95 (Mequinenza, Ribarroja and Flix) are located in the mid-lower part of the river and can
96 cause significant changes in the discharge pattern (Ibáñez et al. 2008). Rivers in
97 Mediterranean climate regions are physico-chemically and biologically shaped by
98 sequential, predictable seasonal events of flooding and drying over the annual cycle
99 (Armengol et al. 1991, Gasith and Resh 1999). Under natural unaltered conditions,
100 plankton densities in these river systems tend to increase from mid to lower river
101 sections and from winter to summer, when slower, warmer and well-lit waters allow
102 maximal phytoplankton development (Vis et al. 2007). Increased discharge during wet
103 periods decreases water residence time, homogenizing water quality conditions and
104 diluting planktonic biomass. These seasonal and longitudinal patterns, though, are
105 dramatically disrupted by the presence of reservoirs. Long term data in the Ebro river
106 reveal that more than 99% of the original sediment load is retained by impoundments
107 along its course (Batalla and Vericat 2011). Abrupt decreases in turbidity, conductivity,
108 chlorophyll *a* and changes in the concentrations of some nutrients are equally associated
109 to the presence of reservoirs, features that seem to trigger the development of
110 differentiated phytoplankton communities between both reaches as well as the massive

111 growth of macrophytes in downstream sites (Roura 2004; Sabater et al. 2008).
112 Moreover, reservoirs in the river have also been shown to cause a change in the use of
113 inorganic and organic phosphorus (Artigas et al. 2012), leading to strong phosphorus
114 (P)-limitation during low water flow periods in waters upstream of the reservoirs. Such
115 wide range of environmental conditions co-occurring in the Ebro river would likely
116 affect bacterioplankton community structure as well. Exploring these relationships may
117 also contribute to the understanding of the mechanisms driving bacterial community
118 composition in complex river systems.

119 We analyzed the changes in bacterioplankton communities in twelve sites located
120 upstream and downstream of the largest reservoir system of the Ebro river. We
121 addressed such variability both from a phylogenetic perspective (through rRNA
122 probing) and a functional point of view, distinguishing among heterotrophic,
123 autotrophic (picocyanobacteria) and photoheterotrophic bacteria (aerobic anoxygenic
124 phototrophs, AAPs, Kolber et al. 2000), providing the first quantitative evidence of
125 significant AAP numbers in rivers. We hypothesized that the two river sections
126 partitioned by the reservoirs would develop distinct heterotrophic microbial
127 assemblages with varying biogeochemical roles, and that the magnitude of the
128 reservoir-driven changes would also change seasonally being lower in winter due to
129 higher discharge and homogenization of water characteristics (Artigas et al. 2012). The
130 contrasting environmental conditions between the sections located up- and downstream
131 of the reservoirs offer a good opportunity to explore relationships between the dynamics
132 of these phylogenetic and functional groups and their physico-chemical environment.

133

134 **Methods**

135 **Study area**. The Ebro river is located in the northern third of the Iberian
136 Peninsula. With a length of 910 km and a basin surface of 85000 km^2, it is the largest
137 Iberian river draining to the Mediterranean Sea. Along the course to its delta, its
138 watershed encompasses diverse climate regimes, landscapes, and land uses (Sabater et
139 al. 2009). The Ebro river is characterized by high precipitation and discharge periods in
140 autumn and spring, while summer rainfall decreases from the NW to the SE parts of its
141 basin. The river shows a highly variable water discharge at the Ebro river mouth
142 (monthly means ranged between 19.5 $m^3 s^{-1}$ to 2470 $m^3 s^{-1}$ from 1912 to 2008, Sabater
143 et al. 2008). The basin has been strongly regulated since the 1940s, and nearly 187
144 reservoirs impound 57% of the mean annual runoff. The largest ones (Mequinenza,
145 Ribarroja and Flix) interrupt the hydraulic continuity in the mid-lower part of the river
146 (Fig. 1). This reservoir system is ca. 140 km long, has a maximum depth of 60 m and
147 presents a relatively long water residence time that oscillates between 1 and 5 months
148 (Roura 2004).

149 **Sampling design.** The study was done in the main middle-low course of the Ebro
150 River. We sampled 6 sites upstream (Zaragoza, Pina de Ebro, Quinto, La Zaida,
151 Sástago, Escatrón) and 5 downstream (Flix, Ascó, Móra d'Ebre, Benifallet, Xerta) of
152 the Mequinenza-Ribarroja-Flix reservoir system, as well as one intermediate site located
153 at the Ribarroja reservoir (Almatret). The studied transect extended for 330 km reaching
154 up to 30 km far from the river mouth (Fig. 1). Samplings were carried out in 3
155 occasions in 2011 during summer (July and September) and winter (December). Water
156 flow (Table 1) was provided by the "Confederación Hidrográfica del Ebro" (CHE) from
157 one upstream site (Zaragoza), one reservoir site (Mequinenza) and one downstream site
158 (Ascó). Surface water samples were collected from the free water zone with 10 L
159 polyethylene buckets. At each station, water temperature, conductivity, pH and

160 dissolved oxygen were determined in situ by means of appropriate probes. Samples for
161 all other parameters were collected in triplicate and processed in the lab.

162 **Chemical analyses**. Triplicate samples for dissolved nutrient analyses were
163 filtered through 0.2 μm pore size nylon filters and frozen at 20ºC until analysis in the
164 laboratory. Concentrations of nitrate, ammonia, reactive phosphorus, dissolved organic
165 and inorganic carbon (DOC and DIC), and total dissolved nitrogen and phosphorus
166 were determined by standard methods as explained in Artigas et al. (2012). Suspended
167 solids were estimated after filtration of 0.2-2.5 L of water and heating in a muffle
168 furnace at 450ºC for 4 h to obtain their ash-free dry weight (AFDW).

169 **Chlorophyll *a* determination.** Chlorophyll *a* (Chl *a*) concentration was
170 determined in triplicate by filtering 0.2-3 L of water on GF/C filters and extracting the
171 pigment in acetone (90% v/v) for 12-20 h in the dark at 4ºC. Absorbance of the pigment
172 was measured with a Shimadzu UV-1800 spectrophotometer.

173 **Prokaryote abundance and biomass**. Heterotrophic prokaryote abundances
174 were quantified in triplicate CARD-FISH filters (see below) by epifluorescence
175 microscopy after staining with 4,6-diamidino-2-phenylindole (DAPI, 1 μg mL^{-1}). A
176 minimum of 10 fields (500-1200 DAPI stained cells) per filter were manually counted
177 in an Olympus BX61 epifluorescence microscope. The presence of filamentous bacteria
178 and particles intensely colonized by bacteria was also quantified in these filters from
179 transects across the section of the filters.

180 Bacterial cell size and biomass were estimated by flow cytometry. Samples of 5
181 mL were preserved with 1% paraformaldehyde and 0.05% glutaraldehyde (final
182 concentrations) and kept frozen at -80ºC until analysis with a Becton-Dickinson
183 FACSCalibur flow cytometer after staining with SYTO-13 (Molecular Probes, Eugene,

184 Oreg.). Bacterial cell size was estimated using the relationship between the average
185 bacterial size and the average fluorescence of the SYTO-13 stained bacteria relative to
186 that of standard beads (Gasol and del Giorgio 2000). Bacterial carbon content was
187 further calculated with the carbon to volume relationship described in Norland (1993).
188 Total bacterial biomass (in μg C L^{-1}) was calculated by multiplying bacterial carbon
189 content by their abundances.

190 **Catalyzed reporter deposition-fluorescence in situ hybridization (CARD-**
191 **FISH).** Triplicate samples of 10 mL were fixed with paraformaldehyde (1% final
192 concentration) at 4ºC in the dark for the determination of the in situ abundances of
193 different bacterial populations by CARD-FISH (Pernthaler et al. 2002). Aliquots of 2-3
194 mL were filtered through 0.22 μm polycarbonate filters (GTTP, 25 mm diameter,
195 Millipore), rinsed with milliQ water, air dried and stored at -20ºC until processing. For
196 the characterization of the bacterial community, we used a suite of seven horseradish
197 peroxidase (HRP)-probes: Eub338-II-III for most *Eubacteria* (Daims et al. 1999),
198 Beta42a and Gam42a for *Betaproteobacteria* and *Gammaproteobacteria,* respectively
199 (Manz et al. 1992), Alf968 for *Alphaproteobacteria* (Neef 1997)*,* CF319 for many
200 clades belonging to the *Bacteroidetes* group (Manz et al. 1996), HGC96a for
201 *Actinobacteria* (Roller et al. 1994) and CYA339 for the photosynthetic cyanobacteria
202 (Nübel et al. 1997). Prior to hybridization, cells were permeabilized with lysozyme
203 (37ºC, 1 h) and achromopeptidase (37ºC, 30 min). Hybridizations were carried out on
204 sections of the filters at 35ºC overnight, and specific hybridization conditions were
205 established by addition of different proportions of formamide to the hybridization
206 buffers (30% for *Actinobacteria,* 45% for *Alphaproteobacteria,* and 55% for the rest of
207 probes). Counterstaining of CARD-FISH filters was done with DAPI (1μg mL^{-1}) and a
208 minimum of 10 fields (500-1200 DAPI-stained cells) was manually counted in the

209 epifluorescence microscope.

210 **Enumeration of aerobic anoxygenic phototrophic (AAP) bacteria.** In
211 September and December samples were additionally collected for the quantification of
212 AAPs. Samples were fixed with 1% paraformaldehyde and 2 mL aliquots were filtered
213 onto 0.22 μm polycarbonate black Nucleopore filters (Whatman). Cells were stained
214 with 4', 6-diamidino-2-phenylindole (DAPI) and counted using an Olympus BX51TF
215 fluorescence microscope equipped with the Olympus UPlanSApo 100°/1.40 Oil
216 objective as described previously (Mašín et al. 2006). Briefly, three fluorescence images
217 were acquired for each frame: one of the cells stained with DAPI in the blue part of the
218 spectrum; one of the fluorescence of Chl *a* in the red part of the spectrum and finally,
219 both bacteriochlorophyll *a* (BChl *a)*- and Chl *a*-containing organisms were recorded in
220 the infra-red region of the spectrum (>850 nm). The red image was used to subtract Chl
221 *a*-containing organisms from the infrared counts. For each sample, 8-10 frames were
222 recorded (>500 DAPI cells) and analyzed semimanually with the Cell F Software
223 (Olympus) to distinguish between heterotrophic bacteria, picocyanobacteria, and AAP
224 bacteria.

225 **Statistical analyses.** Differences in physico-chemical and biological variables
226 were analyzed through two-way multivariate analysis of variance (MANOVA).
227 Samplings (July, September and December) and river sections (upstream and
228 downstream of the reservoirs) were considered as the fixed factors Time (T) and Site
229 (S), respectively. To fulfill the normality assumptions of this test, variables were log-
230 transformed when necessary. Correlations between variables were calculated using
231 Pearson's correlation coefficient. These statistical analyses were performed using the
232 JMP software (SAS Institute). The ordination of the bacterial groups in relation to
233 environmental data was examined by means of multivariate analyses. Transformed data

234 (log or square root transformation) were included in a detrended correspondence
235 analysis (DCA) to determine the length of the gradient for the first two axes. This
236 indicated that the gradient length was lower than 3 standard deviation units (0.5), so that
237 the use of linear ordination techniques was appropriate (ter Braak and Ŝmilauer 2002).
238 Redundancy Analysis (RDA) was applied to find the environmental predictors that best
239 explained the distribution of the different bacterial groups and samples. These analyses
240 were performed using CANOCO version 4.5.

241

242 **Results**

243 **Environmental conditions.** Strong differences in physico-chemical and
244 chlorophyll *a* (Chl *a*) concentration existed between the sections upstream and
245 downstream of the reservoirs as well as between the periods examined (Table 1). Water
246 flow was lower from July to September and increased again in December, though
247 differences were not very high (Table 1). Downstream discharge values were on
248 average 2.5 to 3.8 times greater than those upstream, although they followed similar
249 discharge patterns. July and September were characterized by higher water temperature
250 and conductivity, and much lower dissolved inorganic nitrogen (DIN) concentrations.
251 Temperature ranged from 10ºC in winter to 27.5 ºC in summer (July and September)
252 and was relatively constant throughout the studied river stretch at each sampling period.
253 In winter, though, the temperature in the section downstream of the reservoirs was 2-
254 5ºC higher than that of the upstream sites. The reservoirs also caused an abrupt decrease
255 in conductivity that was maintained in downstream waters; this decrease was smaller in
256 the winter period (Table 1). Soluble reactive phosphorus (SRP) and DIN differed
257 significantly among sections and periods (Time x Site effect, p values <0.0001-0.05).
258 While DIN concentrations were always greater in winter than in summer, and upstream

259 than downstream, SRP showed greater upstream concentrations in July and December
260 and the opposite trend in September, but differences were small. The lowest SRP
261 concentrations occurred at the reservoir site (10.4 and 6.5 µg L^{-1} in July and September,
262 respectively).

263 Suspended matter consistently decreased at the reservoirs by sedimentation,
264 leading to downstream waters of increased transparency. DOC concentrations were
265 often higher in upstream waters, but values were highly variable among sites (Time ×
266 Site effect, $p > 0.05$). Chl *a* concentration varied greatly among sites, sections and
267 periods (Time × Site effect, $p < 0.0001$). The lowest values occurred in winter
268 downstream of the reservoirs, and the highest in summer upstream of the reservoirs
269 (Table 1, Fig. 2A). Chl *a* was positively correlated to conductivity (Pearson's $r = 0.60$, p
270 < 0.0001, $n = 36$) and to suspended organic matter ($r = 0.58$, $p < 0.0005$, $n = 36$).

271 **Impact of reservoirs on bacterial abundances.** Bacteria occurred as free-living
272 cells as well as attached to particles. Abundances of total bacteria ranged from 2.1 to 7.2
273 x 10^6 cells mL^{-1} and tended to be higher in the sections upstream of the reservoirs
274 (Table 1, Fig. 2B). The highest differences occurred in July, when the upstream
275 abundances were 40% higher than those below the reservoirs. In September and
276 December, though, total abundances could have been underestimated due to high
277 numbers of bacteria colonizing particles (Fig. 2C). We quantified the number of
278 particles colonized by bacteria, as it was not possible to accurately estimate the number
279 of bacteria attached to each particle since not all of them were visible in the 3-D
280 aggregate structure. Much higher numbers of particles colonized by bacteria (sized from
281 5 to 100 μm in diameter) occurred in upstream waters than in downstream sites. The
282 number of colonized particles increased from July (range 0-2900 particles colonized by
283 bacteria mL^{-1}) to September (up to 9000 particles mL^{-1} at Sástago), while they

284 decreased again till December (range 65-5900 particles mL^{-1}). The inverse correlation
285 between the abundance of such bacterial aggregates and total bacterial numbers in
286 upstream sites (r = -0.62. $p < 0.01$. n = 18) supports the concept that bacteria could
287 actually be more abundant than quantified at some locations, in particular in September
288 and December. Lower concentrations of aggregates (< 450 particles mL^{-1}) occurred
289 downstream of the reservoirs, where most prokaryotes were free-living bacteria.
290 Colonized particle numbers covaried with the concentration of suspended matter over
291 the three periods (r = 0.76, 0.83 and 0.92 for July, September and December,
292 respectively, $p < 0.005$, n = 12) exhibiting the highest abundances between Quinto and
293 Escatrón (Fig. 2C).

294 Bacterial size estimates indicated that prokaryotic cells were on average larger in
295 upstream sites (0.077- 0.085 μm^3) than downstream (0.066- 0.077 μm^3, $p < 0.05$ for the
296 three samplings) and, accordingly, mean bacterial biomass was higher upstream (0.072-
297 0.117 μg C L^{-1}) than after the reservoirs (0.069-0.064 μg C L^{-1}). These differences in
298 cell size detected with the flow cytometer were visually confirmed under the
299 microscope. However, in upstream waters we also found significant numbers of
300 filamentous bacteria (10-20 μm in size, Fig. 2D). This morphotype was nearly absent
301 downstream of the reservoirs suggesting that the differences in bacterial volume
302 between both sections of the rivers could be even greater, since these large bacteria are
303 not well quantified by flow cytometry. These filaments, most of which hybridized with
304 the probe for *Bacteroidetes* (see below), covaried significantly with increasing Chl *a*
305 concentrations over the three sampling campaigns (r = 0.69, 0.89, and 0.88 for July,
306 September and December, respectively, all $p < 0.02$, n = 12).

307 **Effect of reservoirs on functional bacterial groups.** Besides heterotrophic
308 bacteria, we targeted other two functional groups, the autotrophic picoplanktonic

309 cyanobacteria, and the photoheterotrophic AAPs. Unicellular coccoid cyanobacteria
310 were only observed in July (Fig. 2E), peaking in Sástago (up to 8% of DAPI counts)
311 and showing negligible numbers in most sites. In contrast, filamentous cyanobacteria
312 such as *Planktothrix* sp. and *Geitlerinema* sp. were present in microphytoplankton
313 samples at abundances ranging from 400 to 1900 cells mL^{-1}, and peaking generally at
314 Almatret (M.C. Pérez-Baliero unpubl.). On the other hand, AAPs, which were
315 quantified in September and December but not in July (Fig. 2E), were detected in all
316 sites inspected and showed higher abundances in September than in December (Time
317 effect, $p < 0.005$). The differences were not statistically significant between up- and
318 downstream sites, although the highest percentages of AAPs were found downstream
319 from the reservoirs. In September, AAPs ranged from 5% to 14% of total prokaryotes.
320 Values were highest at both Flix and Benifallet while AAPs contributed only to 5% of
321 total prokaryotes at the reservoir site. Lower proportions of AAPs were found in winter,
322 when the amount of these photoheterotrophic bacteria gradually increased from Pina de
323 Ebro (< 1% of total prokaryotes) to the maximum at Flix (8%) and gradually declined
324 afterwards till Xerta.

325 AAP abundance was negatively related to DIN (r = -0.73, $p < 0.0001$, n = 24) and
326 positively to total dissolved P (r = 0.58, $p < 0.005$, n = 24). AAPs in September
327 decreased with higher Chl *a* concentrations (r = -0.60, $p < 0.05$, n = 12), and in
328 December were negatively related to particulate organic matter (r = -0.78, $p < 0.005$, n
329 = 12). Not a single bacterial group was correlated with AAP relative abundances;
330 however, when upstream and downstream sites were considered separately, a
331 relationship emerged between *Betaproteobacteria* and AAP percentages (r = 0.86, $p <$
332 0.0005, n = 12 for upstream sites, and r = 0.75, $p < 0.01$, n = 12 for downstream sites,
333 details not shown).

334 Overall, the contribution of autotrophic and photoheterotrophic bacteria to total
335 prokaryote abundance ranged from 0 to 7% for the former and from 1 to 15% for AAPs,
336 indicating that the river contained a largely heterotrophic bacterial community.

337 **Effects of reservoirs on bacterial community composition**. The phylogenetic
338 composition of the prokaryotic community was assessed by CARD-FISH (Figs. 3 and
339 4). Most prokaryotic cells hybridized with the eubacterial probes EUB338-II-III (range
340 91-98% of total DAPI counts, Fig. 3A) indicating a basic absence of archaeal groups.
341 Only in winter downstream waters *Bacteria* showed lower numbers (66-72% of total
342 DAPI counts). Typically, the sum of cells hybridized with group-specific probes
343 matched well the total detected *Bacteria* (Fig. 4).

344 Hybridization with specific probes showed a different composition of the bacterial
345 communities in the upstream and downstream river sections, as well as some temporal
346 variability (Figs. 3 and 4). The presence of reservoirs was associated with changes in
347 the dynamics of most groups, yet the most noticeable effects were detected in the
348 summer periods. Overall, bacterial communities were dominated by *Actinobacteria,*
349 which accounted for 22-57% (average 37%) of total DAPI counts (Fig. 3E), and by
350 *Betaproteobacteria* (5% to 43%, average 22%, of total DAPI counts, Fig. 3C).
351 *Alphaproteobacteria* often showed relatively lower percentages (2%-18%, average
352 10%, Fig. 3B) except in downstream waters in July, where they comprised up to 33% of
353 the total community. Both *Bacteroidetes* (Fig. 3F) and *Gammaproteobacteria* (Fig. 3D)
354 ranged between 1% to 23% of total DAPI counts (average 12% and 6% for
355 *Bacteroidetes* and *Gammaproteobacteria*, respectively), but showed different spatial
356 and temporal dynamics. Nearly all the filamentous bacteria observed (Fig. 2D)
357 hybridized with the probe for *Bacteroidetes.*

358 Despite the high cell abundance of *Actinobacteria,* they likely accounted for a

359 small proportion of total bacterial biomass due to their small size in comparison with
360 the much larger *Betaproteobacteria* (details not shown). Indeed, the abundance of the
361 latter was positively related with total bacterial biomass over the three sampling
362 campaigns (r = 0.83, 0.61, and 0. 90 in July, September and December, respectively, p
363 < 0.05, $n = 12$), but *Actinobacteria* only showed a positive relationship in December (r
364 $= 0.75$, $p < 0.05$, $n = 12$), when they dominated along the whole river section (Fig. 4C).

365 The presence of the reservoirs clearly influenced the longitudinal distribution of
366 the bacterial groups, and the changes in community along each section were smaller
367 than the changes between up- and downstream reaches (Fig. 3). *Alphaproteobacteria*
368 and *Actinobacteria* strongly increased their relative abundances at the reservoir site and
369 maintained percentages higher than upstream at all downstream sites (Table 2, Fig. 3B,
370 E). *Betaproteobacteria, Gammaproteobacteria,* and *Bacteroidetes* showed larger
371 proportions in upstream communities and decreased from the reservoirs onwards (Table
372 2, Fig. 3C, D, F). In some occasions, though, this decrease started before the reservoirs,
373 at Escatrón, as was the case for *Betaproteobacteria* in September (Fig. 3C) or
374 *Gammaproteobacteria* in September and December (Fig. 3D).

375 The variations between upstream and downstream sections further depended on
376 the period considered (Time × Site effect, p values < 0.05-0.0001 for all groups except
377 *Betaproteobacteria*). *Alphaproteobacteria* showed the largest differences among
378 sections in July (average 2.5 increase from upstream to downstream sites),
379 *Gammaproteobacteria* decreased 70% after the reservoirs in September, and
380 *Bacteroidetes* decreased by a factor of 4.9 in December. Only *Betaproteobacteria*
381 presented similar magnitude of change among sections regardless of the month
382 considered (47-53% mean decrease between up- and downstream sites, Time × Site
383 effect, $p > 0.05$).

384 Summer upstream communities showed a greater contribution of
385 *Betaproteobacteria* (23-43% of total prokaryotes) and *Gammaproteobacteria* (5-21%),
386 while downstream assemblages were largely dominated by *Actinobacteria* (40-51%)
387 and presented higher proportions of *Alphaproteobacteria* (9-22%). Instead,
388 *Bacteroidetes* only showed significant differences ($p < 0.05$) between the two sections
389 in winter, although in July and September the greatest abundances were reached in Pina
390 and Quinto, both upstream sites (Fig. 3F).

391 Most bacterial groups covaried significantly with each other. The relative
392 abundances of *Alphaproteobacteria* and *Actinobacteria* correlated significantly (r =
393 0.84, $p < 0.0001$, $n = 36$), as well as *Betaproteobacteria* with *Bacteroidetes* ($r = 0.47$, p
394 < 0.005, $n = 36$) or with *Gammaproteobacteria* ($r = 0.36$, $p < 0.05$, $n = 36$). On the
395 other hand, *Betaproteobacteria* were inversely correlated to both *Actinobacteria* (r =
396 0.69, $p < 0.0001$, $n = 36$) and *Alphaproteobacteria* ($r = 0.70$, $p < 0.0001$, $n = 36$).

397 **Distribution of bacterial assemblages in relation to environmental variables.**
398 In order to summarize the environmental variables influencing the composition of the
399 bacterial communities, a Redundancy Analysis (RDA) was performed with all bacterial
400 taxa except *Cyanobacteria* (Fig. 5). In the RDA model, temperature, conductivity, and
401 DIN and DOC concentrations were the environmental variables that statistically best
402 explained the variations in the distribution of the bacterial groups among samples. The
403 explanatory power of the model did not significantly improve when phosphorus was
404 included with the environmental variables, and none of the phosphorus sources (SRP,
405 total dissolved P, dissolved organic P) was selected among the best environmental
406 variables.

407 The RDA model accounted for 64% of the variation in bacterial community
408 composition data. The first two axes explained up to 45% (axis 1) and 14% (axis 2) of

409 the variation. The variables that correlated most strongly with these axes were
410 conductivity, DIN, and temperature. *Alphaproteobacteria* and *Actinobacteria* were
411 associated with lower conductivity and DIN concentrations (typical of downstream
412 sites, Fig. 5A). *Gammaproteobacteria* occurrence was correlated with higher
413 temperatures, DOC, and lower DIN concentrations. *Betaproteobacteria* was associated
414 to higher conductivities and DOC, and *Bacteroidetes,* to higher conductivities and
415 higher nitrogen.

416 Pairwise correlation analyses supported these observations (Table 3). For
417 instance, the relative abundance of all bacterial groups was related to conductivity and
418 nitrate either positively (*Beta- Gammaproteobacteria* and *Bacteroidetes*) or negatively
419 (*Actinobacteria* and *Alphaproteobacteria*). Abundances of *Beta*- and
420 *Gammaproteobacteria* were also associated with higher concentrations of suspended
421 solids, and *Bacteroidetes* did so only in December. Significant correlations between
422 groups and DOC or Chl *a* were more evident in December than in summer (Table 3).

423 These patterns resulted in a clear distribution of the different samples regarding
424 site (upstream vs. downstream) and period of the year (Fig. 5B). The first axis of the
425 RDA separated the bacterioplankton communities characteristic of downstream sites
426 (right part of the graph) from those upstream of the reservoirs. All upstream sites
427 clustered together and were associated with high conductivity, DIN and DOC
428 concentrations, and downstream sites grouped together towards the opposite conditions.
429 The reservoir site (Almatret) was included in the analysis and it often grouped with the
430 downstream sites. The second RDA axis was mostly related to temperature and DIN
431 and separated summer samples (upper part of the graph) from winter samples. Finally,
432 the magnitude of the differences between upstream and downstream communities also
433 varied depending on the month considered, and mainly due to differences in the

434 conductivity values, being larger in July, followed by September and December.

435

436 **Discussion**

437 River regulation through damming has been shown to affect the water physico-
438 chemical conditions, the sediments transported, and the composition of phytoplankton
439 in the sections before and after the dams in major rivers (Roura et al. 2004, Dang et al.
440 2009, Bi et al. 2010). Waters upstream of the Ebro river reservoirs were characterized
441 by lower velocities, higher conductivity and greater concentrations of particulate matter,
442 DIN, DOC, and Chl *a* in comparison to downstream sites, as previously reported for
443 this system (Roura 2004; Batalla and Vericat 2011; Sabater et al. 2008). These different
444 characteristics between up- and downstream waters have been attributed to changes in
445 water residence time (Sánchez-Cabeza and Pujol 1999), and to not fully understood
446 processes occurring within the reservoirs (Roura 2004; Batalla and Vericat 2011). The
447 presence of hypolimnetic dam outlets may also influence the magnitude of these
448 differences over seasons, depending on the degree of mixing between the water from
449 the river and that of the reservoir (Roura 2004).

450 Our study shows evidence that these large impoundments also produce
451 considerable effects in bacterial communities. These effects concern bacterial size, the
452 occurrence of free- and particle-attached bacteria, as well as the longitudinal and
453 temporal patterns of community composition. Thus far, the only study reporting
454 significant effects of damming on bacterioplankton between river sections is that of
455 Dumestre et al. (2001), where different bacterial populations (identified as DGGE
456 fingerprints) occurred between sites upstream and downstream of an equatorial
457 reservoir. Given that different bacterial groups display diverse functional roles (e.g.,

458 Cottrell and Kirchman 2000, Kirchman et al. 2004), the occurring changes in bacterial
459 community structure should have implications in biogeochemical processes in the river.

460 Bacteria attached to particles may constitute as much as 90% of total bacterial
461 numbers and production in riverine and estuarine systems (Bell and Albright 1981,
462 Crump and Baross 2000). In the Ebro, much higher numbers of particles colonized by
463 bacteria were found at upstream sites than in downstream waters, presumably due to
464 sedimentation of suspended particles. Hence, the reservoirs provoked a shift in the
465 proportion of attached versus free-living bacteria, so that downstream bacterial
466 communities were mostly comprised of free-living cells, as observed elsewhere
467 (Kondratieff and Simmons 1985). This is relevant since free-living and particle-attached
468 bacteria are known to differ both phylogenetically and functionally across ecosystems
469 (Karner and Herndl 1992, DeLong et al. 1993, Besemer et al. 2005). For example, while
470 groups like *Bacteroidetes, Gammaproteobacteria,* and *Betaproteobacteria* have often
471 been found associated to particles, *Alphaproteobacteria* usually comprise free-living
472 bacteria (DeLong et al. 1993, Crump et al. 1999, Böckelmann et al. 2000). It was not
473 obvious which particular group dominated the bacterial aggregates in our study, though
474 the abundance of particles was positively correlated with *Beta-*, *Gammaproteobacteria*
475 or *Bacteroidetes*. In contrast, *Alphaproteobacteria* and *Actinobacteria* were negatively
476 correlated to particles, in accordance with their known dominant free-living lifestyle.
477 Several studies have proved that bacteria associated with aggregates exhibit higher
478 ectoenzymatic hydrolysis rates, and sometimes they can account for most of bacterial
479 production (Crump et al. 1998, Crump and Baross 2000). The high abundance of
480 particle-attached bacteria in the section upstream of the reservoirs in the Ebro may have
481 importance for the cycling and flux of elements and energy, yet the fine phylogenetic

482 affiliation of these aggregate-associated bacteria and their biogeochemical role in the
483 ecosystem are still unknown.

484 Overall, upstream water bacteria were larger than downstream cells and the
485 presence of filamentous bacteria was widespread upstream, where up to 6 times more
486 filaments occurred in comparison to downstream waters. Intense grazing by protists
487 triggers the development of large and filamentous (non-edible) morphotypes of varying
488 phylogenetic affiliations as shown in mesocosm and field studies (Pernthaler et al. 1997,
489 Šimek et al. 1999, Pernthaler et al. 2004). Nearly all filaments in our study were
490 identified as *Bacteroidetes* in accordance with the reported ability of this group of
491 organisms to form filaments under high grazing pressure (Pernthaler et al. 2004, Salcher
492 et al. 2005). Different authors have also shown that protozoa, in particular heterotrophic
493 flagellates (HFs), can control bacterial production, abundances and community
494 composition in rivers and reservoirs (Carlough and Meyer 1991, Šimek et al. 1999,
495 Servais et al. 2000). Should the abundance of these filaments in the Ebro river be related
496 to bacterivory, it would indicate a higher grazing pressure on upstream rather than on
497 downstream communities (and consequently, differences in the amount of carbon
498 flowing to higher trophic levels). However, also the morphology of very tiny coccoid
499 cells of the *Actinobacteria* group has been considered a defense strategy against
500 bacterivory (Pernthaler et al. 2001, Jezbera et al. 2006), and their increase downstream
501 of the reservoirs might also indicate an enhanced grazing pressure on downstream
502 bacteria. Finally, prey selectivity might in turn be affected by nutrient availability
503 (Šimek et al. 2003, Jezbera et al. 2006), so it is likely that the changes in bacterial
504 community composition observed between sections might be partially explained by
505 different top-down controlling factors.

506 **Bacterial community composition in terms of functional groups.** Aerobic

507 anoxygenic phototrophic bacteria were observed in the Ebro in what is the first
508 quantification in riverine systems after Mašín et al. (2008), who found minimal numbers
509 of these photoheterotrophs (< 1% of total bacteria) in two low altitude rivers. In the
510 Ebro, AAPs ranged from <1% to 14% of DAPI-positive cells and were more abundant
511 in summer than in winter. These percentages fall within the range previously reported
512 for other freshwater systems (Mašín et al. 2008, 2012). Even though this group is
513 known to be widely distributed across different aquatic environments, very little is still
514 known about their ecological preferences. In a pioneering paper, Kolber et al. (2000)
515 speculated that the capacity to harvest light could be beneficial in nutrient-poor
516 environments, yet diverse studies, most of them from marine environments, have found
517 greater AAP abundances in mesotrophic and eutrophic environments (e.g., Jiao et al.
518 2007, Hojerová et al. 2011). Freshwater environments have been understudied as
519 compared to marine sites for the enumeration of AAPs and so far most studies have
520 been carried out in lakes. Although a tendency for higher abundances towards more
521 oligotrophic conditions was first documented (Mašín et al. 2008), the opposite trend has
522 been found recently (Mašín et al. 2012). Thus, the relationship between AAP abundance
523 and lake trophic status remains unsolved and even less is known about this relationship
524 in riverine systems. In the Ebro river, AAPs were more abundant in downstream waters,
525 yet their values greatly varied among individual sites and AAP abundances and
526 proportions could not be related to the measured environmental variables, indicating
527 that other environmental factors likely determined the survival of particular AAP
528 groups. Light attenuation could be amongst those, since it is remarkably higher in the
529 section upstream of the reservoirs and this could affect their occurrence in comparison
530 with the more transparent downstream waters. Top-down factors, such a bacterivory,
531 could also influence AAP numbers, given that these bacteria have been shown to be

532 fast-growing cells subjected to high grazing pressure (Ferrera et al. 2011).

533 AAPs in freshwater ecosystems have been mainly associated to *Alpha-, Gamma-,*
534 and *Betaproteobacteria* (Salka et al. 2011), the latter being often dominant. Although
535 we did not find a general correlation between AAP abundances and that of any other
536 groups, when upstream and downstream data were considered separately a relationship
537 emerged between *Betaproteobacteria* and AAP relative abundances.
538 *Betaproteobacteria* are phylogenetically diverse, so this might suggest that AAP from
539 up- and downstream waters belong to different *Betaproteobacteria* types adapted to
540 different environmental conditions. Further studies on the abundance, function and
541 phylogenetic composition of these bacteria are required in order to understand their role
542 in freshwater ecosystems.

543 Small unicellular cyanobacteria were only detectable with the CARD-FISH probe
544 in July in upstream waters, but in most sites and seasons they comprised a negligible
545 proportion of prokaryotic communities. Picocyanobacteria are widely distributed in
546 marine and freshwater environments (Stockner et al. 2000, Newton et al. 2011) and can
547 dominate phytoplankton communities also in rivers (Sorokin and Sorokin 1996, Portillo
548 et al 2012). However, Sabater and Muñoz (1990) did not find planktonic chroococcoid
549 cyanobacteria when studying the dynamics of phytoplankton over a period of one year
550 in the river Ebro. Hence, it seems that picocyanobacteria in this system are not major
551 contributors to total primary production, and that the majority of prokaryotes very likely
552 display a heterotrophic lifestyle.

553 **Bacterial community composition in terms of phylogenetic structure**. The
554 influence of reservoirs on bacteria was reflected not only by changes in their
555 abundances, morphotypes, cell sizes and the proportion of attached vs. free-living

556 bacteria, but also in the relative contribution of the different groups considered within
557 the bacterial assemblages.

558 The use of six oligonucleotide probes targeting four phyla (*Actinobacteria,*
559 *Bacteroidetes*, *Cyanobacteria and Protebacteria)* and three classes within the latter
560 phylum (*Alpha-*, *Beta-,* and *Gammaproteobacteria*), identified 84-100% of the bacterial
561 community in the Ebro river, providing the first characterization of the bacterioplankton
562 in this system and one of the few for large rivers. Most of the taxonomic groups
563 enumerated have been shown to be prevalent in freshwater ecosystems (Kenzaka et al.
564 1998, Kirchman et al. 2004, Fortunato et al. 2012). In particular, the high percentages of
565 *Actinobacteria* and *Betaproteobacteria* in our samples are in agreement with the well
566 documented numerical dominance of these two groups in freshwater ecosystems
567 (Stepanauskas et al. 2003, Newton et al. 2011, Warkentin et al. 2011). *Bacteroidetes,*
568 *Alpha-* and *Gammaproteobacteria,* less abundant on average, also showed proportions
569 similar to those described previously (Kirchman 2002, Stepanauskas et al. 2003).

570 The reservoirs generated a clear shift in bacterial communities, yet their
571 composition was also affected by the period considered. Total detected *Bacteria*
572 remained fairly constant across the entire transect in July and September, but showed an
573 average 35% decrease in downstream sites in December. This might indicate that
574 bacteria in these sites and period were less active and thus not visually detected by our
575 probe. Groups like *Betaproteobacteria, Gammaproteobacteria,* and *Bacteroidetes*
576 showed higher proportions in upstream than in downstream waters, while
577 *Actinobacteria* and *Alphaproteobacteria* sharply increased at the reservoir site and
578 maintained greater percentages in downstream sites. In winter, though, these patterns
579 homogenized and only *Betaproteobacteria* and *Bacteroidetes* maintained significantly
580 higher percentages upstream than downstream.

581 **Factors determining the composition of bacterial communities.** The RDA
582 analysis indicated that the temporal segregation of the bacterial communities was
583 mainly driven by changes in temperature, and also by DIN concentrations, which were
584 higher in December. As such, winter communities separated from those of July and
585 September. On the other hand, the clearly differentiated upstream and downstream
586 assemblages co-occurred with strong changes in conductivity, DIN and, to a lesser
587 extent, DOC. The higher variability associated with the longitudinal gradient, indicates
588 that the spatial differences generated by these reservoirs were more important than the
589 temporal variations. It is important to note, though, that the long water retention time
590 within the reservoir system (1-5 months, Roura 2004) might play a relevant role in
591 shaping these differences by allowing new communities to establish, as reported
592 elsewhere (Mašín et al. 2003). Supporting this idea, the reservoir-driven changes were
593 largest in July and smallest in December, when water characteristics along the river start
594 to homogenize because of the greater discharge and shorter water residence times
595 (Artigas et al. 2012). Later in the season, though, when higher rainfall occurs and the
596 patterns of distribution of variables such as conductivity and nitrate are homogenized or
597 even reversed (*Confederación Hidrográfica del Ebro* [CHE], unpubl.), we would expect
598 larger similarity among bacterial assemblages along the entire reach.

599 *Beta-*, *Gammaproteobacteria* and *Bacteroidetes* were positively related to
600 conductivity, while *Actinobacteria* and *Alphaproteobacteria* showed negative
601 correlations to this variable. High conductivity values upstream of the reservoirs are
602 attributed mostly to the large inputs of chlorides and sulfates weathered from tertiary
603 substrata (Torrecilla et al. 2005), and their decrease after the reservoirs is associated to
604 dilution by tributaries and to biogeochemical processes taking place in the reservoirs
605 (Roura 2004). Although large gradients in salt concentration has been shown to be a

606 major environmental determinant for bacterial community composition across diverse
607 environments (Fortunato and Crump 2011; Kirchman et al. 2005; Lozupone and Knight
608 2007), the observed relationship between riverine bacterial groups and conductivity
609 (this work, Rubin and Leff 2007) may not directly be due to mineral composition or
610 total salts abundance.

611 DIN also appeared to have an influence in structuring up- and downstream
612 bacterial communities. In freshwater systems, a relationship between specific
613 phylotypes and DIN has sometimes been observed. Gao et al. (2005), for example,
614 found that *Beta*- and *Gammaproteobacteria* from stream biofilms tended to be most
615 abundant at sites with high DOC and nitrate concentrations, while *Alphaproteobacteria*
616 were more abundant in environments with low DOC and nitrate load, in accordance
617 with our findings. In contrast, none of the chemically different forms of phosphorus
618 influenced the abundance and composition of bacterial communities, despite the
619 previous observations that suggested that summertime plankton communities were
620 strongly limited by P in upstream sites (Artigas et al. 2012). In view of our results, it
621 seemed that in this system nitrate was a more important factor structuring bacterial
622 communities than phosphorus.

623 DOC also covaried with the presence of some bacterial groups, particularly with
624 *Gammaproteobacteria,* their abundances increasing with greater DOC concentrations.
625 However, DOC concentration alone is a poor predictor of bacterial diversity as different
626 groups respond differently depending on DOM quality and lability (e.g., Pérez and
627 Sommaruga 2006). *Gammaproteobacteria* is often comprised of large and fast-growing
628 cells that respond quickly to increases in labile DOM (e.g., Pinhassi and Berman 2003).
629 Algal lysates are presumably rich in labile organic compounds and different bacterial
630 phylotypes are known to prefer exudates from certain phytoplankton species (Sarmento

631 and Gasol 2012). Hence, the decrease in gammaproteobacterial numbers from July to
632 December, and from above- to downstream waters might be partially associated to
633 changes in the availability or origin of DOC derived from seasonally changing
634 phytoplankton assemblages. Downstream bacteria might in turn rely on DOM of
635 macrophyte origin. In this river section the low abundance of phytoplankton is balanced
636 by the mass development of macrophytes. Should downstream bacterial communities
637 depend to some extent on plant primary production, macrophyte loss in winter might
638 explain the decrease in the number of positively hybridized *Bacteria* (which were also
639 related to DOC in the RDA analysis) found in downstream winter waters. Indeed,
640 submersed macrophytes were shown to be a key factor structuring bacterial community
641 composition in a subtropical lake in China (Wu et al. 2007). In any case, riverine
642 bacterioplankton carbon demand is known to be dependant on carbon sources other than
643 primary production, such as terrestrial inputs (Kirschner and Velimirov 1997), and
644 hence correlations between the whole DOC pool and specific bacterial groups should
645 not always be expected.

646 Overall, the contrasting ecological preferences of different bacterial groups
647 translated into negative correlations between taxa from up- and downstream sites. In
648 particular, the antagonistic relationship consistently observed in our samples between
649 the two dominant clades *Betaproteobacteria* and *Actinobacteria* has been also reported
650 by other authors (Glöckner et al. 2000, Pérez and Sommaruga 2006), who suggested
651 that the two groups inhabit separate functional niches defined by DOM quality, water
652 temperature regimes and grazing pressure (Pérez and Sommaruga 2006, 2011). In the
653 Ebro, groups harboring larger and presumably fast-growing bacteria such as
654 *Betaproteobacteria, Gammaproteobacteria,* and *Bacteroidetes* were related to upstream
655 waters of elevated concentrations of nutrients, DOC and suspended matter. This is in

656 accordance with the classification of soil *Betaproteobacteria* and *Bacteroidetes* as *r*-
657 strategists (Fierer et al. 2007), i.e. taxa able to grow rapidly under conditions of high
658 resource availability. Other groups with typically smaller cell sizes, such as
659 *Actinobacteria* and *Alphaproteobacteria*, presumably more efficient at lower nutrient
660 and DOC concentrations, seem to prefer more oligotrophic and/or colder conditions
661 (Jürgens et al. 1999; Pinhassi and Berman 2003; Šimek et al. 2006), potentially
662 explaining their dominance in downstream sites and in winter. In any case, other
663 structuring factors not considered here, such as water retention time, viral lysis,
664 bacterivory or the presence of submerged macrophytes could certainly play a role in
665 shaping the bacterial communities of the Ebro river.

666 In summary, our results suggest that river regulation has a significant influence on
667 the phylogenetic composition of riverine bacterial assemblages. Reservoirs in the river
668 cause an abrupt interruption in most physico-chemical parameters, leading to a niche
669 partition with the development of clearly differentiated bacterial assemblages adapted to
670 such contrasting conditions. Variables such as temperature, conductivity and DIN had
671 an impact on the abundance of major phylogenetic groups, supporting the idea that
672 these major taxonomic groups may share some ecological traits, as suggested elsewhere
673 (Fierer et al. 2007, Philippot et al. 2009, Philippot et al. 2010). In any case, owing to the
674 limitations of the current methods for detecting the entire diversity of microbial
675 communities, it is likely that the patterns observed mostly reflect variations in the
676 dominant taxa and that new ecological trends would certainly emerge if bacterial taxa
677 were targeted at a finer resolution. Future studies using high throughput pyrosequencing
678 of PCR-amplified 16S rRNA genes are necessary for a deeper understanding of the
679 bacterial diversity, the factors explaining their temporal and spatial dynamics along the
680 river, and the potential biological and biogeochemical consequences of river regulation.

681 **Acknowledgements**

682 This study was funded by the Confederación Hidrográfica del Ebro. Additional
683 funds were provided by the project SCARCE (Consolider-Ingenio 2010, CSD2009-
684 00065). We acknowledge the support of Concha Duran throughout the study, and the
685 help from Elisabet Tornes and Carmen Gutiérrez in the field and laboratory.

686

687 **References**

688 Alonso-Sáez L, Balagué V, Sa EL*, et al.* (2007) Seasonality in bacterial diversity
689 in north-west Mediterranean coastal waters: assessment through clone libraries,
690 fingerprinting and FISH. *FEMS Microb Ecol* **60**: 98-112.

691 Armengol J, Sabater S, Vidal A & Sabater F (1991) Using the rescaled range
692 analysis for the study of hydrological records: the River Ter as an example. *Oecologia*
693 *Aquat.* **10**: 21-33.

694 Artigas J, Soley S, Pérez-Baliero MC, Romaní AM, Ruiz-González C & Sabater S
695 (2012) Phosphorus use by planktonic communities in a large regulated Mediterranean
696 river. *Sci Total Environ* **426**: 180-187.

697 Batalla RJ & Vericat D (2011) An appraisal of the contemporary sediment yield
698 in the Ebro Basin. *J Soils Sed* **11**: 1070-1081.

699 Bell CR & Albright LJ (1981) Attached and free-floating bacteria in the Fraser
700 river estuary, British Columbia, Canada. *Mar Ecol-Progr Ser* **6**: 317-327.

701 Besemer K, Moeseneder MM, Arrieta JM, Herndl GJ & Peduzzi P (2005)
702 Complexity of bacterial communitie in a river-floodplain system (Danube, Austria).
703 *Appl Environ Microbiol* **71**: 609-620

704 Bi Y, Zhu K, Hu Z, Zhang L, Yu B & Zhan Q (2010) The effects of the Three
705 Gorges Dam's (TGD's) experimental impoundment on the phytoplankton community in
706 the Xiangxi River. *China Int J Environ Stud* **67:** 207-221.

707 Böckelmann U, Manz W, Neu TR & Szewzyk U (2000) Characterization of the
708 microbial community of lotic organic aggregates (`river snow') in the Elbe River of
709 Germany by cultivation and molecular methods. *FEMS Microb Ecol* **33**: 157-170.

710 Carlough LA & Meyer JL (1991) Bacterivory by sestonic protists in a
711 southeastern blackwater river. *Limnol Oceanogr* **36**: 873-883.

712 Comte J & del Giorgio PA (2010) Linking the patterns of change in composition
713 and function in bacterioplankton successions along environmental gradients. *Ecology*
714 **91**: 1466-1476.

715 Comte J & del Giorgio PA (2011) Composition influences the pathway but not the
716 outcome of the metabolic response of bacterioplankton to resource shifts. *Plos One* **6**:
717 e25266.

718 Cotner JB & Biddanda BA (2002) Small players, large role: Microbial influence
719 on biogeochemical processes in pelagic aquatic ecosystems. *Ecosystems* **5**: 105-121.

720 Cottrell MT & Kirchman DL (2000) Natural assemblages of marine
721 proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and
722 high-molecular-weight dissolved organic matter. *Appl Environ Microbiol* **66**: 1692-
723 1697.

724 Crump BC & Baross JA (2000) Characterization of the bacterially-active particle
725 fraction in the Columbia River estuary. *Mar Ecol-Progr Ser* **206**: 13-22.

726 Crump BC, Baross JA & Simenstad CA (1998) Dominance of particle-attached
727 bacteria in the Columbia River estuary, USA. *Aquat Microb Ecol* **14**: 7-18.

728 Crump BC, Armbrust EV & Baross JA (1999) Phylogenetic analysis of particle-
729 attached and free-living bacterial communities in the Columbia river, its estuary, and
730 the adjacent coastal ocean. *Appl Environ Microbiol* **65**: 3192-3204.
731 Crump BC & Hobbie JE (2005) Synchrony and seasonality in bacterioplankton
732 communities of two temperate rivers. *Limnol Oceanogr* **50**: 1718-1729.
733 Crump BC, Peterson BJ, Raymond PA, Amon RMW, Rinehart A, McClelland JW
734 & Holmes RM (2009) Circumpolar synchrony in big river bacterioplankton. *P Natl*
735 *Acad Sci USA* **106**: 21208-21212.
736 Daims H, Bruhl A, Amann R, Schleifer KH & Wagner M (1999) The domain-
737 specific probe EUB338 is insufficient for the detection of all *Bacteria*: Development
738 and evaluation of a more comprehensive probe set. *Syst Appl Microbiol* **22**: 434-444.
739 Dang TH, Coynel A, Orange D, Blanc G, Etcheber H & Le LA (2010) Long-term
740 monitoring (1960-2008) of the river-sediment transport in the Red River Watershed
741 (Vietnam): temporal variability and dam-reservoir impact. *Sci Total Environ* **408**: 4654-
742 4664.
743 DeLong EF, Franks DG & Alldredge AL (1993) Phylogenetic diversity of
744 aggregate-attached vs free-living marine bacterial assemblages. *Limnol Oceanogr* **38**:
745 924-934.
746 Dumestre JF, Casamayor EO, Massana R & Pedrós-Alió C (2001) Changes in
747 bacterial and archaeal assemblages in an equatorial river induced by the water
748 eutrophication of Petit Saut dam reservoir (French Guiana). *Aquat Microb Ecol* **26**: 209-
749 221.
750 Ferrera I, Gasol JM, Sebastian M, Hojerova E & Koblizek M (2011) Comparison
751 of growth rates of aerobic anoxygenic phototrophic bacteria and other bacterioplankton
752 groups in coastal Mediterranean waters. *Appl Environ Microbiol* **77**: 7451-7458.

753 Fierer N, Branford MA & Jackson RB (2007) Toward an ecological classification
754 of soil bacteria. *Ecology* 88: 1354-1364.
755 Fortunato CS & Crump BC (2011) Bacterioplankton community variation across
756 river to ocean environmental gradients. *Microb Ecol* **62**: 374-382.
757 Fortunato CS, Herfort L, Zuber P, Baptista AM & Crump BC (2012) Spatial
758 variability overwhelms seasonal patterns in bacterioplankton communities across a river
759 to ocean gradient. *ISME J* **6**: 554-563.
760 Gao X, Olapade OA & Leff LG (2005) Comparison of benthic bacterial
761 community composition in nine streams. *Aquat Microb Ecol* **40**.
762 Gasith A & Resh VH (1999) Streams in Mediterranean climate regions: Abiotic
763 influences and biotic responses to predictable seasonal events. *Annu Rev Ecol Syst* **30**:
764 51-81.
765 Gasol JM & del Giorgio PA (2000) Using flow cytometry for counting natural
766 planktonic bacteria and understanding the structure of planktonic bacterial communities.
767 *Sci Mar* **64**: 197-224.
768 Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A &
769 Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals
770 globally distributed phylogenetic clusters including an abundant group of
771 actinobacteria. *Appl Environ Microbiol* **66**: 5053-+.
772 Hojerová E, Masin M, Brunet C, Ferrera I, Gasol JM & Koblizek M (2011)
773 Distribution and growth of aerobic anoxygenic phototrophs in the Mediterranean Sea.
774 *Environ Microbiol* **13**: 2717-2725.
775 Ibáñez C, Prat N, Duran C*, et al.* (2008) Changes in dissolved nutrients in the
776 lower Ebro river: causes and consequences. *Limnetica* **27**: 131-142.

777 Jezbera J, Hornák K & Šimek K (2006) Prey selectivity of bacterivorous protists
778 in different size fractions of reservoir water amended with nutrients. *Environ Microbiol*
779 **8**: 1330-1339.
780 Jiao N, Zhang Y, Zeng Y, Hong N, Liu R, Chen F & Wang P (2007) Distinct
781 distribution pattern of abundance and diversity of aerobic anoxygenic phototrophic
782 bacteria in the global ocean. *Environ Microbiol* **9**: 3091-3099.
783 Jürgens K, Pernthaler J, Schalla S & Amann R (1999) Morphological and
784 compositional changes in a planktonic bacterial community in response to enhanced
785 protozoan grazing. *Appl Environ Microbiol* **65**: 1421-1250.
786 Karner M & Herndl GJ (1992) Extracellular enzymatic activity and secondary
787 production in freeliving and marine-snow-associated bacteria. *Mar Biol* **113**: 341-347.
788 Kenzaka T, Yamaguchi N, Tani K & Nasu M (1998) rRNA-targeted fluorescent
789 in situ hybridization analysis of bacterial community structure in river water.
790 *Microbiology-Uk* **144**: 2085-2093.
791 Kirchman DL (2002) The ecology of Cytophaga-Flavobacteria in aquatic
792 environments. *FEMS Microb Ecol* **39**: 91-100.
793 Kirchman DL, Dittel AI, Findlay SEG & Fischer D (2004) Changes in bacterial
794 activity and community structure in response to dissolved organic matter in the Hudson
795 River, New York. *Aquat Microb Ecol* **35**: 243-257.
796 Kirchman DL, Dittel AI, Malmstrom RR & Cottrell MT (2005) Biogeography of
797 major bacterial groups in the Delaware Estuary. *Limnol Oceanogr* **50**: 1697-1706.
798 Kirschner AKT & Velimirov B (1997) A seasonal study of bacterial community
799 succession in a temperate backwater system, indicated by variation in morphotype
800 numbers, biomass, and secondary production. *Microb Ecol* **34**: 27-38.

801 Kolber ZS, Van Dover CL, Niederman RA & Falkowski PG (2000) Bacterial
802 photosynthesis in surface waters of the open ocean. *Nature* **407**: 177-179.
803 Kondratieff PF & Simmons GM (1985) Microbial colonization of seston and free
804 bacteria in an impounded river. *Hydrobiologia* **128**: 127-133.
805 Leff LG (2000) Longitudinal changes in microbial assemblages of the Ogeechee
806 River. *Freshwater Biol* **43**: 605-616.
807 Leff LG, Brown BJ & Lemke MJ (1999) Spatial and temporal changes in
808 bacterial assemblages of the Cuyahoga River. *Ohio Journal of Science* **99**: 44-48.
809 Levine UY & Crump BC (2002) Bacterioplankton community composition in
810 flowing waters of the Ipswich River watershed. *Biological Bulletin* **203**: 251-252.
811 Lozupone CA & Knight R (2007) Global patterns in bacterial diversity. *P Natl*
812 *Acad Sci USA* **104**: 11436-11440.
813 Manz W, Amann R, Ludwig W, Vancanneyt M & Schleifer KH (1992)
814 Phylogenetic oligodeoxynucleotide probes for the major subclasses of *Proteobacteria*:
815 problems and solutions. *Syst Appl Microbiol* **15**: 593-600.
816 Manz W, Amann R, Ludwig W, Vancanneyt M & Schleifer H (1996) Application
817 of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria
818 of the phylum *Cytophaga–Flavobacter–Bacteroides* in the natural environment.
819 *Microbiology* **142**: 1097-1106.
820 Mašín M, Čuperová Z, Hojerová E, Salka I, Grossart HP & Koblížek M (2012)
821 Distribution of aerobic anoxygenic phototrophic bacteria in glacial lakes of northern
822 Europe. *Aquat. Microb. Ecol*. **66**:77-86
823 Mašín M, Nedoma J, Pechar L & Koblížek M (2008) Distribution of aerobic
824 anoxygenic phototrophs in temperate freshwater systems. *Environ Microbiol* **10**: 1988-
825 1996.

826 Mašín M, Jezbera J, Nedoma J, Straskrabova V, Hejzlar J & Šimek K (2003)
827 Changes in bacterial community composition and microbial activities along the
828 longitudinal axis of two canyon-shaped reservoirs with different inflow loading.
829 *Hydrobiologia* **504**: 99-113.
830 Mašín M, Zdun A, Ston-Egiert J, Nausch M, Labrenz M, Moulisova V &
831 Koblízek M (2006) Seasonal changes and diversity of aerobic anoxygenic phototrophs
832 in the Baltic Sea. *Aquat Microb Ecol* **45**: 247-254.
833 Meyer JL (1994) The microbial loop in flowing waters. *Microb Ecol* **28**: 195-199.
834 Neef A (1997) Anwendung der in situ-Einzelzell-Identifizierung von Bakterien
835 zur Populationsanlayse in komplexen mikrobiellen biozönosen. PhD Thesis, Technische
836 Universität Munchen, Munich, Germany
837 Newton RJ, Jones SE, Eiler A, McMahon KD & Bertilsson S (2011) A guide to
838 the natural history of freshwater lake bacteria. *Microbiol Mol Biol R* **75**: 14-49.
839 Norland S (1993) The relationship between biomass and volume of bacteria.
840 *Handbook of methods in aquatic microbial-ecology* (Kemp PF, Sherr BF, Sherr EB &
841 Cole JJ, eds) pp. 303–307. Lewis Publishers, Boca Ratón, FL.
842 Nübel U, García-Pichel F & Muyzer G (1997) PCR primers to amplify 16S rRNA
843 genes from cyanobacteria. *Appl Environ Microbiol* **63**: 3327-3332.
844 Pérez MT & Sommaruga R (2006) Differential effect of algal- and soil-derived
845 dissolved organic matter on alpine lake bacterial community composition and activity.
846 *Limnol Oceanogr* **51**: 2527-2537.
847 Pérez MT & Sommaruga R (2011) Temporal changes in the dominance of major
848 planktonic bacterial groups in an alpine lake: discrepancy with their contribution to
849 bacterial production. *Aquat Microb Ecol* **63**: 161-170.

850 Pernthaler A, Pernthaler J & Amann R (2002) Fluorescence in situ hybridization
851 and catalyzed reporter deposition for the identification of marine bacteria. *Appl Environ*
852 *Microbiol* **68**: 3094-3101.
853 Pernthaler J, Zollner E, Warnecke F & Jurgens K (2004) Bloom of filamentous
854 bacteria in a mesotrophic lake: Identity and potential controlling mechanism. *Appl*
855 *Environ Microbiol* **70**: 6272-6281.
856 Pernthaler J, Posch T, Šimek K, Vrba J, Amann R & Psenner R (1997)
857 Contrasting bacterial strategies to coexist with a flagellate predator in an experimental
858 microbial assemblage. *Appl Environ Microbiol* **63**: 596-601.
859 Pernthaler J, Posch T, Šimek K*, et al.* (2001) Predator-specific enrichment of
860 actinobacteria from a cosmopolitan freshwater clade in mixed continuous culture. *Appl*
861 *Environ Microbiol* **67**: 2145-2155.
862 Pernthaler J (2005) Predation on prokaryotes in the water column and its
863 ecological implications. *Nat Rev Microbiol* **3**: 537-546.
864 Philippot L, Bru D, Saby NPA, Cuhel J, Arrouays D, Simek M & Hallin S (2009)
865 Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of
866 the 16S rRNA bacterial tree. *Environ Microbiol* **11**: 3096–3104.
867 Philippot L, Andersson SGE, Battin TJ, Prosser JI, Schimal JP, Whitman WB &
868 Hallin S (2010) The ecological coherence of high bacterial taxonomic ranks. *Nat Rev*
869 *Microbiol* **8**: 523-529
870 Pinhassi J & Berman T (2003) Differential growth response of colony-forming
871 alpha- and gamma-proteobacteria in dilution culture and nutrient addition experiments
872 from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. *Appl*
873 *Environ Microbiol* **69**: 199-211.

874 Portillo MC, Anderson SP & Fierer N (2012) Temporal variability in the diversity
875 and composition of stream bacterioplankton communities. *Environ Microbiol* 14: 2417-
876 2422

877 Pozo J, Orive E, Fraile H & Basaguren A (1997) Effects of the Cernadilla-
878 Valparaiso reservoir system on the River Tera. *Regul River***13**: 57-73.

879 Roller C, Wagner M, Amann R, Ludwig W & Schleifer KH (1994) In situ probing
880 of gram-positive bacteria with hight DNA G+C content using 23S ribosomal RNA-
881 targeted oligonucleotides. *Microbiology-Uk* **140**: 2849-2858.

882 Roura M (2004) Incidence of the Mequinenza reservoir in the transport of
883 suspended solids and in the water quality of the River Ebro. PhD Thesis, University of
884 Barcelona, Spain.

885 Rubin MA & Leff LG (2007) Nutrients and other abiotic factors affecting
886 bacterial communities in an Ohio river (USA). *Microb Ecol* **54**: 374-383.

887 Sabater F, Armengol J & Sabater S (1989) Measuring discontinuities in the Ter
888 river. *Regul River* **3**: 133-142.

889 Sabater S, Artigas J, Duran C, Pardos M, Romani AM, Tornes E & Ylla I (2008)
890 Longitudinal development of chlorophyll and phytoplankton assemblages in a regulated
891 large river (the Ebro River). *Sci Total Environ* **404**: 196-206.

892 Sabater S & Muñoz I (1990) Successional dynamics of the phytoplankton in the
893 lower part of the river Ebro. *J Plankton Res* **12**: 573-592.

894 Sabater S, Feio MJ, Graça MAS, Muñoz I & Romaní AM (2009) The Iberian
895 Rivers. *Rivers of Europe,*(Tockner K, Robinson C & Uhlinger U, eds), pp. 113–150.
896 Elsevier, Amsterdam.

897 Salcher MM, Pernthaler J, Psenner R & Posch T (2005) Succession of bacterial
898 grazing defense mechanisms against protistan predators in an experimental microbial
899 community. *Aquat Microb Ecol* **38**: 215-229.
900 Salka I, Cuperova Z, Masin M, Koblizek M & Grossart H-P (2011) *Rhodoferax*-
901 related *pufM* gene cluster dominates the aerobic anoxygenic phototrophic communities
902 in German freshwater lakes. *Environ Microbiol* **13**: 2865-2875.
903 Sánchez-Cabeza JA & Pujol L (1999) Study on the hydrodynamics of the Ebro
904 river lower course using tritium as a radiotracer. *Water Res* **33**: 2345-2356.
905 Sarmento H & Gasol JM (2012) Use of phytoplankton-derived dissolved organic
906 carbon by different types of bacterioplankton. *Environ Microbiol* **14**: 2348-2360.
907 Sekiguchi H, Watanabe M, Nakahara T, Xu BH & Uchiyama H (2002)
908 Succession of bacterial community structure along the Changjiang River determined by
909 denaturing gradient gel electrophoresis and clone library analysis. *Appl Environ*
910 *Microbiol* **68**: 5142-5150.
911 Servais P, Gosselain V, Joaquim-Justo C, Becquevort S, Thome JP & Descy JP
912 (2000) Trophic relationships between planktonic microorganisms in the river Meuse
913 (Belgium): a carbon budget. *Archiv Fur Hydrobiologie* **149**: 625-653.
914 Šimek K, Kojecka P, Nedoma J, Hartman P, Vrba J & Dolan JR (1999) Shifts in
915 bacterial community composition associated with different microzooplankton size
916 fractions in a eutrophic reservoir. *Limnol Oceanogr* **44**: 1634-1644.
917 Šimek K, Hornak K, Masin M, Christaki U, Nedoma J, Weinbauer MG & Dolan
918 JR (2003) Comparing the effects of resource enrichment and grazing on a
919 bacterioplankton community of a meso-eutrophic reservoir. *Aquat Microb Ecol* **31**: 123-
920 135.

921 Šimek K, Hornak K, Jezbera J*, et al.* (2006) Maximum growth rates and possible
922 life strategies of different bacterioplankton groups in relation to phosphorus availability
923 in a freshwater reservoir. *Environ Microbiol* **8**: 1613-1624.
924 Sorokin YI & Sorokin PI (1996) Plankton and primary production in the Lena
925 River Estuary and in the South-eastern Laptev Sea. *Estuar Coast Shelf Sci* **43**: 399-418.
926 Stepanauskas R, Moran MA, Bergamaschi BA & Hollibaugh JT (2003)
927 Covariance of bacterioplankton composition and environmental variables in a temperate
928 delta system. *Aquat Microb Ecol* **31**: 85-98.
929 Stockner JG, Callieri C & Cronberg G (2000) *Picoplankton and non-bloom*
930 *forming cyanobacteria in lakes*. Kluwer Academic Publishers, Dordrecht, Netherlands.
931 ter Braak CJF & Smilauer P (2002) CANOCO reference manual and CanoDraw
932 for Windows user's guide: software for canonical community ordination (version 4.5).
933 Microcomputer Power, Ithaca, New York, USA.
934 Torrecilla NJ, Galve JP, Zaera LG, Retarnar JF & Alvárez A (2005) Nutrient
935 sources and dynamics in a Mediterranean fluvial regime (Ebro river, NE Spain) and
936 their implications for water management. *J Hydrol* **304**: 166-182.
937 Vis C, Hudon C, Carignan R & Gagnon P (2007) Spatial analysis of production
938 by macrophytes, phytoplankton and epiphyton in a large river system under different
939 water-level conditions. *Ecosystems* **10**: 293-310.
940 Warkentin M, Freese HM & Schumann R (2011) Bacterial activity and
941 bacterioplankton diversity in the eutrophic River Warnow: Direct measurement of
942 bacterial growth efficiency and its effect on carbon utilization. *Microb Ecol* **61**: 190-
943 200.

944 Winter C, Hein T, Kavka G, Mach RL & Farnleitner AH (2007) Longitudinal
945 changes in the bacterial community composition of the Danube River: a whole-river
946 approach. *Appl Environ Microbiol* **73**: 421-431.

947 Wu QL, Zwart G, Wu J, Kamst-van Agterveld, MP, Liu S & Hahn MW (2007)
948 Submersed macrophytes play a key role in structuring bacterioplankton community
949 composition in the large, shallow, subtropical Taihu Lake, China. *Environm Microbiol*
950 **9**: 2765–2774.

951 Zwart G, Crump BC, Agterveld M, Hagen F & Han SK (2002) Typical freshwater
952 bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and
953 rivers. *Aquat Microb Ecol* **28**: 141-155.

954

955

Tables

Table 1. Averaged water characteristics in the upstream, reservoir and downstream sections during the three samplings (July, September and December 2011). Values are means ± standard errors of the sites considered (n = 6, for upstream and n = 5 for downstream sites, respectively). Only one site was located at the reservoir. Mean discharge values were obtained from 3 stations (see Methods) and averaged for the days of sampling. Temperature (Temp), dissolved oxygen (DO), conductivity (Cond), pH, suspended matter (Seston, mg dry weight L^{-1}), soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), chlorophyll *a* (Chl *a*) and prokaryote abundances (Prok).

Sampling	River section	Discharge ($m^3 s^{-1}$)	Temp (ºC)	DO ($mg L^{-1}$)	Cond ($\mu S cm^{-1}$)	pH	Seston ($mg L^{-1}$)	SRP ($\mu g L^{-1}$)	DIN ($\mu g L^{-1}$)	DOC ($mg L^{-1}$)	Chl *a* ($\mu g L^{-1}$)	Prok (10^6 cells mL^{-1})
July 2011	Upstream	64.1 (4.6)	25.1 (0.4)	8.2 (0.4)	2203 (68)	8.1 (0)	26.7 (18)	67.4 (6.1)	930 (44)	4.7 (0.6)	13.8 (3.4)	6.2 (0.3)
	Reservoir	51.3 (11.0)	25.9	9.3	846.0	8.2	2.5	10.4	358	3.4	7.9	5.8
	Downstream	160.4 (1.6)	25.4 (0.6)	9.4 (0.5)	1010 (9)	8.5 (0.1)	2.8 (1.5)	48.6 (2.3)	578 (39)	3.3 (0.2)	1.7 (0.3)	3.8 (0.5)
Sept 2011	Upstream	34.3 (4.2)	24.2 (0.6)	7.6 (0.6)	2069 (35)	8.2 (0.1)	30.1 (13.4)	48.2 (9.8)	1118 (52)	3.1 (0.4)	15.1 (5.3)	3.7 (0.5)
	Reservoir	57.6 (7.2)	24.8	9.0	1173.0	8.6	6.2	6.5	480	4.9	6.7	4.9
	Downstream	129.7 (5.2)	26.6 (0.6)	8.6 (0.6)	1363 (12)	8.4 (0.1)	4.6 (2.5)	82.8 (3.9)	519 (14)	3.1 (0.5)	2.2 (0.6)	3.3 (0.3)
Dec 2011	Upstream	52.5 (0.8)	11.0 (0.1)	9.3 (0.2)	1813 (35)	8.3 (0.02)	32.5 (6.0)	74.1 (3.1)	4312 (35)	4.2 (0.2)	2.3 (0.1)	3.6 (0.3)
	Reservoir	77.7 (3.1)	12.8	8	1256	8.3	9.4	53.0	2621	3.2	1.6	3.4
	Downstream	134.39 (2.1)	14.2 (0.5)	9.7 (0.2)	1247 (10)	8.4 (0.02)	5.4 (1.3)	55.0 (2.8)	2098 (24)	3.3 (0.1)	1.2 (0.2)	3.2 (0.2)

Table 2. Averaged percentages of hybridized cells in the upstream, reservoir and downstream sections considering the three samplings together. Values are means ± standard errors of the sites considered (*n* = 18, for upstream, *n* = 3 for reservoir, and *n* = 15 for downstream sites, respectively). *Eubacteria [Eub*], *Alphaproteobacteria* [Alph], *Betaproteobacteria* [Bet], *Gammaproteobacteria* [Gam], *Actinobacteria* [Act] and *Bacteroidetes* [Bctd].

	Fraction (%) of total DAPI counts					
	Eub	Alph	Bet	Gam	Act	Bctd
Upstream	93 (0.6)	6 (0.4)	30 (2)	9 (2)	29 (1)	16 (2)
Reservoir	89 (8)	19 (8)	10 (4)	4 (2)	43 (5)	10 (3)
Downstream	87 (3)	13 (2)	15 (1)	4 (1)	45 (2)	8 (1)

Table 3. Correlation coefficients for significant ($p < 0.05$) relationships between group relative abundances of *Eubacteria [Eub*], *Alphaproteobacteria* [Alph], *Betaproteobacteria* [Bet], *Gammaproteobacteria* [Gam], *Actinobacteria* [Act], *Bacteroidetes* [Bctd], and several environmental variables. [ns] not significant results ($p > 0.05$), $n = 12$ for all cases.

		Eub	Alph	Bet	Gam	Actino	Bctd
July	Conductivity	-0.821	-0.940	0.824	0.848	-0.941	ns
	Nitrate	-0.869	-0.948	0.855	0.799	-0.885	ns
	SRP	ns	-0.616	0.746	ns	ns	ns
	DOC	ns	ns	ns	0.750	-0.776	ns
	Chl *a*	ns	-0.642	ns	0.650	-0.795	0.641
	Suspended matter	-0.870	-0.758	0.611	0.577	-0.714	ns
September	Conductivity	ns	-0.904	0.869	ns	-0.966	ns
	Nitrate	-0.581	-0.876	0.909	0.891	-0.933	ns
	SRP	ns	ns	ns	ns	ns	ns
	DOC	0.687	ns	ns	ns	ns	ns
	Chl *a*	ns	ns	ns	ns	-0.579	ns
	Suspended matter	ns	ns	0.586	0.850	-0.769	ns
December	Conductivity	0.948	-0.638	0.910	0.676	-0.637	0.964
	Nitrate	0.895	-0.669	0.966	0.663	-0.736	0.941
	SRP	0.799	ns	0.756	ns	ns	0.800
	DOC	0.745	-0.679	0.712	0.694	-0.593	0.766
	Chl *a*	0.699	-0.810	0.797	0.739	-0.778	0.800
	Suspended matter	0.970	-0.577	0.914	0.780	ns	0.986

Fig. 1. Map of the Ebro watershed showing the 12 sampled sites. The presence of the reservoirs is indicated by the shaded area. Upstream sites: Zaragoza [Zar], Pina de Ebro [Pin], Quinto [Qui], La Zaida [Zai], Sástago [Sas], Escatrón [Esc]. Reservoir site: Almatret [Alm]. Downstream sites: Flix, Ascó [Asc], Móra d'Ebre [Mor], Benifallet [Ben], Xerta [Xer]. The arrow indicates the location of the sampling area within the Iberian Peninsula. Dashed lines indicate the dams separating the three reservoirs (Mequinenza, Ribarroja and Flix).

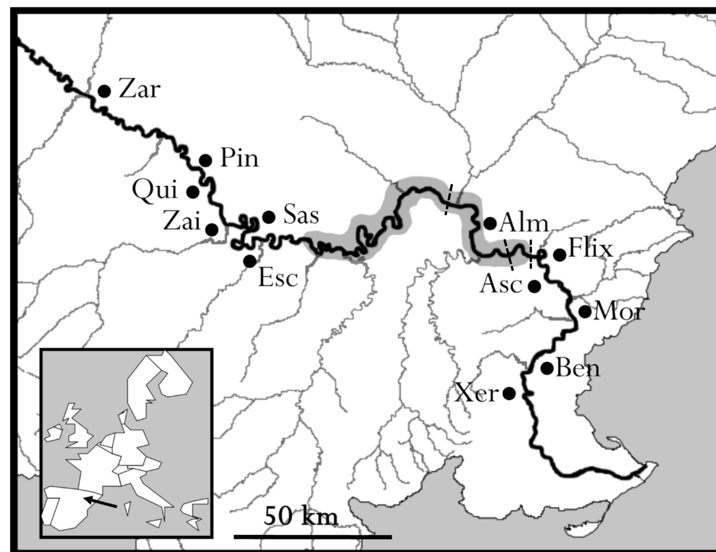

Fig. 2. Temporal and longitudinal patterns in chlorophyll *a* concentration (Chl *a,* A), prokaryote abundances (Prok., B), particles densely colonized by bacteria (C) and filamentous bacteria (D) during the three studied periods. The abundance of aerobic anoxygenic phototrophs (AAPs, E) was not determined in July, and unicellular picocyanobacteria (Cya, E) showed negligible numbers in September and December. Values are means ± standard errors of triplicate samples. Shaded areas indicate the reservoir site. Site acronyms as in Figure 1.

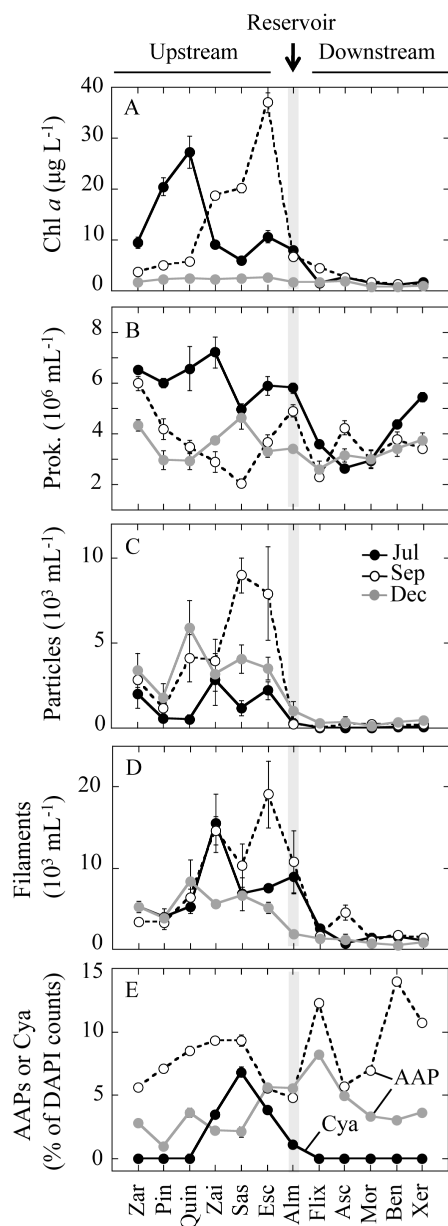

Fig. 3. Temporal and longitudinal dynamics of the relative abundances of the different bacterial groups detected by CARD-FISH probes for *Eubacteria* (A), *Alphaproteobacteria* (B), *Betaproteobacteria* (C), *Gammaproteobacteria* (D), *Actinobacteria* (E) and *Bacteroidetes* (F). Shaded areas indicate the reservoir site, located between upstream and downstream sites. Values are means ± standard errors of triplicates. Site acronyms as in Figure 1.

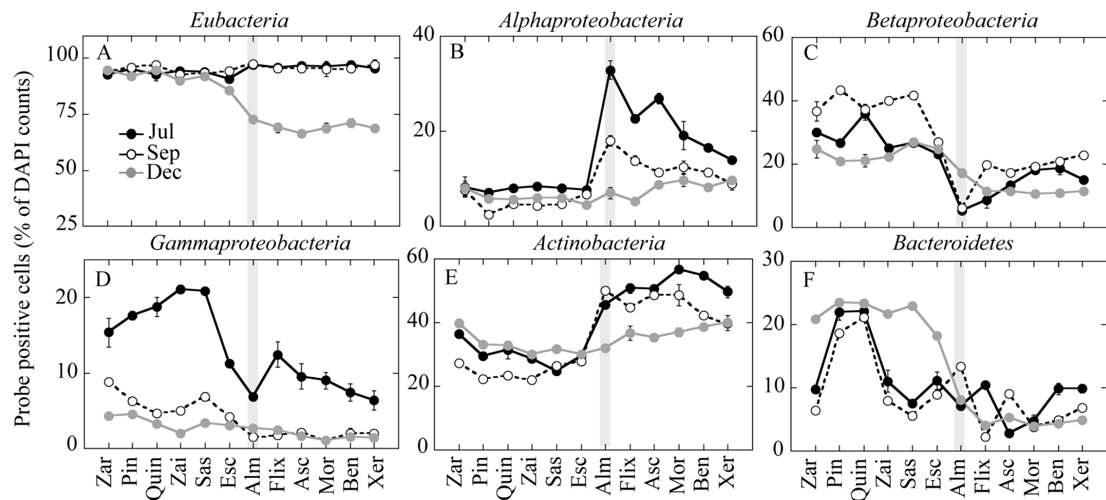

Fig. 4. Composition of the bacterial assemblages present at each site during the three samplings, (A) July, (B) September and (C) December 2011. The relative abundances of each group were calculated with respect to total bacteria. Site acronyms as in Figure 1.

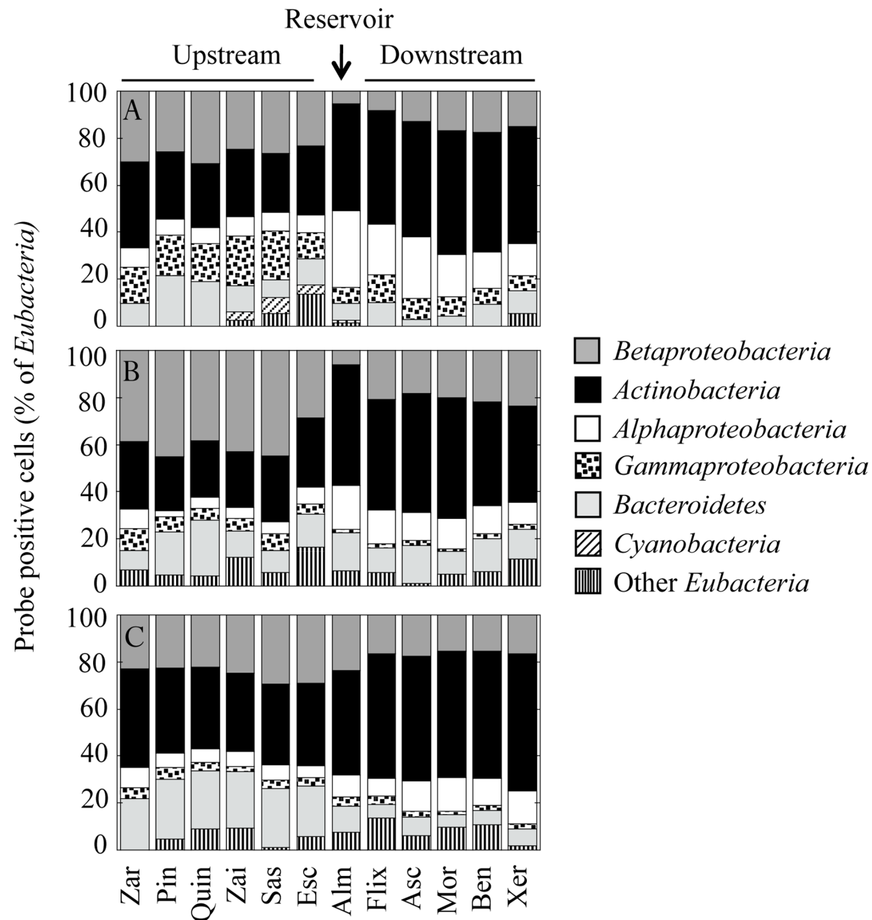

Fig. 5. Redundancy analysis (RDA) biplots. (A) Different bacterial groups (*Betaproteobacteria* [Bet]*, Alphaproteobacteria* [Alph]*, Gammaproteobacteria* [Gam]*, Bacteroidetes* [Bctd]*, Actinobacteria* [Act] and *Eubacteria [Eub*]) in relation to the gradient of the strongest environmental variables: DIN, DOC, Temp (temperature) and Cond (Conductivity). (B) Different samples in relation to the strongest environmental variables. Axis 1 and 2 explain 45% and 13% of the variance, respectively. Site names (as in Figure 1) contain the season (J: July, black dots; S: September, open dots; D: December, grey dots). Upstream sites (grey circles) and downstream sites (dashed circles) are also indicated.