Title
Exploring the links between antibiotic occurrence, antibiotic resistance, and bacterial communities in water supply reservoirs

Author affiliation
Belinda Huerta[a], Elisabet Marti[a], Meritxell Gros[a], Pilar López[b], Marcelo Pompêo[c], Joan Armengol[b] , Damià Barceló[a,d], Jose Luis Balcázar[a], Sara Rodríguez-Mozaz[a], Rafael Marcé[a]

[a] *Catalan Institute for Water Research (ICRA), Emili Grahit 101, 17003 Girona, Spain*
[b] *FLUMEN, Department of Ecology, University of Barcelona, Diagonal 643, 08028 Barcelona, Spain*
[c] *Department of Ecology, USP-IBR, R. do Matão, Travessa 14, 321, Butantã, São Paulo, Brasil*
[d] *Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain*

Corresponding author
Rafael Marcé
Catalan Institute for Water Research (ICRA), Emili Grahit 101, 17003 Girona, Spain
Telephone number (+34) 972183380
Fax number (+34) 972 183248
rmarce@icra.cat

Exploring the links between antibiotic occurrence, antibiotic resistance and bacterial communities in water supply reservoirs

ABSTRACT

Antibiotic resistance represents a growing global health concern due to the overuse and misuse of antibiotics. There is, however, little information about how the selective pressure of clinical antibiotic usage can affect environmental communities in aquatic ecosystems and which bacterial groups might be responsible for dissemination of antibiotic resistance genes (ARGs) into the environment. In this study, chemical and biological characterization of water and sediments from three water supply reservoirs subjected to a wide pollution gradient allowed to draw an accurate picture of the concentration of antibiotics and prevalence of ARGs, in order to evaluate the potential role of ARGs in shaping bacterial communities, and to identify the bacterial groups most probably carrying and disseminating ARGs. Results showed significant correlation between the presence of ARG conferring resistance to macrolides and the composition of bacterial communities, suggesting that antibiotic pollution and the spreading of ARG might play a role in the conformation of bacterial communities in reservoirs. Results also pointed out the bacterial groups *Actinobacteria* and *Firmicutes* as the ones probably carrying and disseminating ARGs. The potential effect of antibiotic pollution and the presence of ARGs on the composition of bacterial communities in lacustrine ecosystems prompt the fundamental question about potential effects on bacterial-related ecosystem services supplied by lakes and reservoirs.

Keywords: Bacterial communities, Pharmaceuticals, Pollution, RDA

1. INTRODUCTION

One of the most significant groups of pollutants within the category of emerging contaminants is antibiotics (Schriks et al., 2010). Antibiotic compounds may be found in different environmental compartments due to their extensive use in human and veterinary medicine and the continuous drainage of surface runoff and release from wastewater treatment plants (WWTP). The presence of these compounds in freshwater systems has attracted increasing attention because of their potential threat to the environment (Gros et al., 2009; Kim and Carlson, 2007). Antibiotics may pose a risk to the ecosystems even at very low concentrations and persistence rates, as they are designed to have a biochemical effect in the microorganisms, and thus have a significant impact in the processes controlled by native biological communities (Maul et al., 2006; Muñoz et al., 2009). To date most research on the impact of antibiotic discharges into the environment are based on the effects on benthic invertebrates and algae (Cleuvers, 2003; Fent et al., 2006). However, little is known about the potential effects on the diversity and functioning of bacterial communities in lacustrine ecosystems, despite its fundamental role as regulators of the processes that define the majority of ecosystem services provided by these freshwater bodies, such as the capacity for self-purification (Bell et al., 2005).

One of the greatest concerns about the presence of antibiotics in the environment is the emergence and dissemination of antibiotic resistance genes (ARGs) due to continuous exposure of bacteria, even at very low antibiotic concentrations (Baquero et al., 2008; Kümmerer, 2009a; Kümmerer, 2009b; Martinez, 2009; Zuccato et al., 2010). Many of these genes are found on transposons, integrons or plasmids, which can be mobilized and transferred to other bacteria, belonging to the same or different species (Allen et al., 2010). Although, antibiotic resistance development has been extensively investigated in cultivable bacteria, especially those of clinical importance (D'Costa et al., 2007), the vast majority of environmental bacteria cannot be cultured using standard laboratory methods (Amann et al., 1995; Kirk et al., 2004; Oliver, 2010). As a result, there is little information about how the selective pressure of clinical antibiotic usage can affect environmental communities (D'Costa et al., 2011; Martinez, 2009) and which bacterial groups might be responsible for dissemination of ARGs in the environment (Allen et al., 2010; Li et al., 2011; Zhang et al., 2009). This is particularly relevant in the case of reservoirs used for abstraction of drinking water and recreational activities, two principal routes for resistant environmental bacteria and ARGs to reach human populations (Taylor et al., 2011). Water supply in Mediterranean countries is frequently based on resources stored in reservoirs located downstream from populated areas, defining these systems as ideal study sites to investigate the effect of antibiotic pollution on natural microbial communities.

The objective of this study was to assess whether the occurrence of antibiotic compounds and ARGs can have a role in the composition of microbial communities from the water column and sediments of three water supply reservoirs (La Llosa del Cavall, Sau, and Foix) located near Barcelona, Spain. Concentrations of nine antibiotic families in water and sediment samples were measured by ultra-high-performance liquid chromatography coupled to mass spectrometry. Real-time PCR was applied for the quantification of four selected ARGs, whereas pyrosequencing was used to analyze the bacterial community composition. This combined approach allowed to draw an accurate picture of the concentration of antibiotics and prevalence of ARGs in the studied reservoirs, to evaluate the associations between ARGs and bacterial communities, and to identify the bacterial groups most probably carrying and disseminating ARGs.

2. MATERIALS & METHODS

2.1 Sampling and *in situ* measurements

Three man-made reservoirs located in Catalonia (Spain) were chosen considering their role in water supply, irrigation, and recreational uses, as well as the intensity of human activity in the upstream watershed. La Llosa Reservoir is located in the headwaters of the Cardener River, and stores 79 hm^3 of water (maximum depth 72 m, mean water residence time (WRT) 333 days) devoted mainly to water supply and irrigation. Sau Reservoir (volume 164 hm^3, maximum depth 50 m, WRT 113 days) is located in a middle stretch of the Ter River, and is a fundamental player in the water supply scheme of the Barcelona Metropolitan Area. Finally, Foix Reservoir is a small reservoir (volume 3.7 hm^3, maximum depth 11 m, WRT 106 days) located in the Foix River devoted to irrigation of local fields and hosts a number of recreational activities. These artificial reservoirs present an increasing pollution gradient from La Llosa Reservoir, which is near pristine conditions, to Foix Reservoir, which is a highly polluted water

body, while Sau Reservoir is in an intermediate situation. The main stressor defining the pollution gradient observed in the reservoirs is the impact of effluents from upstream WWTP: in Foix Reservoir the mean percent of water entering the reservoir that comes from upstream WWTP effluents was 60% for the last decade, in Sau Reservoir was 10%, and in La Llosa Reservoir was 0.3%.

Sampling campaigns were performed in May 2010 and May 2011. Samples were taken at three different points in each reservoir: near the main inflow (tail), in middle reach (mid), and near the dam. The rationale behind sampling in these three different points along the reservoirs is that the input of materials from the river promotes the establishment of heterogeneous bacterial communities along the reservoir (Šimek et al., 2011). During the sampling campaigns, reservoirs were stratified, with surface water temperature around 15 ºC and the bottom water around 8 ºC.

In the three sampling points visited in each reservoir a surface water sample (i.e. 0.5 m depth) was collected with a 5 L hydrographic bottle (except for LC-MS/MS analysis, where an amber glass bottle was used), and a sample from the top 4 cm of the sediments by means of a Ekman-Birge bottom sampler (Hydro-Bios). In the sampling point near the dam an additional water sample one meter above the sediments was collected (i.e., four water samples and three sediment samples for each reservoir in total). ARGs analyses and bacterial community characterization were performed only for samples collected in 2011. At every sampling station profiles of water temperature, conductivity, pH, and dissolved oxygen were measured with a SeaBird 19*plus* SEACAT CTD Profiler. The amount of light down the profiles was assessed by means of a Li-COR spherical Quantum Sensor (LI-193SA), and the light extinction coefficient calculated assuming the Beer-Lambert law. Hydrological data for the reservoirs were supplied by the Catalan Water Agency (ACA).

2.2 General parameters

Water samples were filtered through GF/F filters and analyzed for nitrate and chloride by liquid chromatography in a Konik KNK 500-A, and for soluble phosphate (Murphy and Riley, 1962) and ammonia (Solórzano, 1969) by a colorimetric method. Dissolved organic carbon concentration was quantified by combustion in a Shimadzu TOC-5000 Analyzer after filtration through a GF/F filter and acidification to remove inorganic carbon. Suspended solids were calculated by gravimetry. Total dissolved iron and manganese were measured through the phenantroline method and the persulfate method, respectively (APHA, 1992). Density of live bacteria was assessed after staining water samples with Live/Dead(R) BacLightTM bacterial viability kit. For the top 4 cm of the sediment samples, concentrations of As, Ba, Cd, Ce, Co, Cr, Cu, Ga, Mn, Mo, Nb, Ni, Pb, Rb, Sn, Sr, Th, Ti, V, W, Y, Zn, Zr, and Fe were determined through X-ray fluorescence (Model: Axios advanced-Panalytical) after adhesion using Elvactite 2044 and posterior pressing.

2.3 Antibiotic analysis

A Baker vacuum system (J.T. Baker, The Netherlands) was used to pre-concentrate the water samples using an adapted method (Gros et al., 2012). Briefly, water sample was pH-adjusted to 3 with 1.0 M HCl and 10 ml of 1 M EDTA (4 %, v/v) was added. Oasis HLB cartridges (60 mg, 3ml) cartridges (Waters, Massachusetts, USA) were loaded

with 250 mL of water samples. Cartridges were eluted with 10 ml of methanol. The extracts were evaporated under a gentle nitrogen stream and reconstituted with 1 mL of methanol - water mixture (50:50, v/v). A detailed description of the sediment extraction method can be found elsewhere (Yang et al., 2010). Briefly, 1 g of freeze-dried sediment was sonicated for 15 minutes in 10 mL of 0.2 M citric buffer (pH 4.0) and acetonitrile solution (50:50, v/v). Each extract was centrifuged and the supernatant diluted to 500 mL and purified with SPE following the same procedure as water samples. Extracts were analyzed using the method developed by Gros et al. (Gros et al., 2013) using a UPLC Waters chromatographic system (Milford, MA, USA) coupled to a Qtrap 5500 (Applied Biosystems) mass spectrometer. Chromatographic separation was achieved with an ACQUITY UPLC HSS T3 column (2.1 x 50 mm, particle size 1.8 µm) at 30 °C, supplied by Waters (Milford, MA, USA). Recoveries for the analyzed antibiotic compounds were 50 - 133 % for water samples, and 33 - 101 % for sediment samples.

2.4 Quantification of ARGs by real-time PCR

Water samples were filtered through 0.22µm nylon filters (Millipore) in order to concentrate bacterial cells. The membranes were then resuspended in lysis buffer (1.2% Triton X-100, 20 mM Tris-Cl, 2 mM sodium EDTA, and 20 mg/ml), and DNA was extracted using a standard phenol-chloroform method. Sediment samples were also collected, weighed, and homogenized in phosphate-buffered saline (PBS; 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.2). The homogenates were then resuspended in lysis buffer and DNA was extracted as mentioned above. All samples were collected and analyzed in duplicate.

The ARGs considered in this study were *bla*TEM, *ermB, qnrS*, and *sulI*, which confer resistance to β-lactam antibiotics, macrolides, quinolones (including fluoroquinolones) and sulfonamides, respectively. ARGs were quantified in all DNA extracted samples using real-time PCR. Copy number of the 16S rRNA gene was also quantified for normalization of the data. All real-time PCR assays were amplified in duplicate using the Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent Technologies) on a MX3005P system (Agilent Technologies), with the exception for the *bla*TEM gene, which was amplified using the SYBR® Green Master Mix (Applied Biosystems). The primers sets and the thermal cycling parameters were specific for each gene (Table A.1). After the PCR, a dissociation curve (melting curve) was constructed in the range of 60 ºC to 95 °C. *Escherichia coli* strain 488 (*bla*TEM), *Enterococcus* sp. strain CO4097 (*ermB*), *E. coli* strain J53 pMG306 (*qnrS*) and *Aeromonas* sp. strain P2G1 (*sulI*) were used as positive controls to make the standards by transforming gene-bearing plasmids into *Escherichia coli* using the TOPO cloning kit (Invitrogen). Plasmids were then extracted using the PureLink plasmid kit (Invitrogen), and the concentration was determined using a NanoDrop spectrophotometer (NanoDrop). Ten-fold serial dilutions of purified plasmid DNA were used for the generation of standard curves for each ARG. Gene copies were calculated as described previously (Marti and Balcazar, 2012).

Data (copy number of each ARG) were compared using the one-way analysis of variance (ANOVA) or Kruskal-Wallis test as appropriate, because most data were not normally distributed. Data were analyzed using SPSS for Windows version 17.0 (SPSS, Chicago, IL).

2.5 Analysis of bacterial community composition by pyrosequencing

Purified DNA samples from each sampling point were amplified separately with primers 27F (3'-GAG TTT GAT CNT GGC TCAG-5') and 519R (3'-GTN TTA CNG CGG CKG CTG-5'), and the amplicons were sequenced using Roche 454 GS FLX Titanium chemistry. Raw sequencing reads were quality trimmed using the MOTHUR software package (Schloss et al., 2009). Sequences were restricted to the first 250 bp and aligned using the SILVA reference database (Pruesse et al., 2007). Sequences were then randomly removed from each library to retain a total of 10855 (water samples) and 6058 (sediment samples) sequences for each treatment in order to minimize any bias due to the difference in the total number of sequences. The Ribosomal Database Project (RDP) pipeline and Classifier function (Wang et al., 2007) were used to align and assign identities at a confidence threshold of 80%. MOTHUR was used to assign sequences to operational taxonomic units (OTUs, 97% similarity) and calculate both Shannon's diversity index values (H') and Chao1 richness estimates. The parsimony test, as implemented by MOTHUR, was used to assess whether two or more communities have the same structure. A Bonferroni correction was applied to adjust the significance level for multiple pairwise comparisons. The sequence data from this study have been deposited in the NCBI Short Read Archive under accession number SRA049379.

2.6 Data analyses

To investigate the generality of longitudinal patterns in reservoirs, mean normalized concentrations of antibiotics were calculated to be able to combine data from the different reservoirs in common analyses and figures. Antibiotic concentrations along a given reservoir were normalized by the corresponding average of the whole system, and then data from all reservoirs were used to calculate cross-system means and standard deviations for the tail, mid, and dam regions (water and sediments treated separately). The same procedure was used to calculate mean normalized ARG abundances.

To search for potential links between antibiotic pollution, ARGs, and bacterial communities, a series of Redundancy Analysis (RDA)-based analyses was performed. First, the relative importance of environmental variables (including antibiotic concentrations), space (geographic coordinates) (Van der Gucht et al., 2007), and the presence of ARGs in explaining the bacterial communities in the water column and sediments, separately, was determined. The total variation of the bacterial community matrix was decomposed into unique environmental, spatial, and ARG components with corresponding *p* values using RDA and partial RDA (Borcard et al., 1992; Cottenie et al., 2003; Legendre and Legendre, 1998). This analysis measures the amount of variation that can be attributed exclusively to one set of explanatory (environment, space, or ARGs) variables and the variation explained by correlations between the different sets (shared or common term). The significance of these components was evaluated with a Monte Carlo permutation test (Legendre and Legendre, 1998), and the variation components were bias-corrected (Peres-Neto et al., 2006). Although the set of samples for the analyses was limited (n=12 for water samples, n=9 for sediments), RDA and partial RDA included in the variation analyses never used a number of explanatory variables in excess of n/2 (Borcard et al., 2011) in the case of water samples, and only one RDA failed to fulfill this condition for the sediment set. This was accomplished reducing the number of explanatory variables in each set to the most parsimonious subset of significant variables with a forward selection procedure. This procedure also

prevented over-fitting and co-linearity. The bacterial community data consisted in percentages to avoid the deleterious effect of the different density of bacteria on the analyses, while all explanatory variables were log transformed and standardized. No significant eigenvector or polynomial spatial variable was found, hence only the geographic coordinates were considered in the analyses.

The association between antibiotics and ARGs in the water column and sediments was assessed performing two separate RDA using as explanatory variables the corresponding set of environmental variables measured in this study. Due to the large number of potential explanatory variables, the most parsimonious subsets of significant variables were selected with a forward selection procedure. The association between ARGs and bacterial groups in water and sediments was also tested through RDA, using the ARGs data as the explanatory matrix. Forward selection was used as above to avoid over-fitting and co-linearity. All RDA and partial RDA included in this study were performed with R (R Development Core Team, 2007) and the Vegan library (Oksanen et al., 2007).

3. RESULTS

3.1 Pollution gradient

The general physico-chemical characterization (Table A.2) allowed us to confirm a clear eutrophication and pollution gradient from La Llosa to Foix. Water samples for Foix Reservoir showed extremely high values for ammonia, phosphate, and metals. Nonetheless, maximum values for the light extinction coefficient were very high at Foix Reservoir, according to the largest levels of suspended solids. Chloride concentration, a variable closely related to pollution in reservoirs (Marcé et al., 2008), was also largest in Foix Reservoir. On the contrary, in the case of sediment samples, results did not support the existence of an obvious pollution gradient in this compartment (Table A.2). Among elements that can be potentially attributed to human pollution, only Cu, Pb, and Zn showed mild gradients with largest values at Foix Reservoir.

3.2 Antibiotic occurrence

Antibiotic levels at the water column reached their maximum in Foix Reservoir, where the most abundant antibiotic families found were macrolides and sulfonamides, with erythromycin and sulfamethoxazole as the main family representatives, respectively (Fig. 1a-f, Table A.3). In contrast, very low concentrations were found in water from Sau and La Llosa Reservoirs, where maximum concentrations were in the low ng L^{-1} range. The most prevalent antibiotic compound in these two reservoirs was sulfamethozaxole, present in all the water samples from Sau Reservoir and four samples out of twelve from La Llosa. Antibiotic concentrations in sediment samples were slightly higher in Foix Reservoir (Fig. 1g-l, Table A.4), but this tendency was less evident than in water samples. Whereas macrolides followed the same occurrence pattern found in water, sulfonamides and tetracyclines showed a different behavior, with relatively constant concentrations across reservoirs (Huerta et al, in preparation).

Antibiotic concentration along the water body of all reservoirs sampled in this study (Fig. 2a-f) presented two distinct situations: a) the compounds tetracycline,

sulfamethoxazole, and erythromycin showed stable concentrations along the water body, a similar result to that found for chloride, a conservative ion often used as a tracer, indicating that these substances did not experienced major transformations during transport along reservoirs; whereas b) ciprofloxacin and ofloxacin seemed to be influenced by attenuation processes, as their concentrations decreased significantly from the tail to the dam. Considering the dynamics of chloride, this reduction in the concentrations cannot be attributed to dilution inside the reservoir but to other biotic or abiotic processes such as sorption in particulate matter and photo and biodegradation. Greater variability within each reservoir was present in sediment samples (Fig. 2g-k), where the fluoroquinolones ciprofloxacin and ofloxacin were absent.

3.3 Prevalence of antibiotic resistance genes (ARG)

Quantitative analysis of ARGs in water samples showed that *qnrS* and *ermB* genes had a higher copy number in Foix Reservoir than in Sau and La Llosa del Cavall reservoirs (Fig. 1m-p). In addition, the abundance of the *sul*I gene was significantly higher ($p<0.05$) in water samples from Foix and Sau reservoirs compared with the abundance in La Llosa del Cavall Reservoir. However, no significant differences ($p>0.05$) in abundance of the *bla*TEM gene was found among reservoirs.

For sediment samples, only the abundance of the *sul*I gene was significantly greater ($p<0.05$) in Foix Reservoir compared to the other two reservoirs (Fig. 1q-r). The *qnrS* gene was only detected in sediment samples collected from Foix and Sau reservoirs. However, there was no significant difference ($p>0.05$) in copy number between the two reservoirs. The *bla*TEM and *ermB* genes showed similar copy number in sediment samples from the three reservoirs.

Although the normalized concentration of ARGs in water samples collected at the tail of the reservoirs tended to be larger than in mid and dam regions (Fig. 2 l-s), the differences did not reach the level of statistical significance, with the exception of the *qnr*S gene whose concentration was significantly higher ($p<0.05$) at the tail. A higher concentration of ARGs was indeed observed in sediment samples collected at the tail stations, with the exception of the *qnr*S gene whose concentration did not show any significant difference among sampling points.

3.4 Bacterial community composition

Phylum level affiliations of sequences encountered in each sampling point revealed a diverse bacterial community. The relative abundance of bacterial communities at the phylum level (classes for *Proteobacteria*) showed that the dominant bacterial groups in water samples were *Actinobacteria*, *Betaproteobacteria*, and *Bacteroidetes* (Fig. 3), except for the tail of Foix Reservoir where *Actinobacteria* and *Gammaproteobacteria* were dominant. The bacterial community composition in sediment samples was more variable among the different sampling points (Fig. 3), as measured by the Shannon diversity index and Chao1 richness estimator (Tables A.5-A.8). The dominant bacterial groups in sediment samples were *Actinobacteria, Betaproteobacteria*, and *Gammaproteobacteria* in La Llosa del Cavall Reservoir; *Cyanobacteria, Firmicutes,* and *Planctomycetes* in Sau Reservoir; and *Actinobacteria*, *Firmicutes*, and *Planctomycetes* in Foix Reservoir. A high proportion of unclassified bacteria (mean value for all the sediment samples = 17.41 ± 6.63, $n = 9$) was also observed.

Despite the high degree of similarity shared among the bacterial communities from each sampling point at the phylum level, differences with respect to community structure and composition became apparent at the genus level (97% similarity). Pairwise comparison of each bacterial community harbored a phylogenetic structure that was significantly different ($p<0.01$) from each other, as determined by the phylogeny-based parsimony test (Tables A.5 and A.6). Only water samples collected at the tail, mid, and dam sites from La Llosa del Cavall Reservoir did not show any significant difference in the bacterial community structure.

3.5 Links between antibiotics, ARGs, and bacterial communities

A variation partitioning analysis was performed to identify the environmental and spatial factors that best explain the disparity in bacterial communities found in water and sediment samples (see section 2.6). The analysis included the geographic location of sampling points, the environmental variables measured in this study (including antibiotic concentration), and the ARG abundance data as a third additional descriptor group. As for water samples, the analysis explained the 80% of the variation in the bacterial community data, and yielded 19% of total variance explained by ARG alone, 17% by environmental variables alone, 11% by geographical location alone, and 33% by a common effect (Fig. 4a and Table A.9). In particular, the variables that significantly contributed to explain the overall bacterial community patterns where: geographic location (x coordinate), water temperature, pH, light extinction coefficient, and normalized *emr*B and *bla*TEM copy numbers. In the case of sediment samples, the analysis explained the 74% of the variation of the bacterial communities, but we could not identify any significant isolated component (Fig. 4b and Table A.9). This was most probably because the explanatory matrices were highly correlated, as showed by the large shared component (69%). Geographical location was not significant in this case, being the variables that significantly contributed to explain the overall bacterial community patterns: W, Rb, and Mn concentration, and normalized *emr*B and *sul*I copy numbers.

Potential links between the environmental conditions and the abundance of ARGs in water and sediment samples were explored performing a RDA, using the environmental variables (including antibiotic concentration) as an explanatory matrix. In the case of water samples, the analysis explained 85% of the variability of the ARGs data (Fig. 4c, $p<0.0001$), with a disproportional large effect of macrolides concentration (adjusted $r^2=0.64$, $p=0.001$) and minor contributions of density of live bacteria (adjusted $R^2=0.15$, $p=0.001$) and the light extinction coefficient (adjusted $r^2=0.06$, $p=0.001$). It was noteworthy the association between macrolides concentration and normalized *erm*B copy numbers (Pearson's $r^2=0.77$, $p=0.0002$, $n=12$), and between the density of live bacteria and normalized *sul*I (Pearson's $r^2=0.54$, $p=0.0067$, $n=12$). For the sediments, the analysis explained 50% of the variability of the ARG data (Fig. 4d, $p=0.016$), with a significant effect of macrolides concentration (adjusted $r^2=0.28$, $p=0.036$) and Cd concentration (adjusted $r^2=0.18$, $p=0.044$). Macrolides concentration correlated positively with *sul*I copy number (Pearson's $r^2=0.52$, $p=0.0293$, $n=9$), while Cd concentration was inversely related to *qnr*S (Pearson's $r^2=0.51$, $p=0.0308$, $n=9$).

Finally, associations between ARGs and bacterial groups in water samples were assessed performing a RDA using the ARGs data as the explanatory matrix (Fig. 4e).

The analysis explained 52% of the variability of the bacterial community data ($p<0.0027$), with the *bla*TEM (adjusted $r^2=0.40$, $p=0.011$) and *erm*B (adjusted $r^2=0.12$, $p=0.016$) genes as significant explanatory variables. Although the RDA suggested a positive association between normalized *erm*B copy numbers and the percentage of *Firmicutes*, as well as between the *bla*TEM gene and the percentage of *Actinobacteria*, paired Pearson's correlations did not show any significant result. Remarkably, in RDA analysis for the sediment samples, the bacterial groups related to ARGs in water samples were also involved in main associations found in the sediments (Fig. 4f). Explained variability was 69% ($p<0.0001$), with the *erm*B (adjusted $R^2=0.57$, $p=0.002$) and *sul*I (adjusted $r^2=0.12$, $p=0.019$) genes as significant predictors. The *erm*B gene strongly and significantly correlated with the percentage of *Actinobacteria* (Pearson's $r^2=0.88$, $p=0.0002$, $n=9$), as well as the *sul*I gene with *Firmicutes* (Pearson's $r^2=0.61$, $p=0.0129$, $n=9$).

4. DISCUSSION

Significant associations between the ARGs and bacterial community composition in three water supply reservoirs were detected. Although a correlation exercise cannot confirm the hypothesis that antibiotic occurrence and ARGs is shaping the bacterial communities in the reservoirs sampled, results suggested that this is the most plausible explanation for the associations found. A potential alternative explanation is that correlations were spurious, that is, promoted by an unnoticed common cause. This common cause might be the effect of WWTP effluents containing resistant bacteria (Pruden et al., 2006), which means that all associations found between ARGs and bacterial communities were just promoted by the fact that both bacteria and ARGs are released from upstream WWTPs. Although this may be the main explanation for the presence of the detected ARGs in the resistome of the bacterial communities, this cannot explain associations between ARG and bacterial communities found in this study considering the WRT of the water bodies under investigation. Since WRT in the reservoirs is in the order of months, the bacterial communities are the result of the complex ecological interactions (e.g., competence, predation) among bacteria and the other components of the food web after presumably hundreds or thousands of bacterial live cycles. Although it is reasonable to think that the presence of ARGs in the resistome can be facilitated by direct pollution from WWTP effluents, the most plausible explanation for the associations found between ARGs and the bacterial communities is that ARGs and the resistant bacteria spread in the bacterial community facilitated by the presence of the selective agent (antibiotic).

The association between ARGs and the bacterial communities was more evident in the water column, where ARGs was the unique variation component with the strongest explaining power (19%), despite the dominant role of the variation shared by the three explaining sets (33%). The variation shared by the different explaining sets precluded the detection of any significant unique component in the sediments, but considering the highly significant explanatory power of the ARGs data the effect of ARGs on the bacterial communities cannot be discarded (Table A.8). In any case, the pollution gradient in water samples was more evident than in the sediments, where differences in environmental variables and ARGs were less apparent. Normalized ARGs abundance in water and sediments from the same sampling site were closely related (Figure A.1), suggesting a common process, with the only exception of *qnrS* (related to

fluoroquinolones, the antibiotic family with the most variable concentration in water and absent in the sediment).

Maximum antibiotic concentrations found in this study are frequently reported in systems affected by effluents of wastewater treatment plants (Zhou et al., 2011). In this case, the pollution gradient between reservoirs was mainly determined by the wastewater contribution to the total inflow to the reservoir, which peaked at Foix Reservoir (average annual contribution of 31 %). Some studies have shown that exposure to highly antibiotic-contaminated effluent promotes resistance genes in environmental bacterial communities (Kristiansson et al., 2011), but other studies suggest that levels of antibiotics below the minimum inhibitory concentration (MIC) can also select resistant bacteria (Gullberg et al., 2011). Thus, even though the levels of antibiotics found in this study are, as expected, far below the MIC for bacteria, it cannot be excluded that they might exert a selection pressure enough to maintain the resistome in environmental communities.

Highest concentrations of antibiotics corresponded to macrolides and sulfamides (water), and tetracyclines (sediment), in similar levels (ng/L to μg/L) to those found in some studies carried out in river water and sediments (Tong et al., 2009; Yang et al., 2010). Although RDA identified macrolides concentration as a principal variable for explaining the variability of ARG in both, water and sediment samples, antibiotic concentration was not a significant variable in variation analyses of the bacterial communities. However, this might be a consequence of the incapability of the analyses to assess the presence of all antibiotic compounds present in the samples, including metabolites and transformation products that can have similar or even higher biological activity (Boxall et al., 2004). Thus, the ARG data may be considered as an integrative proxy of the presence of a complex mixture of antibiotic pollutants, better reflecting the impacts of pollution on the bacterial communities. Metals can also be responsible for the presence of ARG due to co-resistance mechanisms (Baker-Austin et al., 2006; Knapp et al., 2011), although no positive association between ARG and concentration of metals was found in this study. However, the presence of metals could be a factor correlated with the transport and accumulation of antibiotics. For instance, fluoroquinolones are known to form complexes with some iron oxides, which suggest a tendency to adsorb onto clay minerals (Gu and Karthikeyan, 2005). Also, the adsorption of tetracyclines on sediments strongly depends on the pH and presence of metals (Zhang et al., 2011). Values of metals in the target reservoirs showed a maximum of fifty-fold higher concentration of Fe^{2+} in water from Foix than from La Llosa Reservoir, what could explain the higher concentration of antibiotics in the sediment of Foix Reservoir, as a result of not only the greater antibiotic concentration but also other pollutants that might increase antibiotic residence time in the system.

One of the most relevant results from this study is that two bacterial groups associated to the presence of ARGs in both water and sediments were identified as *Actinobacteria* and *Firmicutes*. This is not unexpected, because some members of *Actinobacteria* and *Firmicutes* are producers of antibiotics (Allen et al., 2010; Kieser et al., 2000; Mannanov and Sattarova, 2001). Considering that analyses for the different compartments were independent, this suggests that these two groups may be related to the dissemination of ARGs in natural environments like reservoirs. This is in accordance with previous studies, showing that most resistant bacteria belong to Gram-positive group (Mindlin et al., 2008). These results are remarkable considering that

Actinobacteria was the bacterial group contributing the most to the bacterial communities sampled in this study (mean abundance = 22 ± 13 %), suggesting that indeed ARGs may be significantly linked to bacterial community composition in freshwaters affected by antibiotic pollution. The association between ARG and *Firmicutes* was less conclusive, because in some samples the relatively low number of *Firmicutes* may be explained as a residual population coming directly from the upstream WWTP effluents. If this were the case, the association would be entirely spurious, with no implications related to the dynamics of the natural community.

Although the strong and highly significant relationship found in the sediment samples between *Actinobacteria* and the *erm*B gene as well as between *Firmicutes* and the *sul*I gene seems to support an association between particular taxa and particular ARG, this information should be interpreted with caution. Several of the ARGs abundances were significantly correlated both, in water and sediments, and other ARGs not measured in this study might be present in the samples. Thus, the possibility that significant ARGs detected by RDA simply reflect the effect of non-measured ARGs, or that the uncertainty of the measurements resulted in the observed associations, while masking the causal relationships that would include different measured ARG, cannot be discarded. The fact that *Actinobacteria* is related with the *erm*B gene in sediments but with the *bla*TEM gene in the water column seems to point in this direction (the same reasoning is valid for *Firmicutes*, *sul*I, and *erm*B). The association between *Actinobacteria*, *Firmicutes* and the presence of ARGs can be considered robust, but the bacterial group in charge of carrying each ARG cannot be appointed. It is also difficult to ascertain whether other significant environmental and spatial variables in the analyses have a real effect on the bacterial communities or they simply reflect other environmental conditions, which are either not measured or confounded in spatial differences. The presence of significant variables like metals W or Rb concentration seems to point to the latter.

Although the correlations cannot confirm causal mechanisms, the significant role of macrolides, *erm*B, *Actinobacteria* and *Firmicutes* in independent analyses suggests that antibiotic pollution may play a role in the conformation of bacterial communities in reservoirs via the spreading of ARGs. Macrolides are the most prevalent antibiotic family measured in our samples, and the *erm*B gene is an ARG conferring resistance to, precisely, macrolides. Finally, the *erm*B gene had a significant role in explaining bacterial community variability in both water and sediment samples, and at least in sediments showed a clear relationship with abundance of *Actinobacteria*. In fact, a search using BLASTN (Altschul et al., 1990) for the matching sequences in the GenBank database including updates against the sequence for the *ermB* gene (GenBank accession no. AJ972606) as a query sequence revealed the presence of this gene in several bacterial species, including members of *Actinobacteria*, such as *Eggerthella* sp. (GenBank accession no. AP012211) and *Trueperella pyogenes* (GenBank accession no. AY334073). The spreading of the *ermB* gene may be facilitated due to the fact that it is associated with conjugative transposons located in chromosomes as well as on plasmids (Roberts, 2008). Again, although our results cannot prove the existence of such a causal chain, we can speculate about the existence of a selective pressure of macrolides on the bacterial communities, with spreading of ARGs (already present in the community or coming from ARGs pollution from WWTPs) as a convenient mechanism used by particular bacterial groups to out-compete others. But more research in needed to confirm this mechanism.

Although some studies already described impacts of antibiotics on the functionality of bacterial communities in aquatic environments (Bundschuh et al., 2009; Maul et al., 2006), this is one of the few studies to describe significant associations between antibiotic pollution, the presence of ARGs, and the composition of bacterial communities in lacustrine ecosystems. Moreover, the fact that the bacterial communities were analyzed by pyrosequencing avoided the bias obtained when evaluating antibiotic resistance in environmental microbial communities based only on cultivable bacteria. However, we can only speculate about the effects of the shifts of the bacterial communities promoted by antibiotic pollution and ARGs on the functionality of the ecosystem. While it is well known the principal role of bacteria in the ecosystem processes that define most of the ecosystem services provided by lacustrine ecosystems (e.g., organic matter degradation, denitrification), information to anticipate the impact of the combination of antibiotic pollution and the presence of ARGs on the bacterial communities is lacking. Are ARGs conferring resilience against the impacts of antibiotic pollution thus maintaining the community physiological profiles (i.e. the capacity to degrade a number of carbon sources) and growth indices, or some biogeochemical processes will be severely impacted by the community shift? In the light of these results, these kinds of questions deserve much more attention in the future.

Acknowledgments

We thank Carles Borrego, Enrique Navarro, and Veronica Ribé for helpful discussions and comments to the manuscript, and Jaime Ordóñez for help during field work. We also thank George A. Jacoby, Carmen Torres and Joan Jofre for providing us with bacterial strains. This research work was funded by the 1st Interdisciplinary Water Research Projects-ICRA Grants Program 2010 (Project RES^2), the Spanish Ministry of Economy and Competitiveness for its financial support through the project SCARCE (Consolider-Ingenio 2010 CSD2009-00065), and by the European Union through the European Regional Development Fund (FEDER). This work was also partly supported by the Generalitat de Catalunya (Consolidated Research Group: Water and Soil Quality Unit 2009-SGR-965). Prof. Barceló acknowledges King Saud University for his visiting professorship.

References

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. 2010; 8: 251-259.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990; 215: 403-10.

Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Mol. Biol. Rev 1995; 59: 143-169.

APHA. Standard Methods for Examination of Water and Wastewater, 18th ed., Washington, DC, 1992.

Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. Co-selection of antibiotic and metal resistance. Trends Microbiol. 2006; 14: 176-182.

Baquero F, Martínez J-L, Cantón R. Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol. 2008; 19: 260-265.

Bell T, Newman JA, Silverman BW, Turner SL, Lilley AK. The contribution of species richness and composition to bacterial services. Nature 2005; 436: 1157-60.

Borcard D, Gillet F, Legendre P. Numerical ecology with R. New York: Springer Science, 2011.

Borcard D, Legendre P, Drapeau P. Partialling out the Spatial Component of Ecological Variation. Ecology 1992; 73: 1045-1055.

Boxall ABA, Sinclair CJ, Fenner K, Kolpin D, Maund SJ. Peer Reviewed: When Synthetic Chemicals Degrade in the Environment. Environ. Sci. Technol. 2004; 38: 368A-375A.

Bundschuh M, Hahn T, Gessner MO, Schulz R. Antibiotics as a chemical stressor affecting an aquatic decomposer–detritivore system. Environmental Toxicology and Chemistry 2009; 28: 197-203.

Cleuvers M. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicology Letters 2003; 142: 185-194.

Cottenie K, Michels E, Nuytten N, De Meester L. Zooplankton metacommunity structure: regional vs. Local processes in highly interconnected ponds. Ecology 2003; 84: 991-1000.

D'Costa V, King C, Kalan L, Mora M, Sung W, Schwarz C, et al. Antibiotic resistance is ancient. Nature 2011.

D'Costa VM, Griffiths E, Wright GD. Expanding the soil antibiotic resistome: exploring environmental diversity. Curr. Opin. Microbiol. 2007; 10: 481-489.

Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals. Aquat. Toxicol. 2006; 76: 122-159.

Gros M, Petrovic M, Barceló D. Tracing Pharmaceutical Residues of Different Therapeutic Classes in Environmental Waters by Using Liquid Chromatography/Quadrupole-Linear Ion Trap Mass Spectrometry and Automated Library Searching. Anal. Chem. 2009; 81: 898-912.

Gros M, Rodríguez-Mozaz S, Barceló D. Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A 2013; doi: 10.1016/j.chroma.2012.12.072.

Gu C, Karthikeyan KG. Sorption of the Antimicrobial Ciprofloxacin To Aluminum and Iron Hydrous Oxides. Environ. Sci. Technol. 2005; 39: 9166-9173.

Gullberg E, Cao S, Berg OG, Ilback C, Sandegren L, Hughes D, et al. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. PLoS Pathog. 2011; 7: 9.

Kieser T, Bibb MJ, Buttner MJ, Chater KF, DA. H. Practical Streptomyces Genetics. 2nd ed.: Norwich: John Innes Foundation 2000.

Kim S-C, Carlson K. Temporal and Spatial Trends in the Occurrence of Human and Veterinary Antibiotics in Aqueous and River Sediment Matrices. Environ. Sci. Technol. 2007; 41: 50-57.

Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H, et al. Methods of studying soil microbial diversity. J. Microbiol. Methods 2004; 58: 169-188.

Knapp CW, McCluskey SM, Singh BK, Campbell CD, Hudson G, Graham DW. Antibiotic Resistance Gene Abundances Correlate with Metal and Geochemical Conditions in Archived Scottish Soils. PLoS ONE 2011; 6: e27300.

Kristiansson E, Fick J, Janzon A, Grabic R, Rutgersson C, Weijdegard B, et al. Pyrosequencing of Antibiotic-Contaminated River Sediments Reveals High Levels of Resistance and Gene Transfer Elements. Plos One 2011; 6: 7.

Kümmerer K. Antibiotics in the aquatic environment - A review - Part I. Chemosphere 2009; 75: 417-434.

Kümmerer K. Antibiotics in the aquatic environment - A review - Part II. Chemosphere 2009; 75: 435-441.

Legendre L, Legendre P. Numerical Ecology. New York: Elsevier, 1998.

Li D, Qi R, Yang M, Zhang Y, Yu T. Bacterial community characteristics under long-term antibiotic selection pressures. Water Res. 2011; 45: 6063-6073.

Mannanov RN, Sattarova RK. Antibiotics produced by Bacillus bacteria. Chem. Nat., Comp. 2001; 37: 117-123.

Marcé R, Moreno-Ostos E, López P, Armengol J. The Role of Allochthonous Inputs of Dissolved Organic Carbon on the Hypolimnetic Oxygen Content of Reservoirs. Ecosystems 2008; 11: 1035-1053.

Marti E, Balcazar JL. Real-Time PCR Assays for Quantification of qnr Genes in Environmental Water Samples and Chicken Feces. Appl Environ Microbiol 2012; 79: 1743-5.

Martinez JL. Environmental pollution by antibiotics and by antibiotic resistance determinants. Environ. Pollut. 2009; 157: 2893-2902.

Maul JD, Schuler LJ, Belden JB, Whiles MR, Lydy MJ. Effects of the antibiotic ciprofloxacin on stream microbial communities and detritivorous macroinvertebrates. Environ. Toxicol. Chem. 2006; 25: 1598-1606.

Mindlin SZ, Soina VS, Ptrova MA, Gorlenko Zh M. Isolation of antibiotic resistance bacterial strains from East Siberia permafrost sediments. Genetika 2008; 44: 36-44.

Muñoz I, López-Doval JC, Ricart M, Villagrasa M, Brix R, Geiszinger A, et al. Bridging Levels of Pharmaceuticals in River Water with Biological Community Structure in the Llobregrat River Basin (Northeast Spain). Environ. Sci. Technol. 2009; 28.

Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 1962; 27: 31-36.

Oksanen J, Kindt R, Legendre P, O'Hara R. Vegan: Community Ecology Package, 2007.

Oliver JD. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiology Reviews 2010; 34: 415-425.

Peres-Neto PR, Legendre P, Dray S, Borcard D. Variation partitioning of species data matrices: estimation and comparison of fractions. Ecology 2006; 87: 2614-2625.

Pruden A, Pei R, Storteboom H, Carlson KH. Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado. Environ. Sci. Technol. 2006; 40: 7445-7450.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, et al. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 2007; 35: 7188-96.

R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2007.

Roberts MC. Update on macrolide–lincosamide–streptogramin, ketolide, and oxazolidinone resistance genes. FEMS Microbiol. Lett. 2008; 282: 147-159.

Schloss P, Westcott S, Ryabin T, Hall Jr F, Hartmann M, Hollister E, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 2009; 75: 7537-7541.

Schriks M, Heringa MB, van der Kooi MM, de Voogt P, van Wezel AP. Toxicological relevance of emerging contaminants for drinking water quality. Water Res 2010; 44: 461-76.

Šimek K, Comerma M, García J-C, Nedoma J, Marcé R, Armengol J. The Effect of River Water Circulation on the Distribution and Functioning of Reservoir Microbial Communities as Determined by a Relative Distance Approach. Ecosystems 2011; 14: 1-14.

Solórzano L. Determination of ammonia in natural waters by the phenol hypochlorite method. Limnol. Oceanogr. 1969; 14: 799–801.

Taylor NGH, Verner-Jeffreys DW, Baker-Austin C. Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? Trends Ecol. Evol. 2011; 26: 278-284.

Tong L, Li P, Wang Y, Zhu K. Analysis of veterinary antibiotic residues in swine wastewater and environmental water samples using optimized SPE-LC/MS/MS. Chemosphere 2009; 74: 1090-1097.

Van der Gucht K, Cottenie K, Muylaert K, Vloemans N, Cousin S, Declerck S, et al. The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. Proc Natl Acad Sci U S A 2007; 104: 20404-9.

Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 2007; 73: 5261-7.

Yang J-F, Ying G-G, Zhao J-L, Tao R, Su H-C, Chen F. Simultaneous determination of four classes of antibiotics in sediments of the Pearl Rivers using RRLC-MS/MS. Sci. Total Environ. 2010; 408: 3424-3432.

Zhang X-X, Zhang T, Fang HP. Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. 2009; 82: 397-414.

Zhang Z, Sun K, Gao B, Zhang G, Liu X, Zhao Y. Adsorption of tetracycline on soil and sediment: Effects of pH and the presence of Cu(II). J. Hazard. Mater. 2011; 190: 856-862.

Zhou L-J, Ying G-G, Zhao J-L, Yang J-F, Wang L, Yang B, et al. Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. Environ. Pollut. 2011; 159: 1877-1885.

Zuccato E, Castiglioni S, Bagnati R, Melis M, Fanelli R. Source, occurrence and fate of antibiotics in the Italian aquatic environment. J. Hazard. Mater. 2010; 179: 1042-1048.

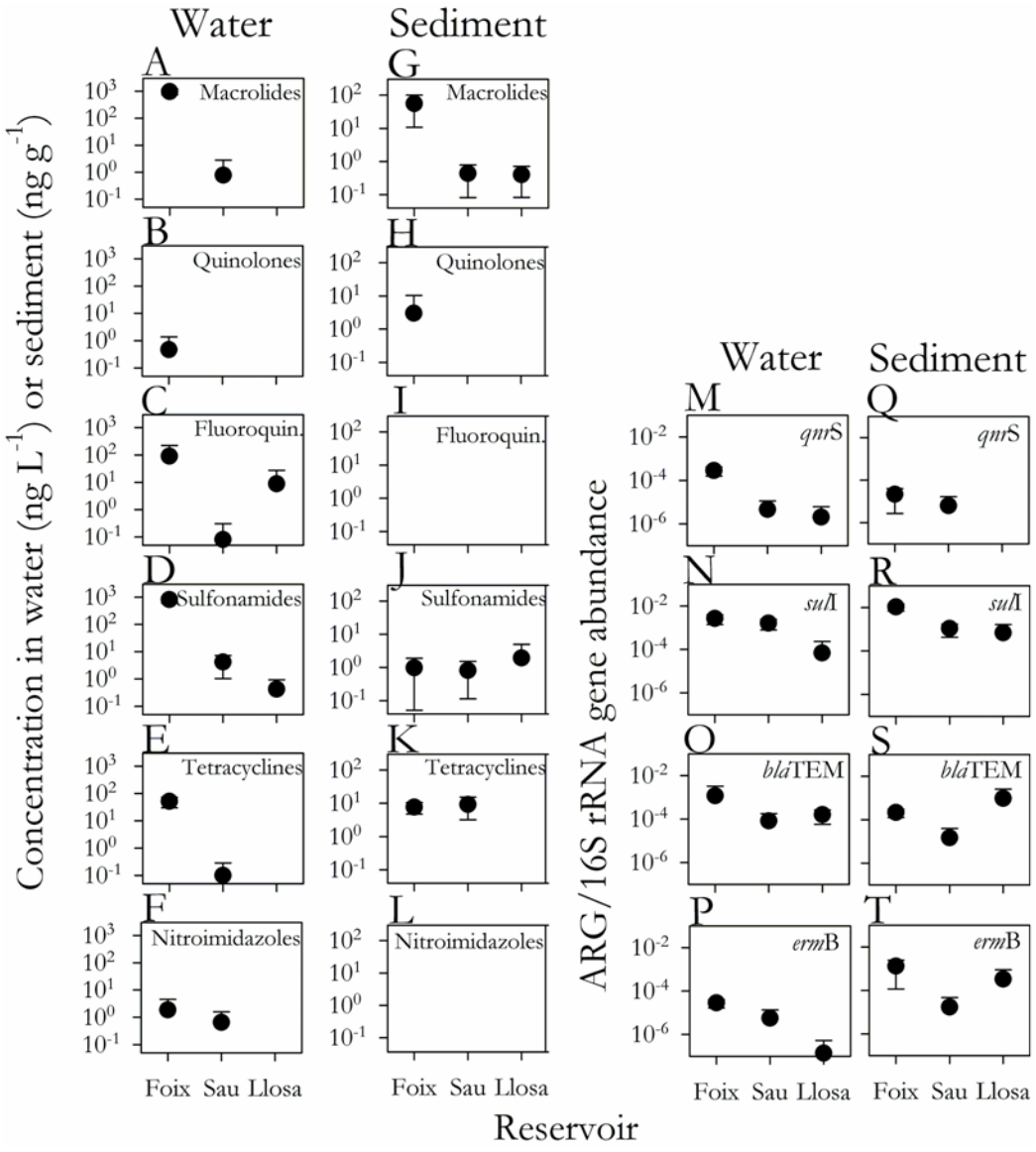
Figure Captions

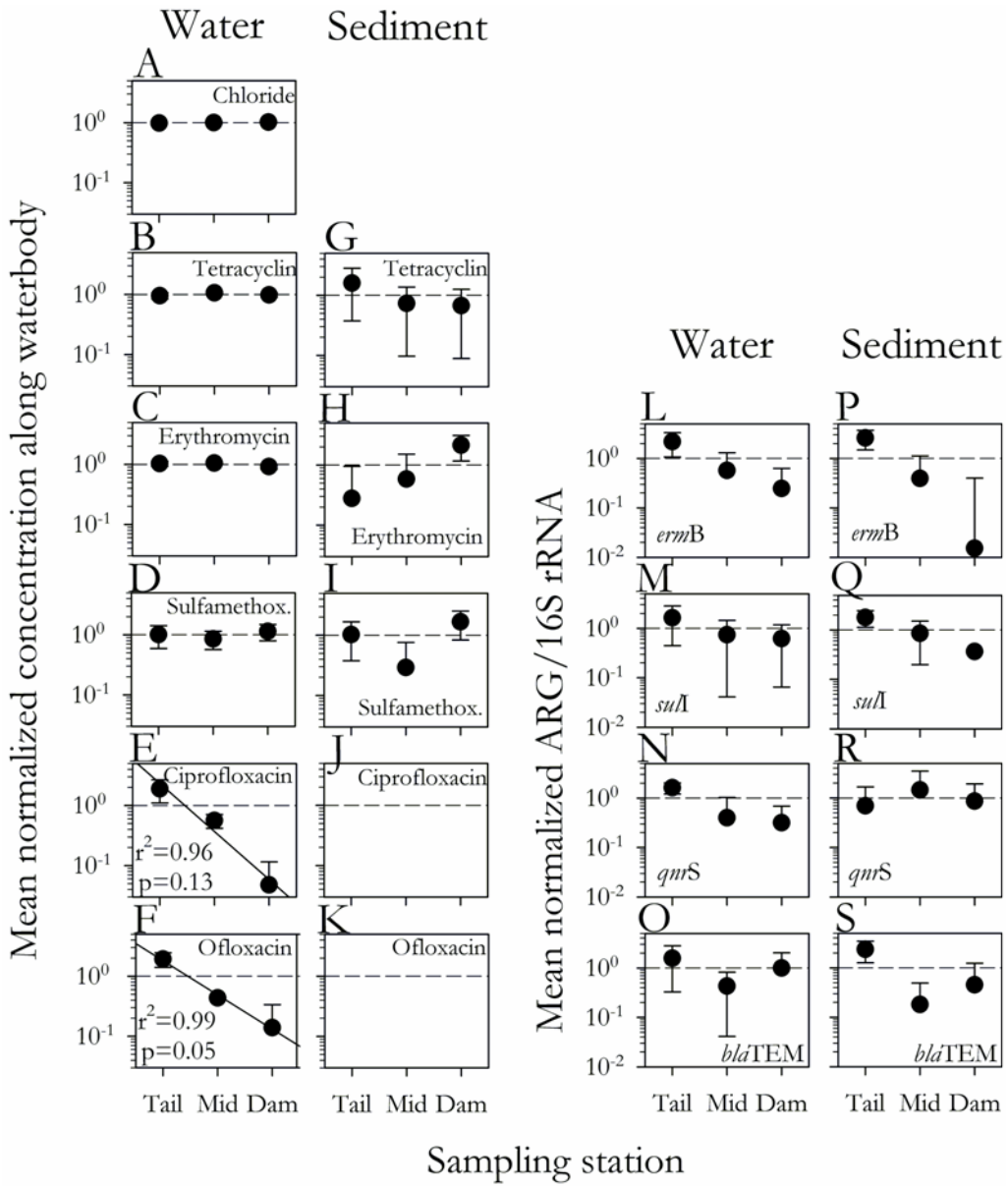
Fig. 1. Average antibiotic concentration and ARG abundance at the water column and sediments found in three water supply reservoirs. For each antibiotic family and reservoir, calculations include all concentrations measured above the limit of quantification from all sampling points. For each ARG and reservoir, calculations included all sampling points. Missing points indicate that the antibiotic family or ARG was not detected. Bars denote standard deviations, and some were not plotted because they extended beyond zero in the logarithmic axis.

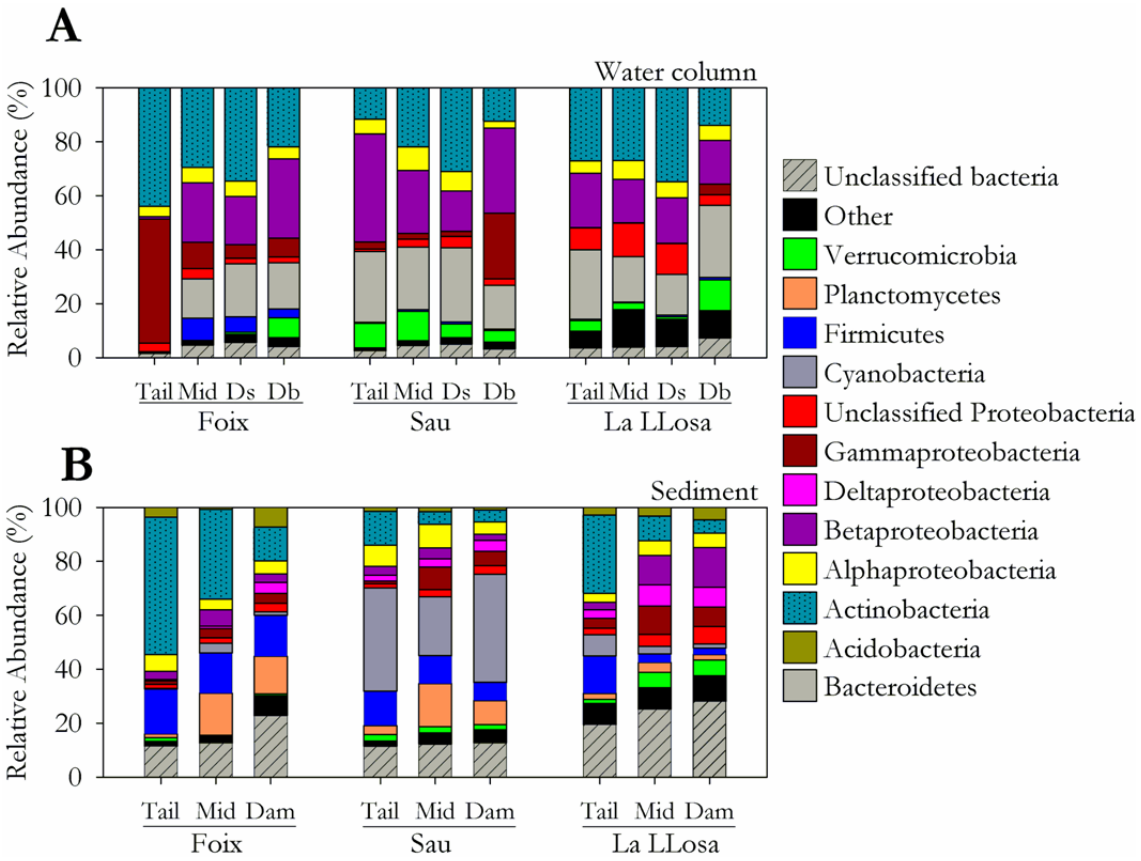
Fig. 2. Occurrence of chloride, selected antibiotics and ARGs in the water column and sediments along the longitudinal axis of water supply reservoirs, expressed as mean normalized concentration (Methods). For reference, the normalized concentration that equals the mean (i.e., one) was indicated as a dashed horizontal line, and we included results of an exponential fit and corresponding curve for fluoroquinolones. Missing points indicate that the antibiotic or ARG was not detected. Bars denote standard deviations, and some were not plotted because they extended beyond zero in the logarithmic axis.

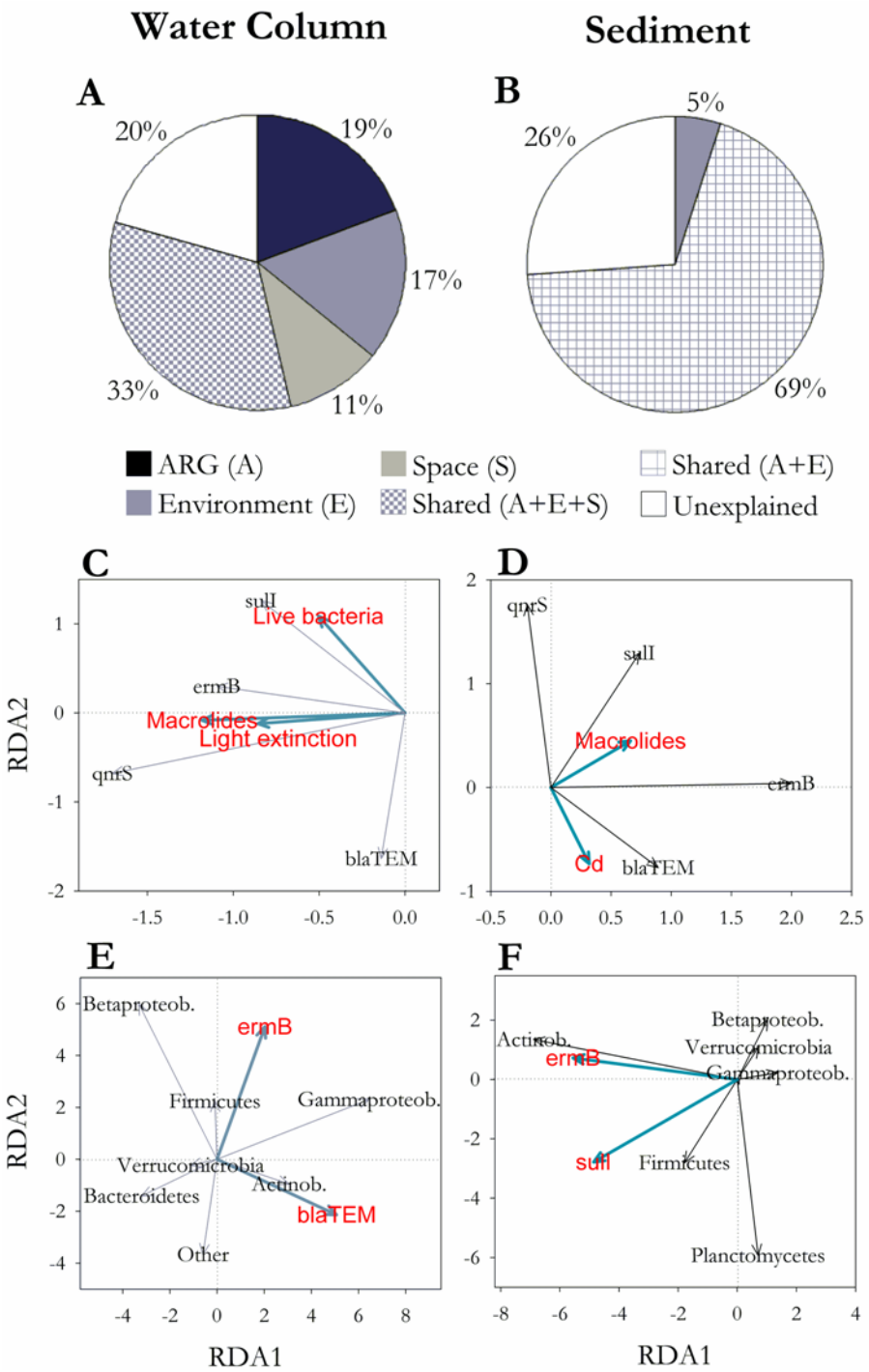
Fig. 3 Bacterial community composition in a) water samples; b) sediment samples from the three reservoirs.

Fig. 4 Analysis of the relationships between environmental variables (including antibiotics), ARG, and bacterial communities in water (left panels) and sediment (right panels) samples: Variation partitioning analyses of the bacterial communities in water (a and b), RDA distance biplots of ARG abundance constrained by environmental variables (c and d) and bacterial community data constrained by ARG abundance (e and f). Explanatory variables in RDA are denoted as bold blue arrows and red marks. We removed unclassified bacterial groups and groups without significant weight in panels e and f to increase readability.


Water
Sediment
A
Macrolides
G
Macrolides
B
Quinolones
H
Quinolones
C
Fluoroquin.
I
Fluoroquin.
D
Sulfonamides
J
Sulfonamides
E
Tetracyclines
K
Tetracyclines
F
Nitroimidazoles
L
Nitroimidazoles
Concentration in water (ng L-1) or sediment (ng g-1)
Foix Sau Llosa
Foix Sau Llosa
Water
Sediment
M
qnrS
Q
qnrS
N
sulI
R
sulI
O
blaTEM
S
blaTEM
P
ermB
T
ermB
ARG/16S rRNA gene abundance
Foix Sau Llosa
Foix Sau Llosa
Reservoir


Water
Sediment
A
Chloride
B
Tetracyclin
G
Tetracyclin
C
Erythromycin
H
Erythromycin
D
Sulfamethox.
I
Sulfamethox.
E
Ciprofloxacin
r²=0.96
p=0.13
J
Ciprofloxacin
F
Ofloxacin
r²=0.99
p=0.05
K
Ofloxacin
Mean normalized concentration along waterbody
Water
Sediment
L
ermB
P
ermB
M
sulI
Q
sulI
N
qnrS
R
qnrS
O
blaTEM
S
blaTEM
Mean normalized ARG/16S rRNA
Tail Mid Dam
Tail Mid Dam
Tail Mid Dam
Tail Mid Dam
Sampling station


A
Water column
Relative Abundance (%)
100
80
60
40
20
0
Tail Mid Ds Db
Foix
Tail Mid Ds Db
Sau
Tail Mid Ds Db
La LLosa
B
Sediment
Relative Abundance (%)
100
80
60
40
20
0
Tail Mid Dam
Foix
Tail Mid Dam
Sau
Tail Mid Dam
La LLosa
Unclassified bacteria
Other
Verrucomicrobia
Planctomycetes
Firmicutes
Cyanobacteria
Unclassified Proteobacteria
Gammaproteobacteria
Deltaproteobacteria
Betaproteobacteria
Alphaproteobacteria
Actinobacteria
Acidobacteria
Bacteroidetes


Water Column
Sediment
A
B
20%
19%
17%
33%
11%
5%
26%
69%
ARG (A)
Environment (E)
Space (S)
Shared (A+E+S)
Shared (A+E)
Unexplained
C
D
sulI
Live bacteria
ermB
Macrolides
Light extinction
qnrS
blaTEM
RDA2
qnrS
sulI
Macrolides
ermB
Cd
blaTEM
E
F
Betaproteob.
ermB
Firmicutes
Gammaproteob.
Verrucomicrobia
Actinob.
Bacteroidetes
blaTEM
Other
Actinob.
Betaproteob.
Verrucomicrobia
Gammaproteob.
ermB
sulI
Firmicutes
Planctomycetes
RDA1
RDA1