

DOCTORAL MEETING
in WATER SCIENCE AND TECHNOLOGY
Book of abstracts



Girona, 15 April 2016
Faculty of Sciences - University of Girona

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Presentation

This book contains ten extended abstracts submitted by PhD students of the Doctoral Program in Water Science and Technology within the framework of our annual meeting. The meeting was addressed to undergraduates, master and doctoral students interested in the ongoing research, current and future applications in the field of water science and technology. With this purpose, doctoral students were asked to summarize their ongoing activities in the format of an outreach scientific paper, with scientific rigor, but attractive, highlighting its innovative and most relevant aspects and potential applications.

This book of abstracts is not an extensive summary of the research training done in the doctoral program; it is the result of a training activity resulting in a sample of the diversity of topics of the program and the different steps followed during the three to four years of a PhD: starting with the research plan; followed by the research activities and completed with the writing and edition of a doctoral thesis. It is organized in research topics: environmental chemistry, genetics, microbiology, ecology and ecotoxicology and chemical engineering, covering a part of the research lines of the program. First year students like Giulia Gionchetta, David Cunillera-Montcusí and Pau Gimeno provide interesting summaries of their research plans, full of ideas and hypothesis that will be responded in the following years. The first set of results is clearly presented by Ruben Vera, Vicente Luis Jiménez Ontiveros, Elena Hernández-del Amo, Laia Planella and Laura Barral, who are in the middle of their thesis. Ruben, for instance, summarizes his first findings on a new type of membranes for chemical speciation studies, whereas Vicente provides evidences demonstrating that microbial diversity in lakes changes over time. We have also two doctoral students presenting a summary of their whole PhD, Imma Nogerola, with interesting findings about the role of bacteria (Epsilon proteobacteria) linking C and S cycling and Baigal-Amar Tuulaikhuu summarizing her findings on the influence of Arsenic on freshwater biofilm and fish.

Helena Guasch, PDCiTA coordinator

Doctoral Students' contributions

ENVIRONMENTAL CHEMISTRY

Design, preparation and characterization of new functionalized membranes as an emerging technique for speciation studies of nutrients and environmental toxic compounds

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Separation processes are of great importance in analytical chemistry and are almost unavoidable when treating either complex or trace level samples. Between the existing possibilities as sample pretreatment, chemical functionalized membranes (CFM) are gaining attention in the last decades. CFM are membranes bearing specific functional groups or that incorporate a specific ligand (also called carrier). In CFM the transport is due to chemical reactions, what is called “chemical pumping”. Among the different kind of functionalized membranes, this research is focused on polymer inclusion membranes (PIMs), which mainly consist on a polymer, to provide mechanical strength, a carrier, which is the responsible of metal ion transport across the membrane, and, a plasticizer, to increase elasticity. Sometimes the carrier acts as a plasticizer and simplifies the formulae of the membrane. PIMs have been extensively used in transport systems of metals such as Cd, Cr and Zn, among others, as well as for organic compounds such as antibiotics. As it can be seen in figure 1, PIMs are homogeneous and without pores in their structure. PIMs are easy to prepare, versatile and a high selectivity can be achieved using the appropriate carrier. However, it is important to take into account that depending on the nature of the carrier it can solubilize in the aqueous phases and thus, affecting the stability of the membrane. For that, membrane stability is a key factor when designing new PIMs.

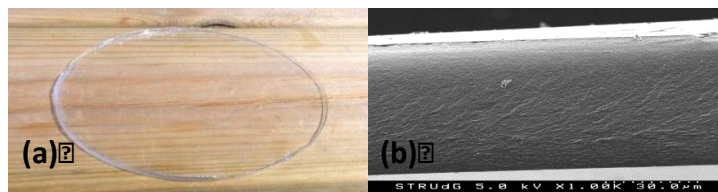


Figure 1. PIM (a) and cross section view on scanning electron microscope (b).

Based on the unique properties of PIMs, in this research we want to explore the possibility of using these membranes as a new tool to perform speciation studies.

Chemical species of the same element have different physicochemical properties, differences in mobility or toxicity. For that, in many cases it is interesting to know not the total amount of a certain metal, but the fraction of this metal available for living organisms. This term is known as bioavailability, and it is of great importance because affects vital issues such as toxicity or nutrient disposal. Thus, in order to predict metal bioavailability by living organisms in natural waters, analytical speciation techniques are required. The most commonly used are based in electrochemical techniques as well as diffusion through a thin film, such as DGT (diffusive gradients in thin films) or through membranes, such as DMT (donnan membrane technique) or PLM (permeation liquid membranes).

As a first step of this research, we have focused on PIMs stability. Therefore, several experiments have been performed to improve PIM's lifetime. We have studied PIMs based on cellulose triacetate (CTA) as a polymer, and derivatives of the reagent trioctylmethylammonium chloride (Aliquat 336) as carrier. This reagent is an ionic liquid (IL) at room temperature and thus, it also possesses plasticizing abilities. New IL have been prepared by exchanging the chloride anion of Aliquat 336 by different anions such as SCN^- , NO_3^- , ClO_4^- . PIMs prepared with these new ILs, have been fully characterized by using different techniques (thermogravimetric analysis, infrared spectroscopy, X-ray photoelectron spectroscopy, membrane potential and scanning electron microscope). Moreover, their stability has been investigated in terms of loss of extractant when weighed pieces of membranes were immersed in both ultrapure water and 0.1 M NaCl solutions. It was seen that the stability of the PIMs followed the trend:

$(A^+ClO_4^-) > (A^+SCN^-) > (A^+NO_3^-) > (A^+Cl^-)$, and it is worthy mentioning that the solubility of the IL was higher in water than in the saline solution. Furthermore, other PIMs containing carriers such as phosphonium based ILs, trihexyl (tetradecyl) phosphonium chloride (TDTHPCl) and trihexyl (tetradecyl) phosphonium bis 2,4,4-trimethylpentylphosphinate (Cyphos IL 104) were also evaluated in terms of membrane stability and As(V) transport. Using a feed solution consisting of $10 \text{ mg}\cdot\text{L}^{-1}$ As(V) and 0.1M as stripping solution, PIMs made of 50% CTA + 50% IL were investigated to As(V) transport. It was found that Aliquat 336 was the most effective PIM to remove As from natural water. However, when anions were present in the medium (natural water), transport of As(V) is affected for Cyphos 104 and TDTHPCl. PIM stability based on these IL, follows the trend: Cyphos 104 > TDTHPCl > Aliquat 336.

As an application study, a device incorporating a PIM has been developed for the preconcentration of As(V) species prior to their determination. Several parameters of the concerning design of the device have been evaluated, such as membrane composition, receiving phase characteristics and membrane thickness, in order to accomplish the preconcentration of arsenic species in a more convenient time scale. Under the best conditions, PIM-based preconcentration method allows the determination of As(V) from well waters samples with satisfactory recovery values.

Other studies have been focused on the use of PIMs to assess Zinc bioavailability. In natural waters, Zinc exists in different chemical forms such as free hydrated ion, inorganic and organic complexes. Zinc bioavailability studies were performed with PIMs containing polyvinyl chloride (PVC) as polymer and D2EHPA (di-2-ethylhexyl phosphoric) as carrier. Influence of different parameters on Zinc accumulation, e.g., membrane composition, pH, concentration of HNO_3 in the receiving phase as well as the presence of organic ligands were performed. Moreover, different experiments with potato plants (*Solanum tuberosum*) were performed in order to assess a correlation between Zinc flux in root cells and to evaluate a PIM flux under same experimental conditions. It has been proven that humic acids and ethylenediaminetetraacetic (EDTA) as organic ligands showed a strong influence on Zinc flux through the membrane.

To sum up, it has been proved that PIM can be an alternative tool not only for metal speciation but also for bioavailability assessment. Our research can expand the range of applicability of this kind of membranes and increase its use for analytical purposes.

The financial support of the research project CTM2013-48967-C2-2-P and the doctoral grant ref. BES-2014-068316 (R.V.) are acknowledged.

GENETICS

Developing new highly variable genetic markers in the blue and red shrimp
(*Aristeus antennatus*)

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The blue and red shrimp *Aristeus antennatus* is one of the most commercially valuable crustacean species of trawling fisheries in the Western Mediterranean Sea. Their populations have been exploited since the 1950s, and global catches have been annually increasing, reaching up to 1851 tonnes in 2013. The species contributes the amount of 12.75 million euros each year to the Catalan harbours which means 10% of the total incomes of the regional fishery.

Several studies have been carried out during the last decades to understand the biology and ecology of this decapod, however, there are still important gaps of knowledge with regards to its reproductive biology, mating strategy or population structure at regional scale. The inability to keep alive adult specimens under laboratory conditions joined with the eurybathic distribution of the species (80 – 3300 m depth) has hampered to progress in many aspects of its biology, which are indispensable to develop a global and long term sustainable management plan.

To fill these gaps we have developed a powerful genetic tool specially designed for the blue and red shrimp in order to infer its genealogy or parentage relationship in a fine-scale resolution. Using Next Generation Sequencing (NGS) to know the genome of *Aristeus antennatus* we searched for all putative microsatellite loci. Microsatellite are high sensitive nuclear markers composed by short segments of DNA that have a repeated sequence such as CACACACA and present high level of polymorphism (more than one allele per loci). Microsatellite have proved its utility for our purpose in other crustacean decapod species such as black tiger shrimp or Norway lobster. Thus, we tested each of the 97 isolated putative microsatellite loci in individuals from the whole

distribution range of the species (Mediterranean Sea, Atlantic Ocean and Indic Ocean) to validate its polymorphism. From 97 putative microsatellite loci, 35 of them were clearly polymorphic. In order to reduce time and cost of the process we set up three Multiplex PCR (Multilocus Polimerasa Chain Reaction), to visualize several polymorphic microsatellite loci (4 to 6 loci) at the same time. This new genetic tool developed in my thesis will be able to answer the following questions:

- Which is the mating system of the blue and red shrimp? For example, males mate with more than one female at each season? Or females mate with more than one male at each season?
- Multiple spermatophores (sperm sack) attached to one female come from the same male or different males?
- The blue and red shrimp fished in Palamós is different from those fished in Roses or Blanes? There is gene flow between Mediterranean populations?

With the purpose to know the mating system of *Aristeus antennatus*, we collected females which had attached a spermatophore to its cephalothorax and adult males from the same fishing ground. Once at the laboratory, we individualized each spermatophore, female and male until DNA extraction. The crucial step of all genetic studies is the extraction of enough quantity and quality DNA to perform subsequent analysis. We found that spermatophores pulled up from female cephalothorax were mixed with female cells. So, DNA extractions were composed by female DNA plus sperm DNA. As a result, we designed a new specific protocol to isolate a clean high-quality sperm DNA from spermatophores attached to female cephalothorax. This new protocol has been successfully applied in all analysed individuals.

Nowadays, we are applying the three PCR Multiplex on females, males and spermatophores to compare their allele at each loci. Thus, will allow us to discover the mating system of the blue and red shrimp, as well as the origin of the multiple spermatophores attached to a single female. If we find that some spermatophores from different females have the same DNA profile at each loci, we could affirm that males mate with multiple females. On the contrary, if males mate only with one female at each

reproductive season, all studied spermatophores will have different DNA profile. On the other hand, if multiple spermatophores attached to one female cephalothorax have the same DNA profile or different DNA profile at each loci, it will indicate that they come from the same male or different males, respectively.

MICROBIOLOGY

Microbial diversity in lakes changes over time

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Lakes have a lot of diversity in them, ducks, fishes, plants... but most diversity can't be seen: lakes have plenty of microbes, i.e. bacteria and microscopic algae. Microbes are important parts of the ecosystem in lakes, where they can affect the processes of the ecosystem severely (Newton et al. 2011). To identify and explain those effects, we have first to know microbial diversity and the processes that affect it in lakes.

We can study biodiversity in several ways. You can take a single snapshot of a lake, what we call a "sample", and try to establish some rules on how the diversity is there. But what if instead of a single photograph of a lake, we take consecutive photos of it, as a film? This is what we call a "time-series", and it allows us to see the dynamics -the changes in time- of biodiversity. One may think that there can't be that many changes in diversity over time, but there are: in lake Zürich (Switzerland), one of our study lakes, we found in total 277 different species of algae, but only as much as 128 species were found at the same time, and the minimum found were 21 species.

But biodiversity is not like a Hollywood actor or actress, that never ages and stays the same for most of their career. Biodiversity evolves, biodiversity is variable, is better suited to one or another condition and this adequacy is transmitted to the following generations. DNA sequences found in a site can tell us about the processes affecting biodiversity, such as the adaptation to saline environments, and so our "film" becomes alive.

In our research project we combine these approaches to study microbial diversity, inspired by the Theory of Island Biogeography (TIB) (Alonso et al. 2015). TIB states that the variation in the number of species in an island is a balance of two processes:

extinction and colonization. These processes underlie the true dynamics of the bacterial species we identify in our samples. If we assume that lakes are like islands, we can count the number of colonization events- when a species that was absent in a sample is present in the next sample of the same site- and extinction events- when a species present in a sample is absent in the next- and calculate colonization and extinction "rates", the fraction of species that colonize or become extinct per unit of time in a site (MacArthur, and Wilson, 2015).

We wanted to test, first, to what extent TIB applies to the communities formed by microbes in lakes, second, if this approach is informative about the processes that govern microbial communities, as competition or the species response to environmental changes, and third, to what extent our approach has predictive power, that is, if we can simulate the temporal dynamics of microbial communities with enough accuracy to predict future changes.

Four lakes of the Pyrenees were the first ecosystems we examined. Three of these lakes were connected by a river, and the fourth one was separated. When we calculated the colonization and extinction rates for bacteria in each lake, we found that the three connected lakes had a similar behaviour, even though the three lakes had some differing species, while the separated lake had a distinct behaviour on its own, a result that is not very surprising. Then, we calculated colonization and extinction rates for different microbial groups in the lakes and the surprise arose. These rates were proportional between them, and we interpret this as a compromise between colonization and competition: groups formed by good colonizers are bad competitors, while groups with low colonization rates are good competitors.

The second system studied was Lake Zürich, a big lake that was heavily contaminated in the seventies and started to recover to a more clean state. We looked at microscopic floating algae, "phytoplankton", for which we had monthly samples for 35 years. We found that the phytoplankton community changes showing four different stages during the recovery of the lake, characterized each one by a single colonization and extinction rate. These four stages were closely related to gradual changes in temperature, silica availability and different forms of phosphorus and nitrogen. Taking these variables into account, we can simulate the phytoplankton at lake Zürich, and we obtain results

resembling those observed, just using the response to the environment of these communities, helping us to predict the response of the community to future conditions.

Saline shallow lakes in the Monegros desert, Spain, are our third study system. These shallow lakes have the peculiarity that when it rains, the salinity of them decreases, but then the lakes start to dry and the salinity increases, reaching values ten times higher than sea water. These changes in salinity might let us see the signal of evolution, because high levels of salinity kills most species of microbes, leaving only those adapted to those conditions until the next rain. Our next step is to analyse this data and try to simulate the evolution of these communities, with all the information that we are capable of gather about them. However, this is yet work to do.

In conclusion, we are trying to understand how microbial communities are organized and how they change and evolve in time. Lakes are good systems to examine these questions, and the answers could let us advance not only in the knowledge of lakes, but also about microbial diversity across systems, and, more generally, our work should contribute to gain understanding about the temporal evolution of ecological communities and their response to different environmental pressures.

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Diversity, dynamics and activity of Epsilonproteobacteria in a stratified karstic lake. Implications in carbon and sulfur cycles

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Karstic lakes exhibit contrasting physicochemical gradients along depth where microbial populations distribute according to their metabolic and physiological requirements. In these environments, oxic-anoxic interfaces are hot spots of microbial diversity and activity where to study the links between community structure and function. Sulfidic redoxclines offer a suitable niche for the development of photo- and chemotrophic populations that obtain energy from the oxidation of reduced sulfur compounds. Among them, members of the class *Epsilonproteobacteria* have long been considered key players in biogeochemical cycling of C and S in aquatic environments where oxygen and sulfide occur (Campbell et al., 2006). It is then somehow surprising that no studies have yet been addressed to elucidate their presence and activity in the Banyoles Karstic System.

To resolve whether or not members of the class *Epsilonproteobacteria* constitute stable populations in Lake Banyoles and to determine their diversity, seasonal dynamics and activity we carried out a molecular survey during three year cycles in a meromictic basin of Lake Banyoles (basin C-III). We applied a complementary array of molecular techniques (CARD-FISH, MAR-FISH, cloning, qPCR and high-throughput sequencing) to resolve the identity and ecological role of Epsilonproteobacteria in the system, with special focus on the contribution of these microorganisms on linking C and S cycles. Our results pointed to a clear seasonality of Epsilonproteobacteria, with maximal abundances at the redoxcline and upper monimolimnion in winter. Furthermore, clone libraries and high-throughput sequencing datasets revealed the predominance of sequences affiliated to genus *Arcobacter* (Noguerola et al., 2015). *In situ* incubations

using radiolabelled bicarbonate and MAR-CARD-FISH measurements clearly indicated that Epsilonproteobacteria actively assimilated CO₂ in the dark thus being responsible of the high rates of dark carbon fixation measured at the redoxcline in winter (Noguera et al., 2015). Molecular analyses targeting key genes of the Arnon Cycle (C fixation) and the multienzyme Sox System (Sulfur oxidation) provided additional evidence that the dominant member of the epsilonproteobacterial community is a chemolithotrophic, sulfide-oxidizing member of the genus *Arcobacter*, distantly related to its marine counterpart *Candidatus Arcobacter sulfidicus* (Wirsen et al., 2002; Noguera et al., 2015; some data not published).

Altogether, our data support the key role of Epsilonproteobacteria in linking C and S cycles and extend their influence to freshwater systems characterized by sharp oxic-anoxic interfaces and euxinic waters. Besides, this study clarifies the ecological role of Epsilonproteobacteria in such systems and identifies the main microorganism responsible for the high dark carbon fixation activity measured at the redoxcline depth by different authors thus solving an intriguing question that has been a matter of debate in recent years (Casamayor et al., 2008, 2010, 2012).

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Ammonia oxidisers on plant root surfaces are driven by oxygen leakage

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Ammonium is one of the most common nitrogen compounds in freshwater systems. It can come from natural sources or from anthropogenic ones, which could become in an excess of ammonium in water. Nitrification is the main biological process to remove ammonium from water. It consists in two enzymatic steps where ammonium is oxidized to nitrate which in turn can be removed from water by nitrate reduction to nitrogen gas. The first step of nitrification is the oxidation of ammonium to hydroxylamine, which can be carried out by two types of microorganisms, namely Ammonium Oxidizing Archaea (AOA) and Ammonium Oxidizing Bacteria (AOB). Ammonium oxidation is encoded by the ammonia monooxygenase subunit A (*amoA*) gene, which is normally used as a gene marker to study AOA and AOB communities.

In freshwater systems ammonium oxidation can occur in the sediment as well as in the roots surface of emergent macrophytes. Several studies demonstrated that nitrifiers are stimulated by the rhizosphere mainly in natural environments (Llirós, Trias, Borrego, & Bañeras, 2013; Trias et al., 2012). However, ammonium reaches higher concentrations in wastewater which requires an efficient biological removal treatment. Constructed wetlands (CW) are an alternative to wastewater treatment which is intended to mimic natural wetlands. Emergent macrophytes have a central role in the functioning of CW. Plant coverage and species selection are significant aspects to be considered in designing CW facilities.

The first aim of this study was to show which representative members of AOA and AOB communities were present in a CW. Furthermore, the second aim was to study the spatial distribution of these microorganisms in different root sections of *Typha latifolia*. Cloning and massive parallel sequencing were used to study in different years the diversity of AOB and AOA in roots from two *Typha* species and sediment. Species from marine groups of AOA were always detected in all the samples which could be consequence of the input of sea water in the studied CW. On the other hand, AOB were only represented by soil groups being this community less diverse than the AOA. Results from the diversity study show that there were significant differences among sample type and temporality.

To study which part of the root presents the highest AOA and AOB activity, oxygen diffusion kinetics, qPCR and Scanning Electron Microscope (SEM) were used. Moreover, 16S rRNA barcode amplicon based Illumina sequencing was used to study the diversity of AOA and AOB at different root sections. Microorganisms could be observed on the entire roots surface in different amounts and shapes. The oxygen diffusion was higher in the middle part of the roots than in the tip or base which coincided with the higher abundances of AOB and AOA. Moreover, total archaea and bacteria were also more abundant in this section. There were no differences between quantifications of ammonium oxidizers; therefore AOA and AOB were equally selected in *Typha latifolia* rhizosphere in the studied CW. Although in the middle section of the root higher densities of ammonia oxidizers were found. This accumulation coincided with a lower diversity of the community, thus suggesting a selection of ammonia oxidizing species.

Overall, we were able to show that plant roots exerted a selection effect on ammonia oxidizers, although this effect may be limited to small portions of the root surface due to changes in oxygen leakage. Oxygen diffusion depends on apoplastic diffusion barriers that may be significantly modified as root grows and suberizes.

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ECOLOGY & ECOTOXICOLOGY

The sensitivity of intermittent' stream microbial communities to drought impacts*Giulia Gionchetta*

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Facing the struggle against the global decline of biodiversity and climate change, the ongoing debate around how microbial diversity and ecosystem functions are related intensified the requirement to deepen knowledge and widen stream ecology research embracing all the protagonists acting in the context.

Global climate change affects running waters not only by increasing water temperatures, but also, by affecting their flow pattern (more variable discharge, more frequent and severe floods and droughts) especially at the headwaters (Marxsen et al., 2010). Drought is among the most important disturbance events for stream ecosystems since it not only modifies the stream hydrology but also the stream biota (Pohlon et al., 2013). Temporary streams lack surface water flow for some periods of the year, their flow regime is shaped by diverse frequency and duration of non-flow phases defined by dry and rewetting alternation. Despite the intermittency, these small streams have an important impact on global biochemical processes and on the preservation of biodiversity. As streams sculpt the continental surface, forming dense and conspicuous channel networks that can be thought of as ecological arteries that perfuse the landscape (Battin et al., 2016), temporary streams would be as ecological blood vessels whose flow draws and feeds the superficial and internal portion of streambed bio-system with intermittent and delicate pulses alternation. Thus, these periodically disconnected reaches might be essential in driving the whole stream ecosystem function, and this intensifies the need to improve our knowledge on their functioning for their proper conservation and management.

Streambed surface and inner zones are pools of biodiversity where microorganisms, originating from the entire tree of life (Eukarya, Archaea and Bacteria), live within aggregates named biofilms. The upper zone of riverbed is dominated by phototrophic life while inside the sediment, where the light is limited, the microbiota is packed together living into a porous extracellular matrix. This complex accrual of microorganisms forms streambed biofilms which are key sites of micro-biota activities related with ecosystem functions and its processes maintenance. Biofilms composition and biodiversity dynamics are not random but driven by environmental factors which seems to design the assemblage of these complex communities (Battin et al., 2016). In this regard, the sensitivity of stream biofilms to climate warming and global environmental change is beginning to be recognized (Zeglin 2015; Febria et al., 2012). Studies up to now have shown that streambed microbial communities in intermittent streams are directly affected by desiccation and rewetting, their ability to degrade, uptake and arrange organic matter available for other organisms could be limited as well as their activities are frequently reduced during flow intermittency (Marxsen et al., 2010). However, little is known about the interplay of the different microbial groups during the distinct drying/rewetting phases and whether their resistance to stress conditions or their capacity to recover would respond fast to unusual, increased or prolonged hydrological depletion.

Our research considers the depth of the streambed acting as a buffer and refuge for the micro-communities during unfavourable water stress conditions. In order to answer whether there is a limit beyond which the taxonomic and functional diversity of streambed microbial communities submitted to intense drought will not be reactivated and to describe the role offered by small headwaters in potentially maintaining the river functions along the river continuum during unusual non-flow periods, we planned to combine field and lab-scale studies which would allow the discovery of unexpected responses driven by the essential role of humidity of the deeper layer of the riverbed or by the potential interactions within the microbial groups studied. Our expectations depend on the portion of sediment considered and the hypotheses would differ within the factor and the group of microorganisms studied. If drought disturbance provokes an effect on microbial biodiversity this could further have an effect on functioning, although this would depend on the level of functional redundancy among species. We

will focus the interest on the temporality thresholds beyond which the loss of taxonomical and functional diversity occurs and whether these limits differ among the most relevant microbial groups in streambed (Archaea, Bacteria, Fungi and Protozoa) (Fig.1).

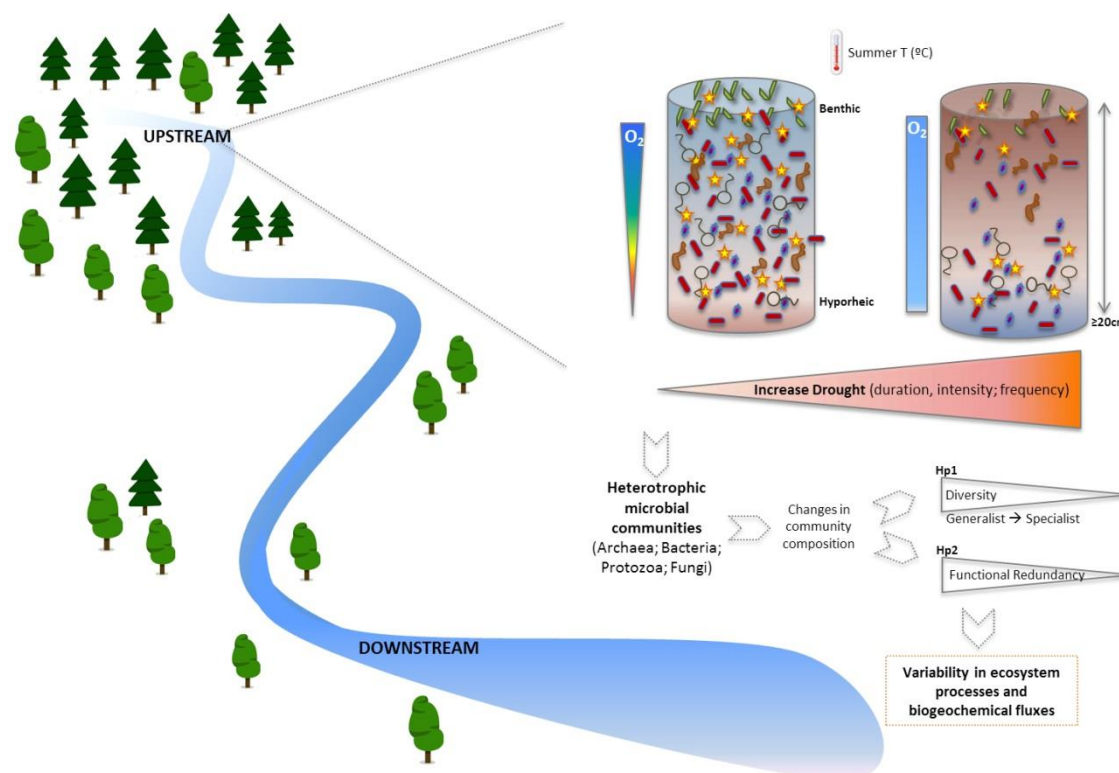


Figure 1 Upstream part of riverbed subjected to increased drought is expected to change its microbial species composition, losing diversity and functionality. These potential transformations driven by the rise of water depletion would potentially vary the entire river ecosystem processes, biota and fluxes.

We hypothesize that, in general, the fragility of the system would increase with longer and more intense drought. The micro-biota living in the superficial zone is expected to be less stable than that from deeper zones since the upper part of the river sediment is more subjected to environmental changes. Aware of field and lab-scale limitations, the contribution to the knowledge of this study can help understand and approach a little more the diversity of the communities most involved in the carbon cycle, and so, on the functioning of temporal stream ecosystems to bring clearer visions of how they are responding to soaring environmental global changes and human needs.

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Burning waters: wildfire effects on aquatic systems*David Cunillera-Montcusí*

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Wildfire is a global natural disturbance that has been present since rise of first plants and plays a key role in world ecosystems distribution and composition. This disturbance is not equally frequent around the world. Climate conditions such as drought or temperatures modulate wildfire impact creating areas where it is more frequent. Historically, wildfires have been studied in relation with their effects on terrestrial plants, their management and how recently burned areas recover. However, wildfire effects go further than terrestrial ecosystems and they can have an impact on aquatic systems.

In contrast to the impact of wildfire over terrestrial ecosystems, aquatic ones are affected by the alterations produced in the watershed after a wildfire (Minshall et al. 1989). Five main consequences on aquatic ecosystems can be observed after a wildfire: First, vegetation loss decreases rainfall uptake and retention, producing an increase in runoff and floods (Vieira et al. 2004). Second, loss of tree coverage increases light incidence in aquatic systems, increasing temperature and microbial activity (Rodríguez-Lozano et al. 2015). Third, ash and debris inputs contribute to increase nutrient concentrations, turbidity and sediment transport, contributing to a loss of water quality (nutrient enrichment) and morphological changes (Spencer and Hauer 1991). Fourth, increase in light, temperature and nutrients enhance algal growth, increasing its biomass and changing the available food in the system, from leaves to algae. Finally, fauna can become affected due to habitat alteration and available food change. Moreover, this can be enhanced by other perturbations favored by wildfire (i.e. floods). Generally, these consequences change the relative abundance of some organisms, being tolerant ones more benefited (Malison and Baxter 2010).

Although these five main consequences are common between aquatic systems some of them can have more relevance than others depending on the type of aquatic system. Running waters (rivers, streams, ditches, ...) are normally more affected by the loss of riparian vegetation and morphological changes, consequences 1 and 2. Thus, faunal community is affected by the shift of resources and floods. In the other hand, lakes (deep standing waters) are more affected by basin changes and nutrient inputs, consequence 3, commonly leading to the loss of water quality (eutrophication) and algal and tolerant groups increase. Wetlands and ponds (shallow standing waters) would be in the middle of these two before-mentioned aquatic systems. Due to their shallowness, they can become dry during drought seasons (such as Mediterranean temporary ponds) so vegetation can burn completely with its associated consequences. At the same time, sediments and ash are not transported downstream leading to a possible loss of water quality (eutrophication). In fact, previous studies reported an increase of biomass and abundance of some groups in burned wetlands but also a fast return to pre-fire conditions.

Despite all these wildfire consequences on aquatic systems, studies suggest that the recovery of pre-fire conditions parallelizes terrestrial recovery lasting for years or decades. However, this recovery could be different depending on the climatic area, being drought adapted areas (i.e., Mediterranean climates) more resistant to such type of perturbations (Verkaik et al. 2013).

Having this general knowledge on the effects of wildfire on aquatic systems in mind, my research aims to study how wildfire can affect Mediterranean temporary ponds. These habitats are very important in Mediterranean regions because they provide habitat to lots of species such as threatened amphibians and unique species of arthropods. The effects of wildfires over these systems are not known although Mediterranean regions are known to have high wildfire frequency. By comparing the fauna from ponds that have been burned and ponds that not, I will try to understand how this disturbance affects these ecosystems.

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An overview of the interactions between arsenic and microbiota in rivers

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Arsenic (As) is a widely distributed metalloid in natural ecosystems whose toxic properties are well-known since ancient times. Most environmental arsenic problems are the result of its mobilization under natural conditions, but were exacerbated by human activities like mining, combustion of fossil fuels, the use of arsenical pesticides and herbicides, among others. An excess of arsenic in the environment, and especially in drinking water, can cause a severe health risk with increased incidences of diseases in many regions of the world. In rivers, water contaminated with arsenic have baseline concentrations with an average of 0.8 µg/L. However, the Aquatic Life Criteria of the United States Environmental Protection Agency (USEPA) establishes in 2014 the limits of arsenic concentration in freshwaters at 340 µg/L for acute arsenic exposure, and at 150 µg/L for chronic exposure.

We can find arsenic in different chemical forms (species). The most common inorganic species in the environment are As^{III} (arsenite) and As^V (arsenate). As^{III} is usually more dangerous to have greater mobility and toxicity compared to As^V, which is the most abundant species in aquatic environments. The change of arsenic chemical forms is crucial in relation to their toxicity, and this change depends heavily on algae and bacteria, that can even transform them into organic species. Therefore, microorganisms play an important role in environmental arsenic toxicity. Furthermore, this speciation process also may vary depending on the presence of phosphate in the medium.

We did some experiments in natural and artificial rivers to check how arsenic affects microorganisms and if their biological activity could change the arsenic chemical forms. Results showed that arsenic causes damage on microalgae: it inhibited their growth and

caused structural changes in their photosynthetic systems. Regarding to arsenic speciation, we detected that, in an arsenic-polluted river with high amount of As^{V} , microorganisms developed on rocks (named periphyton or epilithic biofilm) could change the chemical form by taking this As^{V} and transformed it into a more toxic As^{III} inorganic species and to a less toxic dimethylarsenate (DMA^{V}) organospecies. Then, they could excrete those species into the river water. Therefore, we certify that arsenic is toxic for microorganism, especially for microalgae; however, their biological activity may regulate the toxicity of the arsenic in the environment. We also found that arsenic may be dangerous for freshwater fish, and especially with the presence of microorganisms, what could be due to the excretion to the water of higher toxic species (As^{III} , for instance) by microalgae.

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Arsenic influence on freshwater biofilm and fish

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In Mongolia, mining activities are increasing but little is known about the environmental impacts of this activities. Mining can bring large quantities of sulfides to the surface and break them into small pieces and subject to weathering. As the sulfides break down, arsenic and other heavy elements and metalloids are released. Arsenic in drinking water is a serious public health problem affecting many countries, with millions of people throughout the world causing increased mortality by several cancers. However, the impacts in aquatic ecosystems are not well known. The liberated toxicants can contaminate the entire system, since all processes and components of aquatic ecosystems are tightly interconnected, any disturbance can affect their structure or function. In water, trace elements may bind to particles of decaying plant material or to fecal pellets of animals and to particles and all these tend to sink to the bottom. In the bottom substrates of aquatic ecosystems, there are communities consisting of algae, bacteria and other microorganisms and called biofilm. The biofilms can actively influence and transform the pollutants. It is assumed that toxicity of arsenic highly depends on its oxidation state. In nature, arsenic reducing and oxidizing bacteria coexists and influence arsenic toxicity being different from predictions obtained from simple toxicity assays carried out in the laboratory. So, it is very important to provide as much ecological realism as possible for ecotoxicological studies.

This study aimed to evaluate the destiny and toxicity of a long exposure of biofilm and fish to an environmentally realistic concentration of arsenic.

An experiment with four different treatments: control (C), biofilm (B), arsenic (As) and biofilm with arsenic (B+As) and all included sediment and fish to enhance

environmental realism was conducted. Total arsenic concentration decreased exponentially from 120 µg/L to 28.0 ± 1.5 µg/L during the experiment (60 days), mostly sinking to the sediment and a smaller percentage accumulated in the biofilm.

This concentration of arsenic affected the quality and quantity of the base of the aquatic food chain and the normal functioning of the ecosystem. Fish were also affected directly and indirectly by this low arsenic concentration since exposure had already affected the function of the biofilm. We also highlight the interest and application of integrating some of the complexity of natural systems in ecotoxicology.

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CHEMICAL ENGINEERING

Estimation of pharmaceutical loads in rivers: an integrated model that supports decision-making process

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Urban wastewater systems (UWWS) have the functions of collecting, transport (sewer systems), store (storm water tanks) and treat (wastewater treatment plants, WWTP) the urban wastewater and the stormwater before discharging to the receiving water bodies (river, lake, sea). UWWS not only alter the water quantity and quality of the receiving water bodies, but also significantly affect fluvial ecosystem structure and function and therefore proper management strategies are needed. UWWS discharge organic matter, nutrients and microcontaminants (MCs) into the rivers, compromising the so called “good ecological status” defined under the European Water Framework Directive (WFD). Removal of organic matter and nutrients contained in wastewater is in general not a problem anymore for most of the developed countries. However, MCs have just recently attracted attention by the scientific community. Besides, European Union legislation establishes Environmental Quality Standards (EQS) for 45 Priority substances and a watch list with additional 10 substances that require targeted EU-wide monitoring in rivers to support future prioritization. Six (6) pharmaceutical active compounds (PhACs) are already included in that list (*Diclofenac*, *17-beta-estradiol (E2)*, *17-alpha-ethinylestradiol (EE2)*, *Erythromycin*, *Clarithromycin* and *Azithromycin*). The incomplete removal of PhACs (and their transformation products) leads to a continuous introduction of these substances into the aquatic environment.

This fact results in their widespread and ubiquitous presence, which has raised environmental and human health concerns (Osorio et al., 2012).

New wastewater treatment technologies (i.e. tertiary treatment) are emerging nowadays to decrease MC discharges from UWWS into water bodies, however, research questions oriented towards how to best prioritize investments for MC removal at a river basin scale still needs to be addressed: On which WWTPs within a river basin it would be best to implement tertiary treatments? What type of technology would be the most cost-efficient? To give answers to these questions, environmental decision support tools facilitating the decision-making process on new strategies/investments for MC removal are required.

In addition, conducting extensive sampling campaigns and analysis of MCs within a whole river basin is very expensive and time-consuming. For this reason, the use of a model that successfully represents the fate and removal of MCs through the whole UWWS as well as predicts their concentrations in water bodies can be perceived as a relevant decision support tool.

Thus, the aim of this work is to develop a simple steady-state model capable to describe fate and removal of MCs at a River Basin scale. We selected the Llobregat river basin as a case study and *diclofenac* (common anti-inflammatory drug) as the pharmaceutical model substance. The developed model integrates 3 sub-models: i) a substance-human consumption and excretion model, which estimates load of PhACs that reach the influent of WWTPs; ii) a WWTP model; and iii) a river model. Both the WWTP and the river models describe removal of PhACs using a first order decay equation. Inputs to the model are *diclofenac* human consumption and excretion, inhabitants in each subcatchment, WWTP operational variables, PhAC degradation constant in WWTPs, hydrological variables (flow, velocity and length) provided by Aldekoa et al. (2013) for each river stretch and PhAC degradation constant in rivers. Uncertainty associated to the three most “unknown” parameters (human consumption and excretion of *diclofenac*, PhAC degradation constant in WWTPs and PhAC degradation constant in rivers) are propagated to model predictions in order to identify the relevance of each one. This propagation is done by running *Markov Chain Monte Carlo* simulations of the integrated model (i.e. 10.000 simulations) after defining probability distribution

functions for each parameter (using empirical knowledge from previous measuring campaigns) and randomly sampling from these distributions at each model run. Thus, a range of possible values (i.e. 10.000) for modeled PhAC river loads were calculated as well as their median values for every river stretch. The simulated results for *diclofenac* loads match well the experimental concentrations measured at 9 sampling points in the Llobregat river as well as at 4 sampling points in the influent and effluent of Manresa and Igualada WWTPs respectively, during sampling campaigns conducted in September 2010. Based on model results, the average attenuation of *diclofenac* along river stretches was up to 8% (0.7 (5% percentile) - 20 (95% percentile)) and the average attenuation in WWTPs was up to 37% (17-75). Our results are in agreement with previous experimental studies dealing with the fate of PhACs in WWTPs (Verlicchi et al., 2012). These preliminary results suggest that our model can be used as a support tool to help decision makers to propose more efficient measures at catchment level in order to decrease pollutant concentrations below standards. Specifically, the model can be used to simulate a number of scenarios or alternative measures (for example to increase reactor volumes or add tertiary treatment in WWTPs) to decrease PhAC loads in river basins, as well as to evaluate the suitability of measures including uncertainty ranges.

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