Elephant in Lunz am See

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ARTSCIENCE

Abstract

Biofilms are groups of varied microorganisms[1] that can be found almost everywhere and are involved in many natural phenomena. For this reason, they are the focus of numerous scientific research studies. They can be found forming a microbiota[2] associated to specific parts of bigger organisms, or forming independent colonies,[3] that may also be visible to the naked eye. A new approach to microbiology,[4] focused on the microorganisms colonies *in situ*, rather than *in vitro*, has led us to a new environmental microbiology, also called microbial ecology.[5] New techniques based on genetic engineering, when used along with computational techniques based on software working with databases, allow scientists to characterise the species that are living together in a biofilm, the abundance of each one of them and their role in the sample analysed.

In this context, and in the frame of an experiment run by a group of researchers from the University of Vienna – Department of Limnology & Bio-Oceanography, a sampled biofilm from a streambed in the woods of Lunz am See (Lower Austria) was analysed by the scientists and the genetic information was afterwards processed by the software MEGAN.[6] The sample showed a fairly normal species' composition for such biofilm. The exception was the appearance of one unexpected species in the results: an elephant. Focusing on this result, that may be regarded as an artifact (a spurious experimental result), my aim in the current work is to analyse the scientific methodology used by the researchers, to suggest some hypotheses to explain how this elephant appeared within the data, and finally, and as a complementary part of the written component, to present a video related to a virtual elephant that I encountered in a biofilm during my research.

[1] Microorganisms are very small organisms, that cannot in general be visible to the naked eye. Lens must be used to make them observable. Some examples are bacteria, some algae and fungi, some protozoan. A tiny fraction of them live as singles, but the vast majority prefers to live in aggregates which are collectively termed as biofilms.

[2] I refer here to microbiota (also called *microbiome*) as the ecological community of commensal, symbiotic, and potentially pathogenic microorganisms that literally share the body space of any bigger living being (such as an animal), and which are determinant either for the health or disease of that organism. The human body, for example, contains over 10 times more microbial cells than human cells, although the entire microbiome only weighs about 200 grams. Examples in our body are the microbial film on our teeth, or the microbiota in our guts.

[3] These colonies have different shapes. Inland water biofilms, for example, look like slimy films (jelly material, with variable viscosity and thickness) which occur on peddles of a streambed or, in general, on surfaces exposed to water or humidity.

[4] Microbiology is the discipline of Biology that studies microscopic organisms, either unicellular (single cell), multicellular (cell colonies), or acellular (lacking cells).

[5] This new approach solves some troubles that scientists observed in the lab: i.e. many microorganisms withstand the cultivation methods in the lab, or at least they behave differently. This fact really limits the possibilities of microbiology and biases many of the experimental results. In the particular case of studying biofilms, the environmental approach seems to give more reliable knowledge.

[6] MEGAN is a software developed at the University of Tübingen (Germany) and it is used as a powerful tool to understand the composition and operation of complex microbial consortia in environmental samples through sequencing and analysis of their DNA. A link: http://ab.inf.uni-tuebingen.de/software/megan5/

Acknowledgements

I would like to thank Univ.-Prof. Virgil Widrich and Sen.-Lect. Dr. Bernd Kräftner for the inestimable help they offered me during all the time in this work.

The other part of Art & Science staff played also a determinant role with their knowledge, inputs and logistic tasks to make possible this work to be done. They are Valerie Deifel, Juliana Herrero, Brishty Alam and Sonja Orman. I also thank professor Peter Calvache for his help.

I am also indebted to the Department of Limnology and Bio-Oceanography from the University of Vienna and the whole Tom Battin group in particular for their time to chat and will to collaborate with the project, as well as for the input they offered me all the time, that has been essential for the realisation of the work. I specially mention here Mia Bengtsson and her elegantly conducted scientific work that has been a source of inspiration and a verified source of information that I have been using as a main reference for the current work.

Thanks as well to all my family and friends (Art & Science's colleagues as well) who made possible this work to be achieved by offering their time for the purpose. I also would like to thank Michael Hofinger for his patience and help during the whole study program, Anna Serrano, Sebastià Torreguitart, Helmut Hueber and Christian Reider for their support, Esther Moñivas for giving me multiple references and Alistair Fuller for a proofreading.

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1. Introduction

1.1. Preface

This work is based on the research I did during the time I spent with the department of Limnology & Bio-Oceanography of the University of Vienna[7] (particularly with Tom Battin team, that is the branch working with inland water biofilms) all through the collaboration I was granted with the Master Studies of Art & Science at the University of Applied Arts of Vienna.

1.2. Motivation

As I already mentioned in the abstract, finding an anomalous result in a scientific experiment, such as an elephant depicted in a species' composition of a biofilm, was something impressive for me since the beginning. Of course, the appearance of traces of genetic material depicting such a big animal found in an unexpected place can be regarded as an artifact for the scientists' community.

The mistake would have occurred at a certain point of developing one of the techniques used during the scientific process, for example, whilst taking the samples, when processing the genetic information, or in another step of the experiment. Scientists would probably think that one of these would be the cause of the spurious experimental result and this would be the end of the story: apparatuses in the lab ought to be re-calibrated, samples definitely need to be taken more carefully, etc.

Nevertheless, it can't be now proved whether the genetic information of a real elephant was there when taking the sample of the biofilm for further analysis at the lab. At least there is no full evidence of that. Finding a mystical and big animal in such a tiny place like a biofilm astonished me.

1.3. Collaborating with a scientific institution

I have been engaged for some time with the academic and scientific research at a university level and in some institutions (UB - *Faculty of Biology of the University of Barcelona*, & IRTA - *Research Institute for Food Crops and Biotechnology*, Lleida, Spain). Furthermore, I have also been developing art practice-as-research in other centers (Painting, Graphic Work, Photography, Digital Arts at UPF - *University Pompeu Fabra*, Spain, among other centers).

[7] Department of Limnology & Bio-Oceanography from the University of Vienna on line: http://limbo.univie.ac.at/

Both areas of knowledge have been very interesting for me all along, and specially at this time: a scientific institution as a collaboration partner for a master of arts' student looked as a very good opportunity for me. It was very challenging to rediscover research from another new perspective, in between arts and sciences.

And it has been very fruitful period, indeed: this collaboration with the Department of Limnology in the frame of this new Master of Arts might give me the opportunity to approach and acquire understanding from both arts and sciences, which were put somehow together. This mélange allowed me to deal with both disciplines at the same time and to create with my practice some knowledge and new insights in return.

There are some examples of interdisciplinary collaborations, between artists and scientists, in order to share knowledge and create something unique that none could have achieved by his own. However, there is as well the possibility of wandering as an artist in the field of the sciences and vice versa. From my point of view, this transdisciplinary approach provides a really valuable input for any researcher, too. During this collaboration period, I could experience both.

1.4. My role as an external researcher in a scientific institution

The attitude towards the collaboration with the scientific institution was not very clear for me at the very beginning and it has varied: I thought myself…

(a) "should I be a kind of a visiting scientist-artist who may have a clue about what those scientists are doing in the lab, and who tries to translate it to the rest of the world? Should I make science visualisation with pedagogic purposes out of the scientists' concepts and methods, and divulge them to the other people to raise science awareness?";

(b) "should I stand at that institution as an artist who collaborates with a scientific institution and who encompasses his research for the arts with his own discovery and familiarisation with new tools, materials and methods which typically belong to an experimental sciences' lab context? Should I then apply them to my artistic work?";

(c) "should I behave as a very naïve, open-minded and curious person, student of a master of arts, who visits a scientific institution where a collective of people are doing *something strange* to me? Should I be a sort of student who tries to grasp *what's happening there*, and who is constantly expecting something unexpected from the collaboration to happen?"

At first I behaved as (a). I remember during the first semester I was trying to conceive and design some objects or apparatuses inspired in my initial scientific research on biofilms at the institute. I never executed any of them. The aim of the objects was partly sort of science visualisation and so those objects had intently science pedagogy goals. I conceived them as tools to emulate how the microscopic world of the microorganisms and biofilms *supposedly* work (from my point of view, knowledge and intuition) and how to translate this small world into a bigger scale. Of course, my knowledge was growing all along the collaboration, due to

further readings and conversation with the scientists. This means that the design of the apparatuses was evolving and changing as well.

Would I execute those apparatuses, the result would have been either playful interactive devices (some of them non-interactive as well), that could give an approximation to explain in a larger scale how *invisible* microorganisms do behave in their microscopic scale. I guess it would have been helpful for normal people to grasp the essentials of the tiny world, or, at least, my interpretation of such a small scale world.

This work would have probably belonged to the realm of science popularisation at the same time that would probably never absolutely satisfy the scientists. The examples which I was trying to make science visualisation of were not always so easy to represent: the way they occur in nature is not even completely known, they are based on hypotheses. They cannot always be observable to the naked eye. They are rather deduced from the observation with microscopy techniques[8] and other scientific work based on experiments and data. In addition, other factors may also be important, such as intuitive thoughts or skills of the researcher, etc.

As I said, researchers weren't always fully convinced about the validity of the examples I presented as far as those models weren't based on infographic and digital methods representing "true" data. They were more intuitive, imperfect and pedagogic, in order to serve social or school educational purposes. Anyway, my aim wasn't to build up something *fully* representative from a supposed reality. I also wanted to stamp my own print on the works, and so stain them with my mark, with personal degree of abstraction, that would also fulfill my aesthetic intentions. Let's say that I was in the struggle of fulfilling scientists' expectations, and obeying at the same time my design and esthetic rules.

From those non-executed models, I guess some of them would fit better in a technology museum context, rather than in an art museum. Even if I am not fond on very large explicative captions next to describe the art work, some models I designed would really need in a such a technology museum context, when relating them intentionally to the microscopic world, an explicative caption. Some other designs, though, really needn't.

Anyway, at a certain moment I decided that I did not feel any more like doing science visualisation, in the same way that I was not trying to fulfill a sort of semi-scientific aim. I decided, thus, to investigate further ways of creating knowledge.

Later on I was trying to behave as (b) and I was afraid of ending up doing a kind of bio-art or very "high tech-art". I thought that an excessive use of technology (referring to the use of electronic devices as media) in the works might be regarded both as something very sophisticated, but as well as something not new at all. I must say I didn't have full access to all

[8] At the 6th International Conference on Microbial Biofilms (which I will introduce later in this work) I had a chat at a coffee break with the responsible person of Leica Microsystems' exhibition stall, a man called Heinrich Bürgers. He introduced me to some modern techniques exhibited there, like Stimulated Emission Depletion (STED) and Ground Stage Depletion (GSD), which (the latter) resolve down to 20 nm, much below the wave length of the light. He told me that color blindness is definitely a quite common biasing reality: "*You realise that you are blind for a certain color wavelength when looking through a microscope with someone else, this person suddenly tells you –Can you see it? and you answer –No*". A link to GSDIM technology: http://www.leica-microsystems.com/products/light-microscopes/life-science-research/fluorescence-microscopes/details/product/leica-sr-gsd/

the material, instrumental devices and machines that were in the lab, because the inventory is so vast, and in some occasions, lab materials and electronic devices aren't available.

I finally behaved as (c). And considering myself as an art student, and within the scope of this art research related to a scientific institution, I can say that during my collaboration I have been doing a *research for the arts,*[9] in such a way that I have been getting in contact with objects (lab materials and electronic devices) and procedures typical of a sciences' lab, and so my research and work has been nurtured from an *instrumental perspective*.

For me, it was a big challenge to conceive myself like a "new person" who is experimenting in between the fields of art and science. I must say that the science's lab was not that mysterious for me, since I have been working in it a couple of times in my lifetime. However, I took it as a real challenge, and I considered starting to use the multiple possibilities that this atmosphere offered me. This unknown (at first) territory of action conferred me some new ideas and insights, as I said. From another point of view, I could also say that I have been doing *research in the arts*, from a *performative perspective* as long as I played a role of a performative artist with the scientists when interacting with them in order to receive some feedback.

All along this learning period at the Department of Limnology, as I said, I was *researching for/in the arts*, but not only this, I have been furthermore doing research "*on the sciences",*[10] since I have been engaged in reading several scientific papers, which I got thanks to the researchers I was collaborating with at the Department of Limnology and which helped me to understand the underlying concepts and methodologies in the research frame of biofilms. Due to that, I gained some knowledge about biofilms from the perspective of experimental sciences.

In the frame of the collaboration, I have sporadically attended to weekly meetings at the same institute with this group of researchers. At the meetings, they were discussing methods, results of their experiments, other scientists works and papers, etc. I also visited eventually the research institute to meet up some scientists to ask for advice, to have a chat, etc.

One of the first contacts between biofilms and the *Universität für Angewandte Kunst* has been possible because of the excellent logistic tasks organised by the staff and the great seminars and talks known as *Jour Fixe* that are held in Art & Science. This allowed us to have a speech about biofilms held by Tom Battin, on January, 20th 2012.

[9] Terms from Borgdorff (2005) are used in this paragraph. He distinguishes between *research on the arts* (a), *research for the arts* (b) and *research in the arts* (c). Equivalent terms used by Mittelstraβ (2005) might be *research on art* (a), *research in art* (b) and *research through art* (c). There are also some other references to similar concepts defined with anteriority (Frayling, 1993; Read, 1943).

[10] I am using terms originally coming from Borgdorff but applied now to sciences' research.

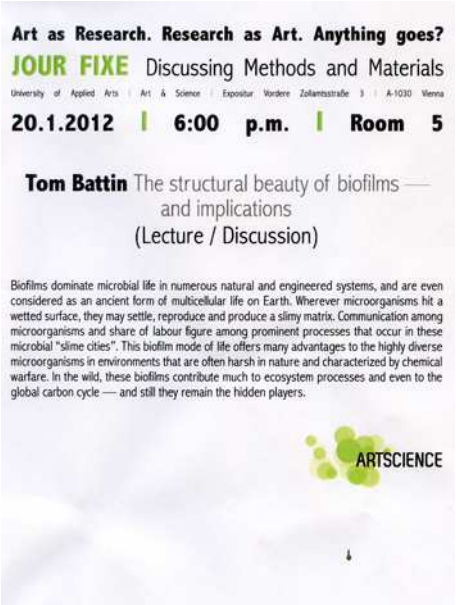

Figure 1.1 *Jour Fixe with Tom Battin. Universität für Angewandte Kunst Wien*

Figure 1.2 *Jour Fixe with Tom Battin. Universität für Angewandte Kunst Wien*

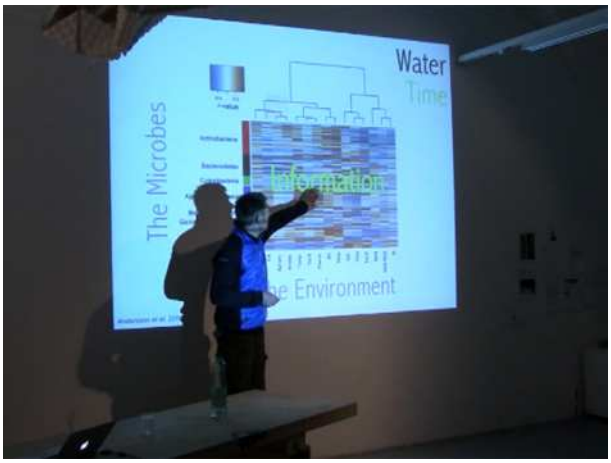

Figure 1.3 *Jour Fixe with Tom Battin. Universität für Angewandte Kunst Wien*

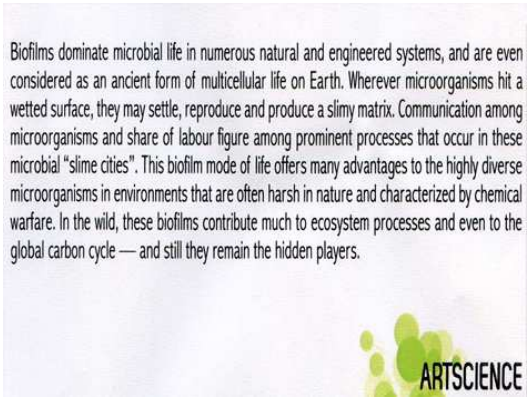

Figure 1.4 *Jour Fixe with Tom Battin. Universität für Angewandte Kunst Wien*

Abstract of the talk he gave.

I also had the opportunity to go to Lunz am See for some days, and to visit the biology institute in there, called *WasserCluster Lunz*[11] *(Interuniversity centre for Aquatic Ecosystem Research)*, a place where the university of Vienna is running a lot of experiments. Tom Battin and his team are quite often there. I went there on a excursion with Sebastian Kienzl (a friend and also student from the Master of Art & Science), Nancy Burns and Amber J. Ulseth (two researchers that are members of Tom Battin's team).

[11] WasserCluster Lunz (WCL) was established to reinvigorate freshwater ecosystem research and education in Lunz and to contribute to the advancement of freshwater sciences. WCL is a nonprofit research center shared to equal amounts by the University of Vienna, the Danube University Krems, and the University of Natural Resources and Life Sciences, Vienna (BOKU Vienna). Read more visiting the link: http://www.wcl.ac.at/

Figure 1.5 *Lunz am See. 4-wheel drive wagon*

Nancy is scrapping the snow from the windshield of the car. This is the vehicle used to move from the streams in the woods to pick samples and to come back to the lab of WasserCluster, next to the lake of Lunz.

Figure 1.6 *Lunz am See. 4-wheel drive wagon*

The vehicle is now parked next to a stream and we are taking a sample.

Figure 1.7 *Lunz am See. Taking samples*

Amber collecting the oximeter-sonds (a device that registers the levels of oxygen in the water for further analyses). In the picture she takes one which was located under the bridge. Sebastian is making a photo of it.

Figure 1.8 *Lunz am See. Poster*

Poster at an office door: “Biofilm Power. No microbe will be left unsequenced”. By “sequencing” scientists and geneticists mean analyse and identify.

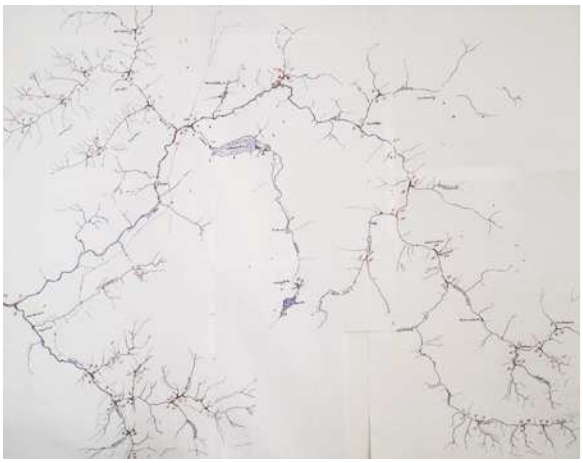

Figure 1.9 *Lunz am See. Hydrological network*

The map is showing a hand-made network with all the small streams drawn by the scientists.

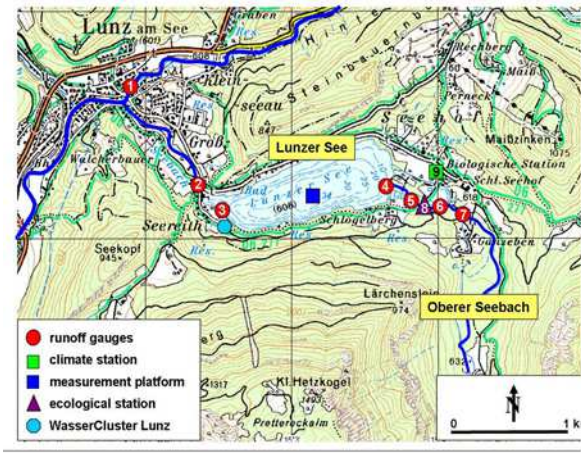

Figure 1.10 *Lunz am See. Hydrological network*

The map is showing the hydrological network. Oberer Seebach is the place where the samples of biofilms were taken for the experiment.

Figure 1.11 *Lunz am See. WasserCluster office*

Some drawers and folders behind a desk in an office at WasserCluster.

Figure 1.12 *Lunz am See. WasserCluster office*

A desk.

Figure 1.13 *Lunz am See. Fieldwork*

Image showing Amber in the river introducing a hose to the basket where the oximeters are, preparing to pump fresh air in order to calibrate them.

Figure 1.14 *Lunz am See. Fieldwork*

Nancy is connecting the pump. Inside the box there is some material they carry together to do this operation.

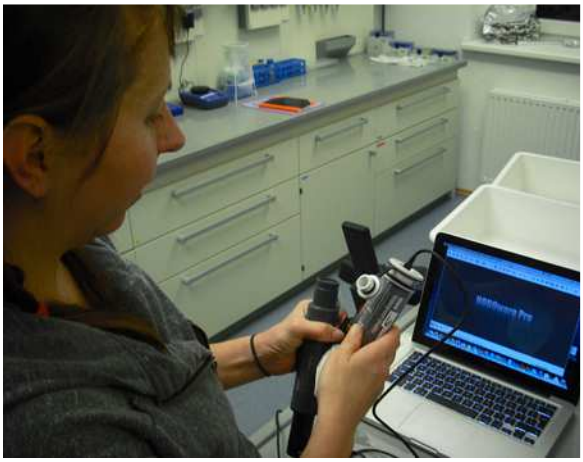

Figure 1.15 *Lunz am See. At the lab*

Amber connects the oximeter to the computer with the help of a cable.

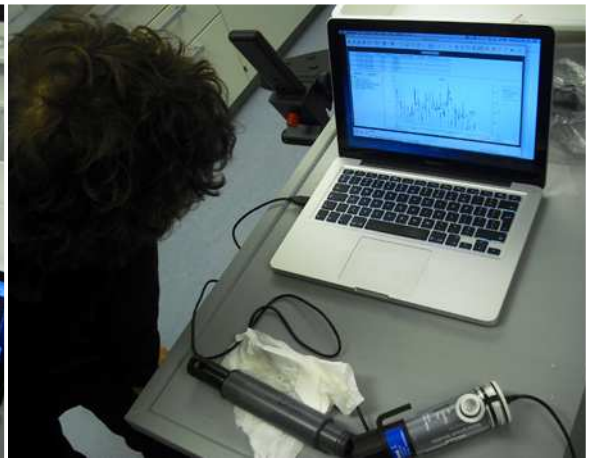

Figure 1.16 *Lunz am See. At the lab*

Sebastian looking at the data on the screen. The graph shows the fluctuating levels of oxygen during the period the oximeters were in the water collecting data.

A guided visit to *Haus des Meeres – Aqua Terra Zoo*[12] with Dr. Mag. Daniel Abed-Navandi, the deputy director and curator of this Viennese institution, allowed me to know more about biofilms and algae cultivation and their importance in aquaria. I went there with Stefanie Koemeda, Solmaz Fahrang and Sebastian Kienzl (friends and students of Art & Science like me).

[12] More information about *Haus des Meeres* can be found in here: http://www.haus-des-meeres.at/

Figure 1.17 *Haus des Meeres. Algal culture stock strains*

Lab with stock strains of green and red algae.

Figure 1.18 *Haus des Meeres. Algal culture stock strains*

Daniel is holding two flasks of red algae stock strains showing the respective color of their cultures in the rear tanks.

Figure 1.19 *Haus des Meeres. Preparation of the phytoplankton*

Daniel is holding two sieves to filter different organisms when preparing the artificial phytoplankton to be added to the aquaria of Haus des Meeres.

Figure 1.20 *Haus des Meeres. Preparation of the phytoplankton*

Daniel measuring a certain amount of water to prepare the feeding solution to be added to the aquaria.

I had as well the opportunity to attend to the *International Conference on Microbial Biofilms – Biofilms 6*[13] (11 – 13 May 2014, Vienna, Austria) with specialists and speakers from all over the world. That allowed me to grasp the state of the arts concerning biofilms and most modern techniques on microscope visualisation.

[13] Information concerning the conference can be found in: https://biofilms6.univie.ac.at/

Figure 1.21 *VI Conference on Biofilms Vienna. Flag at the entry*

Figure 1.22 *VI Conference on Biofilms Vienna. Tom Battin welcoming speakers*

Figure 1.23 *VI Conference on Biofilms Vienna. Back side of the plenary room*

Figure 1.24 *VI Conference on Biofilms Vienna. Presentation of a researcher*

Figure 1.25 *VI Conference on Biofilms Vienna. The coffee break*

Mia from the back chatting with other scientists at the coffee break.

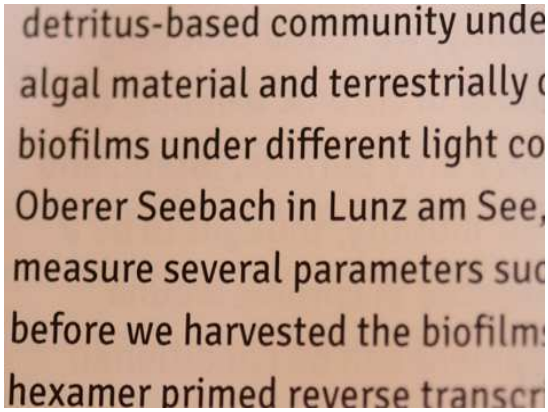
detritus-based community unde
algal material and terrestrially
biofilms under different light co
Oberer Seebach in Lunz am See,
measure several parameters suc
before we harvested the biofilm
hexamer primed reverse transcr

Figure 1.26 *VI Conference on Biofilms Vienna. Catalogue with abstracts of the presentations*

Detail of Mia presentation's abstract based on the experiment done at Lunz am See.

Figure 1.27 *VI Conference on Biofilms Vienna. Exhibitors*

Figure 1.28 *VI Conference on Biofilms Vienna. Exhibitors*

Figure 1.29 *VI Conference on Biofilms Vienna. Posters at the hall*

Figure 1.30 *VI Conference on Biofilms Vienna. Posters at the hall*

Figure 1.31 *VI Conference on Biofilms Vienna. Presentations*

Figure 1.32 *VI Conference on Biofilms Vienna. Closure*

End of a presentation with a “take home message”.

Tom thanks the participants and closes the conference.

2. Biofilms and elephants

2.1. Biology of the biofilms

2.1.1. Definition

In a wide sense, a biofilm is a microecosystem that is formed by different kind of microorganisms, which is attached to a surface and enclosed into a matrix. This definition includes a lot of examples. In particular, the biofilms that are object of study at the Department of Limnology are mainly inland waters' biofilms, which look like slimy films (jelly material, with variable viscosity and thickness). They can be found in every river, i.e. coating the peddles on the streambed. They are basically made of:

(a) a cellular part (lots of different species of microorganisms living in cooperative microcolonies, i.e. bacteria, algae, fungi), and

(b) a non cellular part**,** that consists either of a matrix (formed by different sugar molecules which have stabilizing and vehicular functions between cells) and a void**,** which is represented by pores and empty channels, such as chimneys, full of circulating water, that allow to exchange of nutrients and waste products between the biofilm and its surrounding environment.

Figure 2.1 *Biofilm*

Biofilm in a river, made of a consortium of algae, bacteria, etc.

Figure 2.2 *Biofilm*

Biofilms are found as well on animals' skin, mouth, inside their body, etc.

2.1.2. Components and complexity

In terms of biodiversity, a biofilm is such a complex system, which has a high inner biodiversity referring to number of species it possesses. Specialists prefer to call these species OTU – *Operational Taxonomic Units*. Biofilms may achieve an effective division of work in the microcolony, which is translated in parallel to a varied cell function and so cell specialisation. However, some cells may remain pluripotent and so keep their phenotypic plasticity and some other may even reverse or shift phenotypically backwards, being able to change their shape and functions at crucial moments for the sake of being helpful for the colony.

Microcolonies form together streamers (millimeter long filaments), that are able to flow and oscillate in the water. There is as a communication system or language that interlaces the whole community via signaling molecules, akin to hormones or neurotransmitters in our body. This feature confers the biofilms a category of collective entity, a composite that surprisingly performs in a cooperative way. This language, to which can refer now as a *global sensing* allows the whole colony to work as if it were a super organism, and enables the members of the colony to be interconnected, and so to be a part of a macrostructure that behaves as such.

The hydrodynamic and turbulent conditions of the flux of the streams (a), together with the whole signaling and communication system (b), and the phenotypic plasticity (c) of the cells in the biofilms, make the biofilms quite complex structures that are very difficult to understand from either a physical (a), chemical (b) and biological (c) point of view. In addition, the scales in which these disciplines are mostly determinant are millimetric (a), submillimetric (c) or micrometric (b). This particularity make the biofilms so complex structures and so their study requires a multidisciplinary work team working in different areas to be connected.

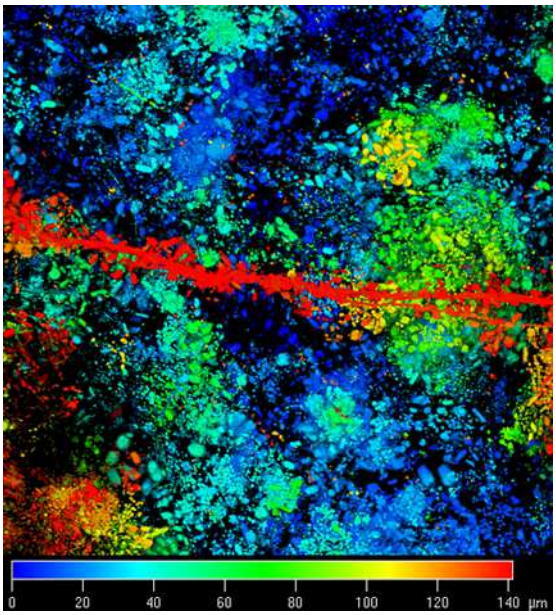

Figure 2.3 *Biofilm*

Microscopy image (provided by Katharina Besemer, member of Tom Battin's research group) of a biofilm with a special technique that colors the entities (algae, bacteria, etc.) in different hues depending on the distance to the viewer.

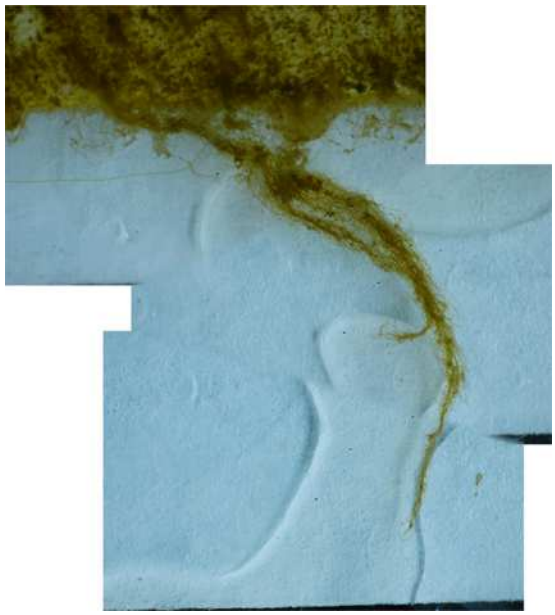

Figure 2.4 *Biofilm*

Composite image (provided by Katharina Besemer, member of Tom Battin's research group) of a biofilm showing a *streamer*, or millimetric structure on a mature biofilm.

2.1.3. Formation of the colonies

There are numerous scientific data showing that biofilms do behave as dynamic structures which evolve and change their shape in time and space. The growing patterns of the colonies of biofilms in inland waters, and the cell distribution in those, deviates notably from randomness, since the first steps of settling down onto a surface, i.e. a peddle on the streambed. This gives an important hint to the researchers: the basis of the growing processes should be based on something different from merely stochastic processes. On the contrary, exposition to turbulence conditions of the flux as well as intraspecies and interspecies symbiotic associative patterns play an important role. This means, at a molecular level that their growing and distribution patterns seem to be guided by different factors, such as the communication system or language I mentioned before, that interlaces the whole community via signaling molecules.

Let's see briefly the main steps of their life cycle:

1) cells hit a surface (some of them have a *flagellum* or *pili,* which are locomotive structures);

2) some of them (primary cells) underlie a phenotypic shift in shape and colonise the surface becoming sessile. They do not distribute randomly on the surface. They adhere both the surface or other already attached cells. Deposition has already started. Interaction and cell cell communication starts as well;

3) slime production begins; proliferation or expansion of the colony begins;

4) microcolonies grow by cellular reproduction producing the "mushroom-like structures". In the mature structure it is possible to identify the canopy (the mushroom cap) and the basis (pores and channels in between the mushroom stalks). This resistance structure provides shell, but also allows an active flow of water through the pores and channels to the top of the structure so there's a flux of nutrients and metabolites;

5) once the colony is mature, some specific cells undergo a reverse phenotypic shift (they get the *flagellum* again) and migrate or detach from the biofilm. There is also a cluster coalescence phenomenon (attachment of cells to already existing clusters). Coalescence and migration determine the size of the distribution in space and they both take place following the direction of the flux (downstream).

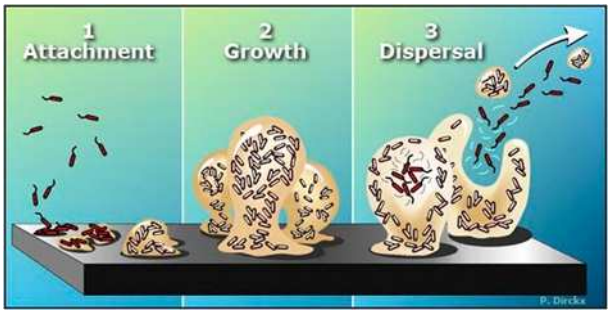

Figure 2.5 *Biofilm growth and life cycle*

Graphic showing a simplified model of the main phases that a biofilm undergoes.

2.1.4. *Talkative* biofilms

Scientists refer to the mature structure of a biofilm as being "talkative" meaning that a communication system is established between distanced cells within it. It means that some bacterial cells from the same colony, even if relatively far distant in space from each other, are able to communicate via signaling molecules.

This system is also called *Cell Cell Communication* or *Global Sensing cell-to-cell*, and would integrate all the members of the colony as if it was a super organism. This particularity enables the colony to be interconnected, and so to "behave" as a macrostructure, a multicellular organism. This bunch of structured cells are organised as *intentionally* regarding to the benefits of a societal division of work, as the *insurance hypothesis*[14] proposes. The colony is able as well to take *group decisions*[15] as a single multicellular organism does.

There are a lot of expectations put upon this language, also called *Quorum Sensing,*[16] which claim that it would play a global role not only within intra-species interaction but also at an inter-species communication level, functioning as a sort of Esperanto. Therefore, it would "connect" all the living forms on Earth,[17] as long as biofilms are ubiquitous on the planet and coexist intimately with all species. Researchers are enlightened by this very *modern* (and *old* at the same time) language of the microorganisms, which is at the moment barely intelligible, although it may be a completely meaningful language. In a scientific race and willing to decipher what messages this language conveys, researchers try to grasp its mode of action by interpreting the code of an encrypted language, that has always been there but never before came into question.

2.1.5. Biological importance

Biofilms are supposed to be the within the first forms of life on Earth, with fossils dating back over 3 billion years. Furthermore, they have a strategic position in some evolutionary theories as precursors of primeval forms of multicellular organisms. They are actually the dominant way of microbial life on Earth nowadays and represent almost 50 percent of the biomass of our planet.

Ecologically, these microecosystems are important for the carbon cycle, which make them crucial concerning the global warming phenomenon.[18]

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[14] See "*Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis*". Shigeo Yachi; Michel Loreau. Source: http://www.pnas.org/content/96/4/1463.full

[15] Other interesting related concepts are *microbial intelligence* and *collective behavior*.

[16] See "*Quorum sensing in bacteria*". Miller, M.B.; Bassler, B.L. (2001). Annu. Rev. Microbiol.

[17] As I will cite at the point [2.3] of this work, Farooq Azam (invited talk at the opening of the conference Biofilms 6 – Vienna) considers the whole ocean as microbial biofilm, and he speaks of it as a big "connectome" or "interactome".

[18] This is so because the algal component in them is responsible for the fixation of a certain amount of the atmospheric CO_2. This means that they are indeed sinks in which the atmospheric carbon can be absorbed and

This is the reason why some modern geo-engineering practices[19] focus on biofilms as a troubleshooter to fight against human impact on Earth. An example based on the "*iron hypothesis*"[20] showing the importance of phytoplankton (water biofilms) follows:

Changes in iron supply to oceanic plankton are thought to have a significant effect on concentrations of atmospheric carbon dioxide by altering rates of carbon sequestration, a theory known as the "iron hypothesis". For this reason, it is important to understand the response of pelagic biota to increased iron supply. Here we report the results of a mesoscale iron fertilization experiment in the polar Southern Ocean, where the potential to sequester iron-elevated algal carbon is probably greatest. Increased iron supply led to elevated phytoplankton biomass and rates of photosynthesis in surface waters, causing a large drawdown of carbon dioxide and macronutrients, and elevated dimethyl sulphide levels after 13 days. This drawdown was mostly due to the proliferation of diatom stocks. But downward export of biogenic carbon was not increased. Moreover, satellite observations of this massive bloom 30 days later, suggest that a sufficient proportion of the added iron was retained in surface waters. Our findings demonstrate that iron supply controls phytoplankton growth and community composition during summer in these polar Southern Ocean waters, but the fate of algal carbon remains unknown and depends on the interplay between the processes controlling export, remineralisation and timescales of water mass subduction.

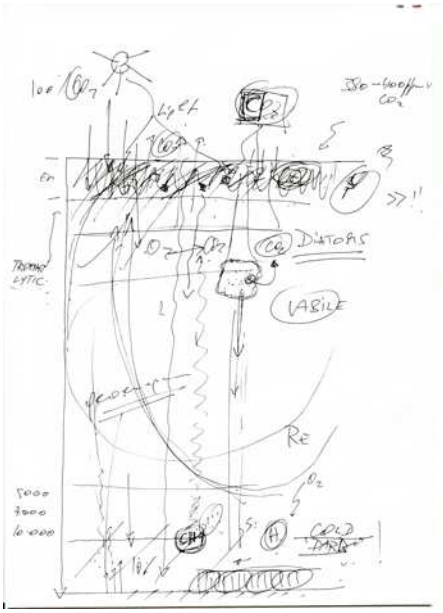

Figure 2.6 *Ocean stratification and geo-engineering*

Tom Battin's drawing of the ocean stratification in order to explain the "iron hypothesis" strategy in geo-engineering.

Even if inland waters' biofilms apparently don't seem to be much relevant concerning the global amount of water reservoirs in our planet, they are. Small inland streams' biofilms aren't negligible and must be considered in the context of a global planetary scale, together with the oceans. This is Tom Battin group's policy.

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trapped, reducing its presence in the atmosphere. Since CO_2 is a determinant greenhouse gas and its production is highly related to human activity, becomes essential to pare attention to biofilms and understand their physiology.

[19] "A review of potential impacts of geo-engineering on ecosystems and biodiversity. Cambridge conservation Initiative – Transforming the landscape of biodiversity conservation" from Smith, R.K., Smith, S., Sutherland, W.J., 2012 is a reference document that summarises all the strategies developed up till our days. There are some practices involving directly biofilms.

[20] This is an abstract from: Boyd, W.B. et al., 2000, A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization, *Nature*, Vol. 407, also cited at point [4], within the in the works.

2.2. About how I found an elephant in a biofilm

2.2.1. The lab: an example of an *in vitro* place. Classical approaches to microbiology

A laboratory is a room or building with scientific equipment for doing scientific tests or for teaching science, or a place where chemicals or medicines are produced. In extension, it might be a place where some research is going on, and even artistic research.

Scientists refer to *in vitro* meaning that they take some live form from its natural place in the nature and put it in on a glass plate, or keep it inside any other container, in a lab. By doing this, researchers keep *captive*, and so *tame* their samples of living forms.

By the end of the 17th century, the Dutch scientist and tradesman Antonie van Leeuwenhoek had a virtual monopoly on microscopic study and discovery. He is commonly known as "the Father of Microbiology", and considered to be the first microbiologist. He is best known for his work on the improvement of the microscope and for his contributions towards the establishment of microbiology. He developed his method for creating powerful lenses and applied them to study of the microscopic world.

In 1665 his contemporary English natural philosopher and polymath Robert Hooke published *Micrographia*,[21] a book describing observations made with microscopes and telescopes. Hooke coined the term *cell* for describing biological organisms. He used this term because of the resemblance of plant cells to monks' cells.

Louis Pasteur was a French chemist and microbiologist renowned for his discoveries of the principles of vaccination, microbial fermentation and pasteurization.[22] His work has been very important for the analysis of causes and preventions of diseases, and his discoveries have saved countless lives ever since.

Although the historical importance of approaching to the microorganisms by observing and characterizing them under the microscope (Leeuwenhoek or Hooke), passing through other approaches based on selecting them and cultivating them *in vitro* (Pasteur), new approaches to microbiology were subsequently developed. They weren't focusing to microorganisms from the classical perspectives of parasitological or pathogenic effects on the humans (or on other animals and plants which may be of human interest), but in a wider range.

This sort of studies and research lines have been also achieved by cultivating in the lab the microorganisms collected from the nature. However, it has been also demonstrated that microorganisms also respond to stress conditions (let us say that they also react to the "white coat hypertension effect") and so the results of scientific research might be biased by this non-natural environment of a lab. In other words, a certain analysed species might behave so

[21] Link: http://en.wikipedia.org/wiki/Micrographia

[22] An interesting reading related to microbes is "Where were microbes before Pasteur", from chapter 5 ("The Historicity of Things"), from the book *Pandora's hope – Essays on the Reality of Sciences Studies*. In this work, the author analyses whether we could say that the microbes did exist before they were discovered. Bruno Latour. Harvard. 1999

differently *in vitro* / *in situ* that somebody could look to it as if it were two different species, when it's only one indeed. Another inconvenient of *in vitro* classical approaches is that many species of microbes and other microorganisms withstand in a certain degree the cultivation methods in the lab, inside a glass container.

Due to these reasons, a modern ecological perspective has been developed by scientists, who focus now on the behavior of the microorganisms in their natural milieu. The new approach tries to evaluate the importance of the microorganisms and the role they play in an ecosystem in which they are interacting with other microorganisms, bigger organisms, or even with inorganic things. This environmental approach provides an optimal framework to analyse biofilms.

2.2.2. The wild: an example of an *in situ* place. Modern approaches to environmental microbiology

A new approach to microbiology, focused on the microorganisms' colonies *in situ*, rather than *in vitro*, has led the scientists' community to a new *environmental microbiology*, also called *microbial ecology*. As I said in the former point, this new approach solves some troubles that scientists observed in the lab: i.e. many microorganisms withstand the cultivation methods in the lab, or at least they behave differently. This fact really limits the possibilities of microbiology and biases many of the experimental results. In the particular case of studying biofilms, the environmental approach seems to be quite optimal.

New techniques like metagenomics, metatranscriptomics, and many other, which are based on genetic engineering, allow microbiologists to obtain more reliable results when identifying the species at the samples *in situ*. Indeed, these two techniques are lab techniques as well, based on PCR[23] methods. However, they are included in this point of the current text, which is related to *the wild*, because they use fresh samples taken from nature (coming from the wild, *in situ*) to be processed. This means that the samples weren't cultivated in the lab. The organisms are only processed in the lab.

Metagenomics are used in molecular phylogenetics[24] and they are based on the recognition of a specific sequence of rRNA[25] which is meant to be specific of a single species. Then, they may be used to recognise the *quality* of the sample analysed, that is how many different species are there in the sample.

[23] PCR or Polymerase Chain Reaction is a biochemical technology in molecular biology used to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

[24] Molecular phylogenetics (also known as DNA taxonomy) is the branch of phylogeny that analyses hereditary molecular differences, mainly in DNA sequences, to gain information on an organism's evolutionary relationships. The result of a molecular phylogenetic analysis is expressed in a phylogenetic tree.

[25] rRNA (ribosomal RNA) referred in here is the one found in the small subunit (called 16 S) of the ribosomes in prokaryotic cells, which is equivalent in eukaryotic cells to one found in their ribosomes' small subunit (18 S). Although we refer here directly to rRNA, this technique is indeed using the PCR-amplified rRNA gene from the cDNA (circular bacterial genome) in the case of bacteria.

Metatranscriptomics is a similar technique based on the recognition of the transcripts[26] (mRNA and tRNA) which are also quite specific for different species, but in addition, they give also information not only about the *quality* of the sample, but also about the *quantity*, or the number of individuals from each species that are in the sample. This *quantity* is equivalent to the amount of activity performed by the microorganisms by producing the transcripts.

The two techniques combined together can give a quite accurate profile of what species of microorganisms are in the sample and how many individuals of each species are there. In other words, they provide an instant picture of the analysed sample, i.e. a biofilm.

Nevertheless, to process and visualise the data gotten from metagenomics and metatranscriptomics, scientists use a special software (i.e. MEGAN) and some available databases in order to display the results in a more intelligible fashion. There are some databases which compile the work of other phylogenetic studies done till now. They provide more reliable information as they are continuously growing. These resources are available on line and they provide information about sequenced genomes. These resources have also applications that allow the user to introduce a genetic sequence and compare it with a database.[27] The dataset must be prepared to be used with MEGAN. [28]

This fruitful combination of techniques (metagenomics, metatranscriptomics and digital data treatment – *bioinformatics*) is a powerful means of assessing the whole *in situ* biological communities and can provide an *accurate*[29] "photography" of a sample, which displays the three domains of life that are living together in that sample. The domains are *Archaea* (1) or primeval bacteria, *Bacteria* (2) or all the other bacteria, and *Eukarya* (3) or the rest of living beings.

Like the authors from MEGAN say in their website:

The first three basic computational tasks for such [big amount of] *data are taxonomic analysis, functional analysis and comparative analysis. These are also known as the "who is out there?", "what are they doing? " and "how do they compare?" questions. They pose an immense conceptual and computational challenge, and there is a great need for new bioinformatics tools and methods to address them.*

[26] As the central dogma of molecular biology figures it out, proteins are the result of gene expression, and transcripts are mRNA (messenger RNA) and tRNA (transfer RNA). In a first step of gene expression, called *transcription*, they are formed out of a single string of the double DNA helix that serves as a template. They will be used, in the second step of gene expression, called *translation*, to ensemble proteins. tRNA will become part of the ribosomes and mRNA will be used for the ribosomes as a blueprint to create proteins.

[27] Some examples are BLAST (Basic Local Alignment Search Tool) by NCBI (National Center for Biotechnology Information). Links: http://blast.ncbi.nlm.nih.gov/Blast.cgi and http://www.ncbi.nlm.nih.gov/

[28] To prepare a dataset for use with MEGAN, one must first compare the given reads against a database of reference sequences, for example by performing aBLASTX search against the NCBI-NR database. The file of reads and the resulting BLAST file can then be directly imported into MEGAN and the program will automatically calculate a taxonomic classification of the reads and also, if desired, a functional classification, using either the SEED or KEGG classification, or both. The results can be interactively viewed and inspected. Multiple datasets can be opened simultaneously in a single comparative document that provides comparative views of the different classifications.

[29] *Accurate* is used in here in a special way. As the developers of MEGAN put it in a paper from 2007, which is cited at point [4], the sensitivity and power of MEGAN directly relies on the amount and quality of existing databases: "*Goals of metagenomics studies include assessing the coding potential of environmental organisms, quantifying the relative abundances of (known) species, and estimating the amount of unknown sequence information (environmental sequences) for which no species, or only distant relatives, have yet been described.*"

2.2.3. Concrete experiment settings and context of the finding

In the frame of an experiment run by a research team of the University of Vienna – Department of Limnology, a sampled biofilm coming from Lunz am See (Lower Austria) was analysed by the scientists. The aims of that concrete experiment were to obtain a species profile about the microscopic communities of the biofilms and their metabolic activity.

Two different samples were coming from a stream: the first sample was taken from an area under a shade and the second from an area that wasn't under shade, so it received directly the solar radiation. The aim of the study was to analise whether there are significant differences in species composition in both cases. After extracting the genetic material and preparing it for the subsequent analysis, genetic information was processed by the software MEGAN. The results yielded an unexpected biofilm composition: a member of the taxonomic family *Elephantidae* (an elephant) surprisingly appeared within the species profiled by the software.

I hereby attach the abstract about this experiment a member of Tom Battin team, Mia Bengtsson, who presented this work for the 6th International Conference on Microbial Biofilms –Vienna 2014 in a presentation included within the section "Omics": [30]

Monday, May 12th 2014

08:30-09:50 Omics for the study of biofilm structure and function – Jean-Marc Ghigo (Chair)

09:35 Mia Bengtsson (Univeristy of Vienna) Impact of shading on the food web structure of phototrophic stream biofilms elucidated by metatranscriptomics

Team: Bengtsson, Mia (Department für Limnologie und Ozeanographie, AUT); Wagner, Karoline (University of Vienna, Vienna, AUT); Urich, Tim (University of Vienna, Vienna, AUT); Burns, Nancy (University of Vienna, Vienna, AUT); Schwab, Clarissa (University of Vienna, Vienna, AUT); Battin, Tom (University of Vienna, Vienna, AUT)

Phototrophic stream biofilms are highly diverse communities featuring multiple trophic levels with organisms representing all three domains of life. It has previously been difficult to study natural biofilms in their entirety due to their inherent diversity of microscopic organisms. Whereas molecular techniques such as PCR amplification of ribosomal RNA genes and subsequent sequencing has shed light on mainly bacterial community members, eukaryotic microorganisms such as protists and fungi have been overlooked due to the lack of suitable PCR primers. In addition, microscopic identification of eukaryotic microorganisms, including algae, is often difficult and laborious. Therefore, we designed an experiment to study community dynamics in natural stream biofilms using metatranscriptomics, targeting ribosomal RNA transcripts of all three domains of life.

We hypothesized that manipulation of light, the primary resource of these biofilms, would alter the structure of the foodweb, shifting the community from an algal-dominated state under high light conditions, featuring organisms adapted to grazing on and degrading fresh algal material, to a more detritus-based community under low light conditions, with organisms adapted to utilization of dead algal material and terrestrially derived dissolved organic matter. We grew phototrophic stream biofilms under different light conditions ranging from full light to 73% light exclusion in the stream Oberer Seebach in Lunz am See, Austria. Transferring the biofilms to microcosms allowed us to measure several parameters such as gross primary production, nutrient uptake and DOC uptake before we harvested the biofilms for RNA extraction.

Extracted RNA was subjected to randomhexamer primed reverse transcription into cDNA, which was sequenced using Ion Torrent technology. We generated in total 10.4 million RNA reads and used the small subunit (SSU) rRNAs

[30] Omics refers to genetic engineering techniques, such as metagenomics and metatranscriptomics, as they put it in the catalogue of the 6th International Conference on Microbial Biofilms, 11-13 may 2014, Vienna, Austria.

for deep 3-domain community profiling with the CREST toolbox. 68% of SSU rRNA transcripts originated from Eukaryota, 31.8% from Bacteria and <0.1% from Archaea. Diatoms of the genera Achnanthes, Nitzschia and Navicula were the most abundant phototrophic organisms closely followed by Cyanobacteria related to the genus Leptolyngbya. Among the protists, the Cercozoa dominated.

The shading treatment had clear impacts on the composition of the phototrophic portion of the community and also had cascading effects which were evident on certain bacterial groups, for example the Planctomycetes. Overall however, the biofilm foodweb structure was relatively stable across the different light treatments, indicating a robust assemblage that is to some extent independent of light availability.

This study shows that metatranscriptomic techniques are extremely powerful when analyzing complex biofilm communities. Further, the compositional robustness of our studied biofilms raises interesting ecological questions concerning a homeostatic versus dynamic nature of microbial communities.

Let's see now the representation of the species' composition done by MEGAN:

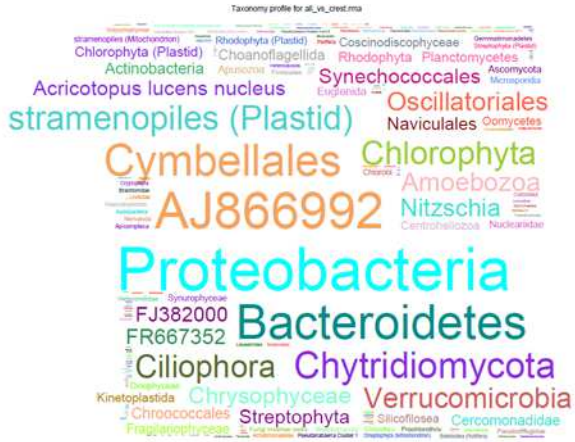

Figure 2.7 *Species' composition by Megan*

Names cloud mode representation done by MEGAN. Due to the size, it's not possible to see "*Elephantidae*". However, this graph was originally a vectorial draw (pdf) and I will present a zooming presentation in the next figure so the reader can find the word "*Elephantidae*". In here, "*Elephantidae*" is represented in a very small typography (in this graph the size of the species' typography is proportional to the number of members it has) and it's almost invisible.

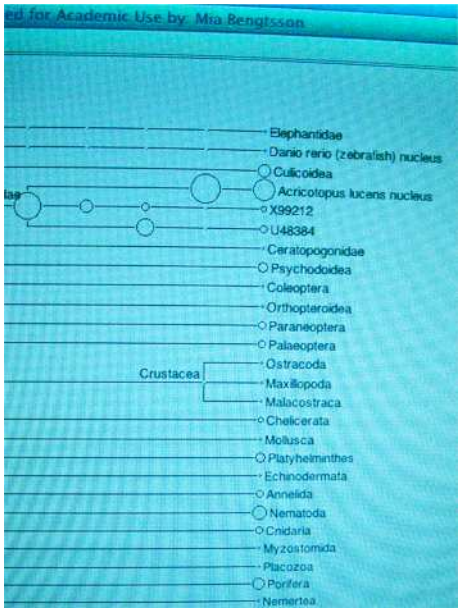

Figure 2.8 *Species' composition by Megan*

Screenshot of the cladogram mode representation done by MEGAN. "*Elephantidae*" appears at the top.

Figure 2.9 *Species' cloud by Megan. Zooming presentation*

This composition shows the names cloud of the species identified by the software MEGAN in Mia's experiment. It is zooming from the left-up figure to the right-down figure in order to detect "*Elephantidae*".

I remembered I was very surprised when I discovered an elephant in a MEGAN graph which Mia displayed in her computer. That day, just some time before, we were having a chat about those techniques I just presented before: metagenomics and metatranscriptomics. I had some questions about ribosomes and genes and about the trustworthiness of the software and existing databases or libraries with genomes. I am not worried about whether a computer program which compares strings and detects levels of significant differences works reasonably well. It might work well, and it saves time, for sure. It might have a mistake as well, which haven't been reported yet. However, computers' job is to read through and compare simple nucleotide strings, not grammatical sentences with hidden messages.

If the two Omics-methods here presented are analysed more in detail, somebody probably may re-think about the possibilities of finding an elephant in a biofilm. Let's consider on the one hand the first Omics-technique, metagenomics. First, it is really fascinating how scientists categorise life forms in a sample by analysing the genome of the whole sample at once, working with hundreds of species mixed together. This is a kind of "*environmental DNA* approach",[31] which makes me think about contamination of water by the feces, urine or skin cells of a "real" elephant. Anyway, scientists are chasing concrete sequences of genome, not external appearances of the cells.[32] Remember the message of *Figure 1.8* ("*Biofilm Power. No microbe will be left unsequenced*"). The genetic approach is faster than any observational. When organisms are classified by their genome (no matter how different in size and shape are the organisms), no other information is required, but their genomic sequence.[33] Then, as a corollary, the more different the whole genomes are, the more different the organisms look from each other. However, in evolutionary biology, a group of organisms share common descent if they have a common ancestor. There is strong evidence that all living organisms on Earth are descended from a common ancestor, called the *last universal ancestor* or LUA (or *last universal common ancestor*, LUCA).

[31] Originally, *environmental DNA* refers to a new approach to monitoring species in freshwater environments. The technique is based on the fact that all animals that live in water leave DNA behind via their feces, urine or skin cells. This DNA dissolves in the water and becomes diluted as it spreads over a larger area. This external DNA is called environmental DNA (eDNA). By taking water samples and analysing them for eDNA, it is possible to show the presence of a species without actually needing to catch individuals or even see them. The great advantage of using the environmental DNA technique is that generally the chance of detecting a species is higher than with traditional surveying techniques, such as visual observation, electro-fishing, dip-netting or using fykes. Moreover, it costs much less time and effort (and therefore much less money) to confirm the presence of a species or generate a list of species.

[32] Notice that this method is opposite in fashion to the method some lab technicians apply, who count number of cells per cubic milliliter of a fluid with the help of a microscope. They base their experience on recognising the external shape of the cells. Sometimes, properly stained and cultivated strains of bacteria, makes their work easier and yields more reliable results.

[33] Isolated genomic sequences are indeed not so complex in terms of how many combinatorial elements are combined together. They are sequences of only 4 elements (nucleotides) repeated in a mysterious fashion. However, from a point of view of some complexity theories, the phenomenon of live is more complicated. Two examples: a) the Algorithmic Complexity (AC, or also called Algorithmic Information Content - AIC, which was independently developed by Kolmogorov, Solomohownoff, and Chaitin) of a given system, would categorise a genome as a quite disordered system, so quite low compressible, and with a quite high information content; b) the Effective Complexity of a given system (EC, as defined by Gell-Mann) measures the complexity of a system in terms of relevant information content, but not in terms of size nor randomness. In this case the genome would be a super complex structure, which is neither a very disordered (fully random) nor very ordered (fully structured) system.

To have an idea about how much computerised is the process of discovering, let's have an example: I remember that Mia told me, once she saw the results from MEGAN: -"Let's see what this species looks like: *B342190,* or *Kinetoplastidia*,… Ah, it's an algae!" or whatever, and so on. She didn't have a reference of all the species depicted by the software (which are included in the databases). Instead, she had to look every time to google images to get a representative image of such a species, to recognise it, or, at least, to classify it.

On the other hand, and concerning the second Omics-technique presented here, metatranscriptomics, it has important implications when detecting what species and how many individuals are in a biofilm. This technique evidences the presence of the organisms that are active in the sample analysed. In other words, this technique is not that sensitive to depict the species that are there in the sample but somehow aren't active for any reason. However, the combination of the two Omics seems to provide a quite fairly species' composition.

A corollary of this is that scientists count the microscopic species not only in relation to their genomic presence, but in relation to their "performative presence",[34] too. This raises the observation that a microorganism is more likely to be observed when it is *doing something*, that is, performing an action, i.e. interacting with its environment in a metabolic way. Translated into cell biology words, it implies that its ribosomes are active. And, at this point, I must say that I was sceptic about the supposed correlation between the number of ribosomes and the rate of gene expression (*translation*) of the microorganisms. The ribosomes of a cell provide a machinery to produce proteins. That is true, but does it exist a so fixed correlation between the number of ribosomes or their rate of activity and the amount of proteins generated by them? I don't know the answer, and needs further investigation. The central dogma of molecular biology confers an important role to the ribosomes, but of course there is a whole cascade of events in the regulation of their activity, and so a lot of unknown steps.

As I said in the beginning of the current text ("Motivation", at point [1.2]), there was the option to just forget about the result of the elephant, not taking it to consideration, due to its low probabilistic significance and/or real relevance for the results of the experiment. Anyway, and as a further step into my aim of making sort of science visualisation in the very beginning of the collaboration, I felt very attracted about that elephant and decided that I should somehow show my finding.

"What if an elephant could really be in there?" –Should be then a small one, a microminiature[35]. What a paradox: the elephant is the heaviest land animal on Earth and there was one in such a small place where only bacteria and other microscopic organisms normally can live. Otherwise, should have been traces of its genetic fingerprints which interfered into the process? Environmental DNA interference? Waste DNA that somehow got there from Viennese Schönbrunn Zoo? I thought as well that the elephant was there, in the woods, some

[34] Microorganisms which are more active (meaning that they intake more nutrients or produce more proteins) are the ones that can be profiled in the results of metatranscriptomics or, at least, to not being neglected.

[35] Such a small elephant would be a good candidate to become part of a "cabinet of wonders", to be displayed in the section of miniatures, next to a microminiature of a waving John Paul II in full Papa regalia which is built alongside the eye of a needle. (Reference to marvels in between art and science from "A Wish upon a Piece of Hair" from Part I of Lawrence Weschsler's book *Mr. Wilson's Cabinet of Wonder*. Vintage. New York. 1995.)

hours before scientists went there to get the sample, and it escaped some minutes before perceiving the arrival of the scientists.

"An error occurred!" –someone would have thought. However, in case of being a mistake caused by a contamination of the sample, shouldn't have been more evident to have a human being profiled in the results, rather than a rare elephant? Mia said that *this* must have been an error. Nevertheless, she also thought that in case of being a mistake caused by a contamination of the sample, it should have been more evident to have a human being in the plot, rather than an elephant.

To finish with this point, I would like to say that other mistakes might have occurred during the whole process of identification of species, but weren't probably perceived, like it happened with the elephant. And why not? Because there isn't a clear idea about *the exact* and vast spectrum of different species that are present in a concrete sample of a biofilm.[36] An example: some of the species are quite unknown species, not so common. In case the software committed a mistake by characterising a species of a rare bacteria instead of another one, which is also not very likely to be found in a biofilm, nobody would have notice it. I don't know with precision what mistakes is the software likely to commit. This needs further investigation. Of course, I quite blindly rely on technology, except in cases when I stumble upon something *too* strange.[37]

As I already mentioned in a footnote (*28,* at point [2.2.2]), MEGAN recognises and compares sequences which are then assigned with a high probability to a certain species. The assignments are done within a pool of known species (already identified species). This means that there are only matches with known species, and not assigned DNAs remain without being assigned to any group. This is a metaphor to illustrate that "scientific knowledge is constructed upon scientific knowledge." The authors of MEGAN put it the following way, as comparing it to "environmental forensics", so meaning that "there must be an actor behind every action":

Metagenomics has been defined as "the genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms" (Handelsman 2004), and its importance stems from the fact that 99% or more of all microbes are deemed to be unculturable. Goals of metagenomics studies include assessing the coding potential of environmental organisms, quantifying the relative abundances of (known) species, and estimating the amount of unknown sequence information (environmental sequences) for which no species, or only distant relatives, have yet been described. It is useful to extend Handelsman's definition to also include sequences from higher organisms as well as just microorganisms, thus opening the door to "environmental forensics."

[36] As I said at the point [2.1.2] about "Components and complexity", scientists usually speak of OTUs (Operational Taxonomic Units) instead of "species" when referring to biofilm species' composition. The boundaries between two different species is not so clear in such a dynamic and "phenotypically plastic" system.

[37] I remember that Mia, reflecting on the elephant finding, told me during a coffee break at the 6th International Conference on Microbial Biofilms, that "*you* [She meant *herself*, but this sentence can be applied to any scientist] *should use these methods with proportion and you have to use your biological knowledge to verify your findings because, of course, I know that there are no elephants in Lunz, then I can adjust the parameters, so I don't see an elephant.*"

2.3. Reflections

Even if a scientific mind would probably first think of an error occurred during manipulation, or calculation, or within the databases, I thought I shouldn't *get rid of* the elephant so fast. It could give an important hint. It was an unexpected finding, and like always happens, it brings a lot of information along.

In this fashion, I would like to mention here the work "Arbitrary hypotheses" (by Stefanie Koemeda, Solmaz Fahrang, Sebastian Kienzl and me) presented at the Crucial Experiments in occasion of Vienna Art Week 2013.[38] The aim of the exhibition was to re-enact scientific experiments that are considered as crucial. In our particular work, the way in which the hypotheses were formulated provided very specific insights into topics that are probably not considered as essential by traditional experimental culture or common intuition. Their design aims at showing what a reality is not like, rather than showing what it is like. The approach was based on a Popperian concern that avoids to come to a certain conclusion by means of inductive reasoning, but proposes an alternative course of action: formulate any hypothesis and then try to falsify it by finding a contradictory example. Accordingly, a hypothesis can be unequivocally falsified and assigned to the realm of the "non-real". Every hypothesis that is not thoroughly disproven, however, is still part of the "potentially-real" where it remains until it is falsified. The "real" thus becomes a collection of verified possibilities.

The elephant, that could be regarded as a spurious experimental result, may also be also regarded as an anchor point to develop further debate and knowledge. It was an unexpected and serendipitous finding, in a remote place somewhere in between a lost place in the woods of Austria and a lab in the city of Wien. A marvel that appeared all of a sudden.[39] A totemic figure, a virtual one, arose from some place in between the captive and the wild.

To finish with a further reflection about biofilms, I would like to mention the abstract of the invited talk by Farooq Azam for the opening of the 6th International Conference on Microbial Biofilms –Vienna. Azam's approach considers the ocean as a biofilm. In such a "poetic" way, the oceans (and so the biofilms) are the bridge (the "connectome") that connects different living forms in the water. The text follows in the next page.

[38] A link to *Crucial Experiments* in Art & Science website: http://artscience.uni-ak.ac.at/jart/prj3/art_science/main.jart?rel=de&reserve-mode=active&content-id=1348219249457&projekt_id=1377499933131&page=2

[39] In the novella *The Little Prince*, from Antoine de Saint-Exupéry, the six years-old protagonist sees a picture in a book called "*True Stories from Nature*" in which a boa constrictor is swallowing an animal. He, then, pondered deeply and made a drawing of an elephant which had been already swallowed by a boa constrictor and was being digested in its stomach. Afterwards, he showed his drawing to the grown-ups and asked whether they felt frightened of such a drawing. As far as the grown-ups interpreted a kind of panama-hat represented in the drawing, they answered by saying that why should be they scared of a simple hat. Chapter 1 of the book. First edition from 1943.

Ocean as Microbial Biofilm

Azam, Farooq (Scripps Institution of Oceanography University of California San Diego, La Jolla,USA)

Microbes are a major component of ocean biomass, abundance, biodiversity and metabolic activity, therefore, it is important to understand how their in situ activity structures the ocean ecosystems, and response to climate change. Despite major strides in describing microbial diversity and genomic predictions of activity we lack knowledge of microbes' interactions with other microbes and the architecture of the habitat in which these interactions play out. Considerable data now show that seawater is replete with nanometer to millimeter scale gels, fiber networks and refractory organic matter creating an organic matter continuum within which seemingly free bacteria and Archaea as well as the traditional organic matter hotspots are dynamically embedded. This architecture is created and maintained by microbes' interactions and EPS [Extracellular Polymeric Substances: high-molecular weight compounds, mainly exopolysaccharides and proteins, secreted by microorganisms into their environment] *production—constituting the ocean's "connectome" and "interactome". We suggest cumulatively conceptualizing the ocean's microbiome and organic matter continuum as a having basic characteristics of a biofilm. Indeed, the global ocean's biofilm may have been an evolutionary force throughout much of history of life in the sea. This context may help understand and predict the structure and biogeochemical dynamics of the future ocean in response to climate change.*

3. Complementary work (artistic component)

3.1. Description

A video-installation is the complementary material for this work. It will be exhibited in occasion of the Diploma Final Presentations Summer Semester 2014 at the room *ZG 21 B, Universität für Angewandte Kunst, Expositur Vordere Zollamtsstraße, 3 A-1030 Vienna*.

The video-installation focuses on scientific research and analyses that place *between the lab and the wild*, a place where the heaviest land animal on Earth can fit into such a tiny environment surrounded by microbes.

The installation consists of a delimited space in the room, where mirrors and water are used to reflect the projection of a beamer onto the wall. The blurry and distorted reflection of the projection on the wall is used as a metaphor of a mirage, of something illusory. The images projected on the wall may evoke an elephant, as if trying to depict something which falls between the realms of the unreal and the real.

More in detail, the installation consists of a video projected by a beamer, which is set upon a platform leaning towards the floor and pointing to a container, that is a squared pool filled with water. Its dimensions are 90 x 90 x 5 cm, and it contains a mirror of 85 x 85 cm, which function is to reflect the liquid image to the wall. The water level is upon the mirror. For the day of the presentation of this work, a small performance will be done by me, in order to move the water and create special effects to be seen on the wall.

3.2. Used media

The video is made out of different images and sound sequences edited by me. The images consist of a footage shot by me in Lunz am See, an animated image made with dots, and some animated text masks made out of diatoms' names (algae). I have cut and edited the video with video editing software. The dots' image has been done out of an image of an elephant coming from the web ("*Angry elephant ears*", from "Mister-E" [Chris Eason], 2008, Creative Commons 2.0 Generic). My processed image consists of a squared grid of 200 circles (40 x 40) in which every circle is monochromatic. Its color resumes all the color pixels of the neighbouring area on the original image. To do so and animate the apparition of the dots randomly on a black background, I used special softwares for programming and rendering special effects. The sound of the video comes from my footage, but there are also sequences of "*Processed Sounds of Water in Hot Spot*", original material from Takanobu Hoshino, that I took from sound libraries on internet. I finally flipped vertically the video and warped it to transform the trapezoid-shape into a square when displaying it on the wall.

Besides of that, a platform to hold the beamer, a beamer, a pool, a mirror and a wall are required. The room has to be darkened by sealing light-proof the windows.

3.3. Related works

Installations based on reflections on water:

http://art-agenda.com/shows/bill-viola-at-the-nordic-watercolour-museum/
The Messenger, by Bill Viola, 1996. Video-sound installation. Color video on vertical wall screen in darkened room.

http://i-ac.eu/fr/collection/107_bad-der-verspiegelten-tautropfen-bain-de-rosee-miroitante-REBECCA-HORN-1985
Bad der verspiegelten Tautropfen, by Rebecca Horn, 1985.

http://outofice.org.uk/elizabeth-ogilvie/selected-works/
Bodies of water and other works. Elizabeth Ogilvie, 2006, 2007, …

http://ruthhoggerreflectivejournal.blogspot.co.at/2013/02/installation-research-making-water.html
Immersive, mesmerising and hypnotic installation experience that makes the viewer observe ripple patterns created by light and water. Technique used: a vessel filled with water and placed on a opaque projector.

http://www.brita.net/art_in_the_surface_of_the_water.html , or http://youtu.be/D_urrt8X0l8
The reflecting pool, by Bill Viola. 1977-1979.

http://www.edp24.co.uk/news/water_filled_installation_reflects_on_the_world_s_most_precious_resource_1_3595367
Tipping Point (sound and vision art installation) by Kathy Hinde.

http://www.essl.museum/en/exhibitions/exhibition?article_id=1367496240909&event_id=1368177007435
Abstract Film No. 1, by Valie Export, 1967.

http://www.ntticc.or.jp/Archive/1997/Chaos/Works/water.html
Water Garden, by Yoko Takahashi, 1997.

http://youtu.be/Mn9Hl2cMsnc
Notion Motion, by Olafur Eliasson, 2005.

http://youtu.be/S8EkqHa7R8c
He Weeps for You, by Bill Viola, 1976.

https://vimeo.com/35594708
Lake Orumiyeh, by Mana Salehi, 2012.

Selection of works *evoking* elephants:

http://youtu.be/37b9wmsES30 , or http://youtu.be/sl1T9DUfCRk
Shadow play of an elephant made of many people. Pilobus shadowland. First long video. Second at (01:49).

http://youtu.be/UYQDw5POFu0
Little girl showing how to make an elephant's truck with water and foam. Spanish.

http://youtu.be/vjnCUzUblX4
Chinese shadows of an elephant made with both hands at (2:09).

http://youtu.be/vS7qC2fLSU8
Chinese shadows of an elephant made with both hands.

Selection of works *depicting* elephants:

http://youtu.be/wUPp2GIBfX0
Richard Symonds - Wildlife Paintings and Drawings – Elephant - oil on canvas in real size.

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