Microbial Mediations
Cyber-Biological Extensions of Human Sensitivity to Natural and Made Ecologies

BY
YANIV JACOB TURGEMAN
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Signature of Author: Signature redacted
Yaniv Jacob Turgeman
Department of Architecture
Department of Civil & Environmental Engineering
May 21, 2015

Certified by: Signature redacted
Terry Knight
Professor of Design and Computation
Thesis Supervisor

Certified by: Signature redacted
Eric Alm
Associate Professor of Civil & Environmental Engineering
Associate Professor of Biological Engineering
Thesis Supervisor

Accepted by: Signature redacted
Takehiko Nagakura
Associate Professor of Design and Computation
Chair of the Department Committee on Graduate Students

Accepted by: Signature redacted
Heidi Nepf
Professor of Civil & Environmental Engineering
Chair of the Department Committee on Graduate Students

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ABSTRACT

As natural and human made environments become increasingly monitored and modulated by embedded digital technologies, we are presented with a staggering flow of information reverberating between the scales of the made, the grown, and ourselves. In this thesis, I use two projects as cases for interrogating the material, computational, and biological architecture mediating this information. Common to these projects is the move towards a novel cyber-biological system, which couples physical and digital processes, and utilizes microbiota as natural and programmable agents. I posit that microbes are well suited to mediating between humans and our environments, especially when they are exploited for informing on, or intervening in, phenomena that occur in temporal and spatial scales towards which we are not sensitive. In the thesis, I theoretically frame cyber-biological systems as instantiations of a post-ecological cybernetic vision, collapsing the divide between the made and the natural. As creative interventions, I describe projects designed to provide community and individual access to participate in this hybridized ecology, and suggest future research to explore microbes as sensors, living visualizations, and deployable interventions. The projects I discuss figure the microbiome as an enabling substrate for the extension of our sensitivity to natural ecosystems (*Waterfly*), the built urban environment (*Underworlds*), ultimately suggesting a similar extension of sensitivity to our bodies (*Everybiome*). My analysis of these projects culminates with ideas for future research (Biological Homeostat, Biovisualizations). By exploring realized cyber-biological systems alongside more speculative ones, I establish scientific and technological challenges in their deployment and future development. Through a critical analysis of these projects, I crystallize a design attitude towards creating sensitivity to environments, and juxtapose it with the optimization-oriented problem solving common to the discourse on "responsive" and "smart" environments. Ultimately, I aim to contribute an interdisciplinary synthesis of a scientific paradigm that is emerging between the domains of biological engineering, computation, and the design of our built environment. In promoting new connections between bits, bricks, and biology, I hope to inform contemporary practices of how we design technologies that extend the reach of our senses to the phenomena in our hybridizing made and natural world.

Thesis Supervisors:

Terry Knight
Professor of Design and Computation

Eric Alm
Associate Professor of Civil & Environmental Engineering
Associate Professor of Biological Engineering

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Chapter 1: Introduction

1.1 BITS, BRICKS, AND BIOLOGY

In 1990 Mark Weiser, chief scientist in Xerox Park, speculated on the future of computing, and envisioned a world in which every physical object would have a computing device or tab attached to it.[1] In such a world, acquiring information about things would become, as Weiser put it, "trivial."[2] Today, the affordability, miniaturization, and interconnectivity of sensors and mobile devices has turned Weiser's conjectures about the ubiquity of computing into a technological reality.

Computing and communication capabilities are being embedded in all types of objects and structures in the physical environment,[3] producing what Helen Gill from the National Science Foundation coined as "cyber-physical systems."[4] These "physical and engineered systems whose operations are monitored, coordinated, controlled and integrated by a computing and communication core"[5] build on the capacities previously developed for embedded systems. While numerous research groups worldwide explore the dynamics of such systems, an elaborate infrastructure of cabled and wireless sensor-based communication-enabled technologies is forming the backbone of a rapidly emerging network of *smart*

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[1] Weiser, M. 1991. "The Computer for the Twenty-First Century". In *Scientific American* 265 (3): 94–104. doi:10.1038/scientificamerican0991-94

[2] Ibid.

[3] Ibid

[4] Lee E.A., Seshia S.A. (2011) *Introduction to Embedded Systems - A Cyber-Physical Systems Approach*, LeeSeshia.org, Berkeley: University of California.

[5] Rajkumar, R., Lee, I., Sha L., Stankovic J., (2010). Cyber-Physical Systems: The Next Computing Revolution. In *Design Automation Conference (DAC), 2010 47th ACM/IEEE.*

objects. The term introduced by Marcelo Kallman and Daniel Thalmann categorized smart objects as those that can "describe their own possible interactions."[6] Media theorist Lev Manovich later defined them as "objects that can sense their users and display 'smart' behavior,"[7] in reacting optimally to input.

Sensor embedded 'smart' devices have become ubiquitous,[8] covering a vast range of fields and activities, from large urban-scale transportation systems to a web of personal health monitoring wearables. Dialogic networks of "smart objects" harvest information about human and environmental parameters in an ever-growing variety of contexts. Realtime feedback from these devices is making possible interactions between people and our 'things', while also opening conversations between the objects themselves. Interconnected networks of these digital devices are currently blanketing our world, and are transforming the way we interact with our physical environment, which in itself has been transformed so that interactions constitute much of its physical core.

As natural and human made environments are increasingly monitored, modulated, and mediated by embedded digital technologies, they are producing an immense amount of instantaneous information. Kevin Ashton's *Internet of Things* scenario[9] has recently been recast through a techno-optimistic lens as the *Internet of Everything*; the logical end of an automated network of objects capable of instantly translating interactions with their environments or users.

A new generation of data scientists structure these digital flows to curate perspectives that were previously impervious to human scrutiny. They give us access to an unprecedented amount of realtime information about phenomena outside the scale we are naturally sensitive to, "from the nano-world to large-scale wide-area systems of systems."[10] This feedback interpolated to the human senses invites individuated access to processes that are otherwise distanced from us in time, space, and size. Extending our personal sensitivity to the events that shape our world and our individual lives promises we will no longer be abstracted from our world by scale.

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[6] Kallman, M., Thalmann D., 1998. "Modeling Objects for Interaction Tasks" *Springer*: 73–86.

[7] Manovich, L. 2006. "The poetics of urban media surfaces". *First Monday* (Special Issue #4: Urban Screens: Discovering the potential of outdoor screens for urban society). Available at: www.firstmonday.org/htbin/cgiwrap/bin/ojs/index.php/fm/article/viewArticle/1545/1460. Last accessed May 15, 2015

[8] Pun intended with the trend of "ubiquitous computing," which contrary to desktop computing, refers to computing happening everywhere and anywhere.

[9] Wood, A. "The internet of things is revolutionising our lives, but standards are a must". *theguardian.com*. The Guardian. Retrieved 31 March 2015.

[10] Rajkumar, 2010. Op. cit.

The technological architecture that mediates this new human perspective includes three central features: (i) sensing at an unprecedented temporal and spatial resolution, (ii) the dialogic capacity of connected devices, and (iii) the emergent control of this intricate massive web through processes of self organization and auto-regulation.

It seems that with these capacities, made systems are increasingly incorporating properties of biological systems. The possibility of distributed realtime feedback is introducing processes of auto-regulation and adaptation in to the made environment at scales that were previously reserved for natural systems. Processes of self-organization are emerging in made systems, and are integrated as designed strategies of made systems.

This is also evident in today's computational design processes of made objects, which are incorporating biological strategies of evolution by selection. The notion of speculative design science, as postulated by Buckminster Fuller, suggested an iterative 'mutation' of existing ideas, positing them in the world for 'selection'.[11] Going beyond this biomimetic logic, today's design tools computationally model biological evolution in generative algorithms, imitating selective pressures to arrive at a desired solution. In the self-organizing context of dialogic objects, a design process of "directed evolution" becomes conceivable outside of the purely digital space, whereby a human-made object is designed to calibrate its function or form through active interaction in the cyber-physical world.

Parallel to these "natural" dynamics emerging in the made world, a complimentary trajectory appears in the natural world. While methods of evolution by design date back about 10,000 years to early agriculture,[12] new methods in biological engineering are extending design into the realm of the living. Today, the technological acceleration of our ability to modify living beings has reached genome scale modification. Recent years have seen the rapid increase in the capacity to read and write DNA. The

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[11] Buckminster F., Kuromiya K., (1992). *Cosmography: A Posthumous Scenario for the Future of Humanity*. New York: Macmillan.

[12] Piperno, D. R.; Ranere, A. J.; Holst, I.; Iriarte, J.; Dickau, R. (2009). "Starch grain and phytolith evidence for early ninth millennium B.P. maize from the Central Balsas River Valley, Mexico". *PNAS* **106** (13): 4957–4958.

proliferation of these biological technologies is illustrated in the *Carlson Curves.*[13,14] This ability to convert from digital computation to genomic generation is inviting biological interventions well beyond the "nature-inspired" forms of biomimicry. With the connection between bits and biology, the design of the made is merging with the generation of the grown. In this respect we are introducing the evolution by design methods of the human made into the biological. The ability to imagine in the human mind, and then make is now being applied to the design of living things.

A clear instantiation of these hybridizing design strategies is manifested in the modern manipulation of microorganisms. Microbes are the intense focus of study and engineering aimed at conferring them with functions beneficial to humans. Simultaneously, scientists use microbes to better understand ecological events in every environment. Both evolving by selection and design, modified microbes are tasked with a vast array of sensing, computing, and actuating tasks in, and increasingly out of the lab.

1.2 CYBER-BIOLOGICAL SYSTEMS

I see these trends in the things we are making, the processes we are employing in order to make them, and the processes these things are enabling, culminating in an instantiation of a post-ecological cybernetic vision, which seems to be collapsing the distinction between the natural and the human-made. In this context it is not just the artificial that is naturalized, but also nature itself. Natural and human interventions operate on a continuum, with converging and complimenting processes of making, reproducing, and creating. Design by evolution and evolution by design have emerged as co-evolutionary forces.

This hybridized ecology spanning the digital, built, and grown intertwines bits, bricks, and biology. More than ever before, human agency both as designer and participant depends on tuning in to the staggering flow of information reverberating between all scales of the made and the grown. This thesis revolves around the material, computational, and biological architectures mediating the flows of realtime information extending our reach into our hyper-connected environment. These architectures

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[13] Carlson, R.B., (April 2011). *Biology Is Technology : The Promise, Peril, and New Business of Engineering Life*. Cambridge, MA: Harvard University Press; Carlson R.B., (September 2003). "The Pace and Proliferation of Biological Technologies." In *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science* (Mary Ann Liebert, Inc.) 1 (3: 203-214). doi: 10.1089/153871303769201851

[14] The Carlson Curve is a graph based on the empirical observation of the diminishing cost per base of sequencing DNA over time. It was termed by the publication The Economist, and named after biologist Robert Carlson, who predicted the biotechnological equivalent of Moore's law, named after cofounder of Intel Gordon Moore, who successfully predicted 40 years ago that the transistor density of integrated circuits would double about every two years. Tritton, T. (November 5, 2009). "Carlson Vs. Moore" In *Chemical Heritage Foudantion (CHF)*. Available at: http://www.chemheritage.org/discover/media/periodic-tabloid/archive/2009-11-05-carlson-vs-moore.aspx. Last accessed: May 21, 2015.

are related to how we sense and experience the world around us, as they mediate between the information produced and the sensory input through which we construct meaning out of the world.

With the aim of extending our sensitivity to events in this hybridized context, I consider an architecture that exploits this convergence, and look at agents that act as made sensing objects on a biological backbone. Extending the promise of the 'Internet of Everything' as it manifests in cyber-physical systems, I propose cyber-biological systems as those which couple physical and digital process with networks of microbes utilized as natural or programed agents capable of sensing, computing, and actuating. Microbes have become a real-world instantiation of co-evolutionary design strategies, and present possibilities for intervention and mediation of hybridized ecologies, calling designers into the lab and biological engineers to the drawing board.

My motivation is to generate feedback from these hybridizing ecologies as an opportunity to tune our individual sensitivity into phenomena that are of concern to us, and to make them meaningful to our lives through individual access and shared participation in them. The sensitizing-oriented architecture I propose is a complement to the event-driven bottom-up service-oriented architecture that currently mediates the design of digital technologies. Ultimately, the vision driving these interactive processes between the individual and the living world around and inside ourselves, implicates a shift from sustainability by optimization to sustainability by sensitivity.

1.3 THESIS ROADMAP

In the Background Chapter, I share additional material supporting my thesis claim. I review contemporary thought regarding ecology and human agency. Through the theories of philosophers and cultural theorists, such as Vilém Flusser, Michel Serres, Slavoj Zizek, and Bruno Latour, I consider the present moment as one that allows us to understand the made and the grown on a continuum, and our own interventions as part of an ecology of agents collectively shaping the world. Then, I describe current advancements in microbiology and biological engineering which position microbes as ideal mediators in the hybridized context described above. Finally I present a short review of theoretical material regarding sensory ecology and the nature of human sensing. In particular I examine Jakob von Uexküll's framing of the "Umwelt" in its relevance for my claim of designing microbially-mediated cyber-biological systems as extensions of our sensory envelope, opening up new horizons of enhanced integration of humans and their environment.

In the Projects Chapter, I address my concerns and claims by interrogating two projects which I conceived and developed during my time at MIT. These projects will serve as case studies that demonstrate initial proof-of-concept systems in which microbes can mediate phenomena that happen in the world at other scales of time, space, and size than the ones we are naturally sensitive to. These projects revolve around the idea of using the microbiome as an enabling substrate for the extension of our sensitivity to natural ecosystems, the built urban environment, ultimately suggesting a similar extension of sensitivity to our bodies.

First, I discuss a cyber-physical network of aerial UAV vehicles called "Waterfly," designed to scan bodies of water, identify indicators of water pollution with high precision and in realtime, and map their expansion over time. This ultimately allows people to better sense changes in aquatic ecology, correlate these changes with other geographical and temporal factors, and finally yield a potential change in behavior with regard to their relationship with the ecosystem. Second, I discuss the ongoing "Underworlds" project, a realtime sampling platform comprised by robots distributed in the city sewage. This project aims to develop the science and technology needed to build a proof-of-concept system to track microbial indicators of health and disease in realtime, through the city's "collective gut."

Finally, I briefly present work which has started as future research. I describe "Everybiome," a 'smart toilet' system for passively collecting personal health biomarkers from the stool in the home, with the aim of monitoring personal disease and health. The "Bio-Homeostat" is a toy environment for modeling self organizing dynamics between microbes and humans when mediated by a cybernetic infrastructure. And finally, "Microbe-fluidics: Living Biovisualization" which involve the design of 3D printed bio-reactors to utilize cultures of microbial communities as living visualizations of otherwise imperceptible environmental phenomena.

Background

ABSTRACT

Cyber-biological technologies with living microorganisms at their core, offer to extend the human sensorium to stimuli, processes, and phenomena beyond the reaches of our sensory physiology. This expansion can reshape the construction of our perceptual world, as well as our behavior in it. In this chapter, I provide an overview of the context in which this transformation occurs and some of its likely consequences. First, I examine how today's world constitutes a product of a long process in which natural and human creative forces have co-evolved. A co-evolution that has lead to an accelerated convergence between the natural and the human-made world leading to a collapse of the subject-object divide, and the emergence of a hybridized natural – made ecology. Second, I describe microorganisms as information-carrying agents with a unique potential to mediate between the natural and the made. These agents enable effective sensing, computing and are readily manipulated, extending the human sensorium and serving as one of the foremost instantiation of the above described collapsed dichotomy. Finally, I explore one of central anticipated outcomes of this transformative technology. Our perception of the world is created by and limited to the stimuli that we are sensitive to. Humans interpret the environmental signals we sense into an understanding of the world around us, both in a biological and semantic sense. In this context, I suggest the design of microbially-mediated cyber-biological systems as extension of our sensory envelope, opening up new horizons of enhanced integration of humans and their environment.

Francisco Goya, Fight with Cudgels', (c.1820–23. Oil mural transferred to canvas. 123 cm × 266 cm. Museo del Prado, Madrid)

Background

2.1 HYBRIDIZED ECOLOGY: THE COLLAPSE OF THE HUMAN MADE – NATURAL DIVIDE

2.1.1 The act of making as a co-evolutionary force

Over a vast period of time, from the emergence of the first single-cell microbial life-form four billion years ago, to our early hominid ancestors 2.5 million years ago, the information of life propagated and evolved through increasingly complex natural, biological structures. Over that time, our senses were honed by a lengthy evolutionary process. Like most of our physiological features our senses are deeply connected with other living things on Earth, through shared ancestry and history of interactions.

Our first hominid ancestors were well calibrated to extract signals from the environments: their sense of sight was in tune with movement signals produced by potential prey or predators; their sense of taste and smell were in tune with signals separating the edible from the poisonous. Sensing was not limited to the external world, but also provided meaningful cues regarding the state of the internal environment – their sense of hunger provided signals that were in tune with the effort that was required to hunt and gather. Our ancestors were both in tune *with* and tuned *into* the signals in the natural world around them, providing a type of intuitive direct access to the environment and their bodies.

Approximately fifty-thousand years ago a dramatic change occurred; language emerged and with it *making* proliferated at an unprecedented rate. From a nonlinguistic, non symbolic ancestor, the species of *homo sapiens* emerged unique in their possession of language and symbolic consciousness.[1] This is when we start seeing cave art, and concurrently an immense diversification of tool making.

Preceding this crucial point of transition, evolution was fundamentally biological. The *born* evolved with its environment. Since, and most pronouncedly, for the last few centuries, the *made* has emerged as a powerful co-evolutionary force, radically changing the world, through increasingly complex human-designed, made structures.

In his essay "The Factory," Vilém Flusser argues that above all names assigned to our species, the term *hobo faber*,[2] which is "less zoological than anthropological," best describes humans as "those kinds of anthropoids that manufacture something."[3] The factory, the place of manufacturing, produces not only things, but also kinds of human: from the hand-man, to the tool-man, then the machine-man, and now

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[1] Tattersall, I. (2008). "An Evolutionary Framework for the Acquisition of Symbolic Cognition by Homo sapiens" *Comparative Cognition & Behavior Reviews*. v3: 99-114

[2] Flusser, V. (1999). *Shape of Things: A Philosophy of Design*. London: Reaction Books Ltd.

[3] Ibid: 43

the robot-man,[4] who will in Flusser's words "overcome the crazy alienation from nature and culture, such as it was at the height of the machine revolution [...] and bring neurophysiological and biological theory and hypotheses into play."[5]

2.2.2 "Ecology without Nature"

There has been wide philosophical debate around the perceived *divide* between the *natural* and the *made*. In 2006, French philosopher and author Michel Serres, articulated the inherent complexity of the concept of ecology.[6] First, it refers to a scientific discipline that studies the interactions of living beings with their environment. Second, it encapsulates "the controversial ideological and political doctrine varying from author to author or group to group that aims at the protection of the environment through diverse means."[7]

Ever since the production of what Serres calls *world-objects*, the traditional concept of ecology, as well as its categories of objectivity, subjectivity, knowledge, and action — both individual and collective— have fallen apart. These categories were instituted on the basis of an assumed divide between an acting human subject acquiring knowledge about a static object (nature). This divide is being fundamentally challenged by the existence of tools and technologies whose dimensions are comparable with those of natural phenomena: "a satellite for speed, an atomic bomb for energy, the Internet for space, and nuclear waste for time. We now live in those world-objects as we live in the world,"[8] Serres posits. And so, in today's world there is a new object-subject distribution: the subject (human) becomes an object and the object (nature) becomes a subject as it reacts to our actions.

The Object has been redefined, as the Earth is no longer the obscure background of our being-in-the world: we have seen it whole from outer space, we can communicate with the world through the internet, and we can act on the whole earth, affecting its climate, through the use of our techniques of survival. The Subject is also redefined as a global 'we', anew *collective global subject* formulated facing the new *natural global object*. In this context, Serres suggests that a new contract emerges of symbiosis

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[4] Ibid: 44

[5] Ibid: 46

[6] Unless noted otherwise, all quotes included in the section from page 17 until page 18 should be noted as: Serres, M. (2006). Revisiting the Natural Contract. In *CTheory.net*. ed. Arthur and Marilouise Kroker. Available at: http://www.ctheory.net/articles.aspx?id=515. Last accessed May 20, 2015.

[7] Ibid

[8] Ibid

between the global Earth and the totality of actors. We are currently shifting to a time where a new nature is being reborn, "new for our globalized knowledge and acts."[9]

Focusing on the reactionary efforts to reverse these shifts in our relation to our environment, Slavoj Zizek in his 2007 lecture entitled "Ecology without Nature," raised ecology to the ideal candidate for hegemonic ideology.[10] Zizek claims that the concept of ecology has become a new opium for the masses. It takes the religion's old fundamental trick of installing an unquestionable authority which can impose limits, only now that authority is not a deity but rather Nature itself. The underlying message reflects a fundamental distrust and a deeply conservative perspective: any human-generated change in nature can only be a change for the worse.[11]

Zizek places himself in opposition to the ecological ideology that regards nature as an ideal object which we have destabilized and we now have to somehow restore.[12] He dismisses as erroneous the very basic presupposition that there is such a thing like Nature, in the sense of a maternal deity, a harmonious system of balance and reproduction, which is destabilized by the human desire to manipulate and potentially dominate it. The fallacy in such a schema is found in the very assumption that we humans exist on a separate Cartesian subjectivity (thought separated from the material world). In other words, that we are somehow outside of nature, and nature is an object of manipulation. Zizek suggested that the core lesson of Darwinism is that nature itself is not natural, but naturalized. As an answer to the ecological 'religion', Zizek proposes a radical ecology that denaturalizes nature and accepts its open and imbalanced character. The first premise of a truly radical ecology should therefore be that nature does not exist.[13]

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[9] Serres' renowned invocation of Goya's "Fight with Cudgels" painting is iconic of this new condition. The painting depicts two enemies fighting on quicksand. He makes an analogy with the dualistic arrangement of the painting, assigning one of the two opponents to the Cartesian subject, and the other one to Nature. "With every blow dealt to the adversary, their legs sink into the sludge, ever deeper as the energy spent in combat increases. Since the dawn of history, we have only seen the belligerents in the grand spectacle of the battle and have only been interested in the question of who will win or lose, who will become the master by subduing or killing the slave?"

[10] He explained that the seductive power of ideology is that it deals with very real problems, but gives them mystifying twists.

[11] Zizek refuted the thesis that the ultimate cause of our ecological problems is modern technology, and invited contemporary thought to accept that the abstract scientific reification and objectivization of nature to so-called natural mechanism is the cause of problems. What we should rediscover is that nature is not out there, an object of manipulation.

[12] For Zizek, ideology at its purest is precisely this idea that we humans are disturbing some pre-existing balance. Extending Lacan's motto "The big Other doesn't exist" to Nature, he claimed that Nature is not a scientific object for observation and manipulation, but our background. We are wired to nature, embedded in it, he says.

[13] In a provocative example, he claimed that the natural equilibrium of our earth is already to such an extent accommodating pollution, that if it radically disappeared this would now cause a great imbalance anew, as nature already includes our pollution into its reproduction. The ultimate human hubris and violence is imposing the view of nature as a stable system of patterns.

Referring to biogenetics, Zizek posited that the principle consequence of scientific breakthroughs in biogenetics is "the end of nature" — once we know the rules of the construction of a natural organism, natural organisms are transformed into objects amenable to manipulation. Nature —human and inhuman— is in this way deprived of its impenetrable density. Biogenetics, through synthetic biology, therefore aims not only to manipulate existing beings through interventions, but to create new forms of artificial life, what Zizek calls "a life number two." Natural life therefore loses its natural character and becomes "life number one." Nature becomes a transparent mechanism.

Disruptive as these philosophical notions may seem, even they struggle to keep up with the pace of transformation. The frontiers between the made and the natural are shattered on a daily basis, raising increasingly complex ethical considerations and challenges. Thus, evolution by design and evolution by selection have become so intertwined as to constitute a single stream of change, carrying humans and their environment into completely uncharted territories, and "life number two" has begun. As one of the simplest organisms, microbial life forms have naturally become the testing ground for the birth of this new perspective, through advances of synthetic biology towards the creation of the first completely artificial life form.[14]

2.2 MICROBES AS SENSING, COMPUTING, ACTUATING AGENTS

2.2.1 Introduction

Microbes – microscopic single-cell organisms – are the most abundant form of life. They inhabit nearly every habitat on earth, from 400C hydrothermal vents at the ocean floor to kilometers beneath the antarctic frozen ice sheet.[15] We interact with microbes in everything we touch, the air we breathe, the food we eat. They drive the earth's biogeochemical cycles, cycling key nutrients such as nitrogen, phosphorous, and carbon, producing oxygen, and decomposing organic matter. They provide essential functions for life to all individual species and whole ecosystems on earth.

Microbes respond and adapt to the conditions of their environment. This can happen through physiological changes, such as the sensing of nutrients inducing changes in flagellar motion. Such

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[14] Gibson D.G.,Glass J.I., Lartigue C., Noskov V.N., Chuang R-Y., Algire M.A., Benders G.A., Montague M.G., Ma L., Moodie M.M., Merryman C., Vashee S., Krishnajumar R., Assad-Garcia N., Andrews-Pfannkoch C., Denisova E.A., Young L., Qi Z-Q., Segall-Shapiro T.H., Calvey C.H., Parmar P.P., Hutchison III C.A., Smith H.O., Venter J.C.,(2010). "Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome." In *Science* Vol.329, no.5987, pp.52-56. DOI: 10.1126/science.1190719. Web. 12 May 2015.

15 Christner B.C., Priscu J.C., Achberger A.M., Barbante C., Carter S.P., Christianson K., Michaud A.B., Mikucki J.A., Mitchell A.C., Skidmore a., Vick-Majors T.J., & the WISSARD Science Team. (2014). "A microbial ecosystem beneath the West Antarctic ice sheet." In *Nature* 512, 310–313 (21 August 2014) doi:10.1038/nature13667. Web. 21 May 2015.

changes can generally be described as the behavior of the organism. Alternatively, adaptation can happen through evolution. This happens through a process of mutation and selection acting on the microbial DNA, which contains the genetic blueprint for the organism. Naturally occurring mutations can add new genes, or delete existing ones, change the regulation of gene expression, or alter the function of an expressed gene. These different alterations create genetic variation, and can confer new functions to an organism. If an improved function creates a fitness advantage for the organism in it's environment, it will have a greater likelihood of propagating the new genotype to progeny populations, a process known as selection.

Microbes adapt through these mechanisms at a vast range of time scales. Physiological responses take place on the order of milliseconds, while genetic adaptation takes place at generational time scales ranging from over 250 million years[16] to under a half hour. For example, *E. coli*, one of the most well studied bacteria, reproduces every 20 minutes. As *E. coli* has served as a model organism for laboratory studies, scientists hoping to finish their experiments faster 'naturally' select for variants of the bacteria that reproduced faster. Today's common lab strain has a generation time that is less than half[17] compared to that of the strains first discovered in 1885.

In addition to the adaptation of individual microorganisms, microbial communities adapt via the changes in abundance of member species. Most microbes live in complex communities comprised of tens to thousands of species in the same environment. These ecosystems of microorganisms are known as a microbiomes. Within a community, microbes interact competitively and cooperatively, vying for resources and sharing metabolic capabilities to process nutrients. These processes shape the community's structure relative to it's specific environment. The structure of a community is therefore an indicator of the conditions of its environment. For example, the constellation of bacteria living in the human gut changes in response to gut inflammation in a predictable manner. Patients with active state inflammatory bowel disease show decreased levels of *Akkermansia* and increased levels of *Escherichia*.[18] The changes in community structure are responding to changes in mucosal tissue in the gut environment.

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[16] Guinness World Records. http://www.guinnessworldrecords.com/world-records/oldest-living-bacteria. lol: Guinness claims that Bacillus 2-9-3 is ten times older than the previous oldest revived bacteria. Nature also cites this.

[17] Novak, M. et al. "Experimental Tests for an Evolutionary Trade-Off Between Growth Rate and Yield in E. Coli.." In *The American naturalist.* 168.2 (2006): 242–251. Web. 21 May 2015.

[18] Papa E., Doctor M., Smillie C., Weber S., Preheim S.P., Gevers D., Giannoukos G., Ciulia D., Tabbaa D., Ingram J., Schubert B.D., Ward D.V., Korzenik J.R., Xavier R.J., Bousvaros A., Alm E.J. (2012). "Non-Invasive Mapping of the Gastrointestinal Microbiota Identifies Children with Inflammatory Bowel Disease." In *PLOS Collections*. (June 29,2012) Web. 21 May 2015.

2.2.2 Enabling Technologies

The study of microbes and the relationships between their functions and environments was limited until recently to what could be observed through microscopy and culture based methods. This only allowed us to collect information about a very small subset of bacteria through time intensive methods. The past decade has seen a dramatic increase in the reading of DNA largely due to massively parallel high-throughput sequencing methods.[19] These methods enabled a shift in the scale of data acquistion, not only through improved speed, but also through a precipitous reduction of the cost per megabase (one million sequenced base pairs of DNA) from ten thousand dollars in 2001 to one cent in 2014.[20] This has led to the unprecedented rapid discovery of thousands of new microbial species,[21] gene functions, and ecological associations including their essential role in human health. This year, a portable hand held sequencer[22] has been introduced that heralds a near future where realtime genomic analysis will happen in small devices outside of the lab.

The ability to quantify the products of microbial communities is another way of 'reading' microbes to link community structure to function and environment. High throughput methods for reading the products of microbial communities, such as metabolites and proteins, can monitor specific chemical transformations and measure the concentrations of biological compounds in the environment.[23] Additionally, biological engineering has been able to modify bacteria to produce fluorescence proteins which act as reporters for events the bacteria have encountered. This fluorescence reporter can be designed to be the output of synthetically engineered genetic circuits in bacteria, allowing the result to be read optically.[24]

Engineering has not only produced microbial genetic circuits with optical readouts, but also methods to write and directly edit DNA. Techniques such as CRISPR/Cas9 allow biological engineers to design

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[19] Carlson, R.B., (April 2011). Biology Is Technology : The Promise, Peril, and New Business of Engineering Life. Cambridge, MA: Harvard University Press; Carlson R.B., (September 2003). "The Pace and Proliferation of Biological Technologies." In Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (Mary Ann Liebert, Inc.) 1 (3: 203-214). doi: 10.1089/153871303769201851

[20] Ibid

[21] Brady A., and Salzberg S.L. (2009) "Phymm and PhymmBL: Metagenomic Phylogenetic Classification with Interpolated Markov Models." *Nature methods* 6.9: 673–676. *PMC.* Web. 21 May 2015.

[22] Oxford Nanopore Technologies, MinION <https://www.nanoporetech.com/products-services/minion1>

[23] Haiser H.J., Gootenberg D.B., Chatman K., Sirasani G., Balskus E.P., Turnbaugh P.J. (2013). "Predicting and Manipulating Cardiac Drug Inactivation by the Human Gut Bacterium Eggerthella lenta." In Science (19 July 2013) Vol. 341 no. 6143 pp. 295-298 DOI: 10.1126/science.1235872. Web. 21 May 2015.

[24] Elowitz M.B., & Leibler S. (2000). "A synthetic oscillatory network of transcriptional regulators." In Nature 403, 335-338 (20 January 2000) | doi:10.1038/35002125; Received 6 July 1999; Accepted 9 November 1999. Web. 21 May 2015.

functions into microbial genomes[25] in a manner more akin to the design of human-made objects than naturally evolved life forms. The possibilities of these nascent methods are just being discovered, and already they have yielded groundbreaking living designs.

The power of these technologies has recently attracted the maker and DYI movements. With the convergence of 3d printing and cheap rapid electronics prototyping, at home kits are moving bioengineering techniques out of the institutional lab and into the home kitchen. The continued development of such technologies and their democratization implies that tinkering with designer microbes engineered in the garage is not far off. In fact it's already happening.

2.2.3 Microbes as Computation & Communication

In order to sense and respond to their environment, microbes, along with all living cells, need to perform sophisticated computational tasks. To do this, they integrate signals using simple logic gates,[26] such as AND, OR and NOT, and combine these pieces into complex regulatory circuits.[27] This raises the possibility that we can program microbes to perform human-specified tasks. In fact, scientists have already programmed functions in microbes using both analog[28] and digital circuits.[29] They have also programmed permanent genetic memories into microbes, encapsulating the past experience of microbes as permanent records in their DNA.[30] [31] We can read these historical records using DNA sequencing, giving us a written record of their prior exposures and experiences. In these ways, microbes are similar to computers that can live and propagate in virtually any environment. However, one additional feature of computation in microbes is that it is distributed. Although each microbe exists as a single unit and does its own computation, microbes also communicate using a system of quorum

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[25] Ran F.A., Hsu P.D., Lin C-Y., Gootenberg J.S., Konermann S., Trevino A.E., Scott D.A., Inoue A., Matoba S., Zhan Y., Zhang F. (2013). "Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity." In *Cell* Volume 154, Issue 6 (12 September 2013): Pages 1380–1389. Web. 21 May 2015.

[26] Voigt C.A. (2006). "Genetic parts to program bacteria." In *Science Direct* Vol.17, Issue 5 (October 2006): Pages 548-557. Web. 21 May 2015.

[27] Moon T.S., Lou C., Tamsir A., Stanton B.C., & Voigt C.A., "Genetic programs constructed from layered logic gates in single cells." In *Nature* 491, 249–253 (08 November 2012) doi:10.1038/nature11516. Web. 21 May 2015.

[28] Daniel R., Rubens J.R., Sarpeshkar R., & Lu T.K., "Synthetic analog computation in living cells." In *Nature* 497, 619–623 (30 May 2013) doi:10.1038/nature12148. Web. 21 May 2015.

[29] Voigt 2006. Op.cit.

[30] Yang L., Nielsen A.A., Fernandez-Rodriguez J., McClune C.J., Laub M.T., Lu T.K., Voigt C.A. (2014). "Permanent genetic memory with >1-byte capacity." In *Nat Methods*. 2014 Dec;11(12):1261-6. doi: 10.1038/nmeth.3147. Epub 2014 Oct 26. Web. 21 May 2015.

[31] Farzadfard F., Lu T.K., "Synthetic biology. Genomically encoded analog memory with precise in vivo DNA writing in living cell populations." In *Science*. 2014 Nov 14;346(6211):1256272. doi: 10.1126/science.1256272. Web. 21 May 2015.

sensing. By communicating in this way, microbes can share the results of their computations, reducing the noise and making decisions in aggregate. Thus, microbes represent an ideal system for distributed environmental computing.

2.2.4 Microbes as Sensors

To take advantage of these unique computational abilities, researchers have begun to use microbial cells as environmental biosensors. Unlike previous man-made devices, these biosensors are ubiquitous, distributed, can self-replicate, perform complex computations and report on microscopic environmental conditions that extend well beyond the human senses. For example, one active area of research is to use microbes to sense environmental pollutants.[32] Following the Deepwater Horizon oil spill,[33] scientists were able to detect the presence of minute quantities of oil in the ocean by sequencing the microbes that degrade this oil directly from the environment. In similar ways, microbes can sense pH, copper, arsenic, phenols, glucose, ethanol and cholene, among hundreds, if not thousands, of small molecules.[34] At the same time, microbes are efficient indicators of soil[35] and air quality and can even be used as robust markers of human disease states, such as obesity,[36] malnutrition,[37] colorectal cancer,[38] and inflammatory bowel disease.[39]

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[32] Shin H.J. (2011). "Genetically engineered microbial biosensors for in situ monitoring of environmental pollution." In *Springer* Vol. 89, Issue 4 (February 2011): Pages 867-877. Web. 21 May 2015.

[33] Mason O.U., Hazen T.C., Borglin S., Chain S.G.P.,Dubinsky E.A., Fortney J.L., Han J., Holman H-Y.N., Hultman J., Lamendella R., Mackelprang R., Malfatti S., Tom L.M., Tringe S.G., Woyke T., Zhou J., Rubin E.M., and Jansson J.K. (2012). "Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill." In *The ISME Journal* (2012) 6, 1715–1727; doi:10.1038/ismej.2012.59; published online 21 June 2012. Web. 21 May 2015.

[34] Su L., Jia W., Hou C., Lei Y. (2011). "Microbial biosensors: A review." In *Science Direct* Vol.26, Issue 5, (15 January 2011): Pages 1788–1799. Web. 21 May 2015.

[35] Garbisu C., Alkorta I., Epelde L. (2011). "Assessment of soil quality using microbial properties and attributes of ecological relevance." In *Science Direct* Vol. 49, September 2011, Pages 1-4. Web. 21 May 2015.

[36] Turnbaugh P.J., Ley R.E., Mahowald M.A., Magrini V., Mardis E.R., & Gordon J.I. (2006). "An obesity-associated gut microbiome with increased capacity for energy harvest." In *Nature* 444, 1027-1031 (21 December 2006) | doi:10.1038/ nature05414; Received 8 October 2006; Accepted 7 November 2006. Web. 21 May 2015.

[37] Smith M.I., Yatsunenko T., Manary M.J., Trehan I., Mkakosya R., Cheng J., Kau A.L., Rich S.S., Concannon P., Mychaleckyj J.C., Liu J., Houpt E., Li J.V., Holmes E., Nicholson J., Knights D., Ursell L.K., Knight R., Gordon J.I. (2013). "Gut Microbiomes of Malawian Twin Pairs Discordant for Kwashiorkor." In *Science* 1 February 2013: Vol. 339 no. 6119 pp. 548-554. DOI: 10.1126/ science.1229000. Web. 21 May 2015.

[38] Dejea C.M., Wick E.C., Hechenbleinker E.M., White J.R., Mark Welch J.L., Rossetti B.J., Peterson S.N., Snesrud E.C., Borisy G.G., Lazarev M., Stein E., Vadively J., Roslani A.C., Malik A.A., Wanyiri J.W., Goh K.L., Thevambliga I., Fu K., Wan F., Llosa N., Housseau F., Romans K., Wu X., Vogelstein B., Kinzler K.W., Pardoll D.M., Sears C.L. (2014). "Microbiota organization is a distinct feature of proximal colorectal cancers." In *PNAS* 2014 111 (51) 18321-18326. Web. 21 May 2015.

[39] Sartor R.B., (2008). "Microbial Influences in Inflammatory Bowel Diseases." In *Science Direct* Vol. 134, Issue 2 (February 2008) Pages 577-594. Web. 21 May 2015.

2.2.5 Microbes as Actuators

Microbes not only sense environmental conditions, but also respond to their surroundings in complex ways. For example, upon reaching high population densities, some microbial populations will form dense biofilms that share valuable resources and exclude competitor bacteria from gaining access. Intriguingly, genome editing now allows us to rewire these microbial responses, associating environmental inputs with human-specified outputs. For example, one high-impact research aim is to program microbes to sense cancer cells and to destroy them.[40] Other examples of programmed environmental actuation include the addition of nitrogen fixation capabilities to cereals,[41] the synthesis of biofuels from switchgrass,[42] and the engineering of pathogen resistance in agricultural crops.[43]

Microorganisms are information-carrying agents with a unique potential to mediate between the natural and the made. These agents enable effective sensing, computing, and actuation, and are readily manipulated with current methods in biological engineering. As such, I propose the consideration of microbes as a core component of cyber-biological systems designed to extend our sensitivity to phenomena that occur at scales we cannot naturally perceive with our biological senses.

2.3 HUMAN SENSOME AND UMWELT

2.3.1 Sensory Ecology and the Human Sensome

A human sense is commonly defined as a group of sensory cell types that receive stimuli and information about the environment and our bodies, and provide these signals to a corresponding region within the brain, so that we can respond to a specific physical phenomenon. Humans have a multitude of senses. The five traditionally recognized ones are: sight (ophthalmoception), hearing (audioception), taste (gustaoception), smell (olfacoception or olfacception), and touch (tactioception). However, there

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[40] Anderson C.J., Clarke E.J., Arkin A.P., Voigt C.A. (2006). "Environmentally Controlled Invasion of Cancer Cells by Engineered Bacteria." In *Journal of Molecular Biology*. Vol.355. Issue 4, 27 January 2006, Pages 619-627. doi:10.1016/j.jmb.2005.10.076. Web. 21 May 2015.

[41] Geddes B.A., Ryu M.H., Mus F., Garcia Costas A., Peters J.W., Voigt C.A., Poole P. (2015). "Use of plant colonizing bacteria as chassis for transfer of N2-fixation to cereals." In the *US National Library of Medicine National Institutes of Health*, 32: 216-222. doi: 10.1016/j.copbio.2015.01.004 . Web. 21 May 2015

[42] Bokinsky G., Peralta-Yahya P.P,, George A., Holmes B.M., Steen E.J., Dietrich J., Lee T.S., Tullman-Ercek D., Voigt C.A., Simmons B.A., Keasling J.D..(2011). "Synthesis of three advanced biofuels from ionic liquid-pretreated switchgrass using engineered Escherichia coli." In the *US National Library of Medicine National Institutes of Health.* 108(50):19949-54. doi: 10.1073/pnas. 1106958108. Web. 21 May 2015

[43] Blümke A., Sode B., Ellinger D., Voigt C.A.. (2015). "Reduced susceptibility to Fusarium head blight in Brachypodium distachyon through priming with the Fusarium mycotoxin deoxynivalenol." In the *US National Library of Medicine National Institutes of Health.* 2015 Jun;16(5):472-83. doi: 10.1111/mpp.12203. Web. 21 May 2015.

are difficulties in defining where the borders between different responses to related stimuli lay. What constitutes a distinct sense is a matter of debate.[44]

The human body is actually capable of a wider range of sensory functions, which means that it can detect stimuli beyond those governed by the five most broadly recognized senses. In other words, our bodies have a sensory system that monitors a wider range of changes in the body and its environment. Receptors are distributed throughout human anatomy, giving rise to our general senses. To provide a provisional sketch, these include temperature (thermoception), kinesthetic sense (proprioception), pain (nociception), balance (equilibrioception), vibration (mechanoreception), and various internal stimuli, such as the different chemoreceptors for detecting salt and carbon dioxide concentrations in the blood. More specialized sensory organs give rise to the specialized senses. These organs include the tension sensors, the stretch receptors, the chemoreceptors, and receptors for monitoring thirst, hunger, and time.

Regardless of these categorizations and the debate around what constitutes a distinct sense, it is this biological sensory apparatus, or sensorium, that describes the sensory envelope of what signals we are naturally capable of tuning into. This sensory envelope of signals representing stimuli can be thought of as the human "sensome."

Our senses emerged from evolutionarily successful semiotic interactions with our environment. Biologists have recently coined the term *sensory ecology* to broadly describe "the acquisition of information and the ways in which an organism responds to sensory information to organize its interaction with its environment."[45] This concept can be invoked here to suggest the evolutionary process through which humans developed particular sensing mechanisms related to specific types of phenomena. This information is related to actual environments and habitats as experienced by an animal in its own individual life.

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[44] In an article entitled "Humans have a lot more than five senses," Daven Hiskey writes: "Even in the case of the traditional five senses, sight is technically two senses given the two distinct types of receptors present, one for color (cones) and one for brightness (rods). Taste is sometimes argued to be five senses by itself due to the differing types of taste receptors (sweet, salty, sour, bitter, and umami), but generally is just referred to as one sense. Touch has been found to be distinct from pressure, temperature, pain, and even itch sensors. Hearing refers to the ability to detect vibrations along some medium, such as air or water that is in contact with your ear drums. And finally the sense of smell sense combines with taste to produce flavors." In *Today I found out: Feed your Brain.* July 16, 2010. Available at: http://www.todayifoundout.com/index.php/2010/07/humans-have-a-lot-more-than-five-senses/ Last accessed May 20, 2015.

[45] Barth, F.G. Schmidt, A. (2001). *Ecology of Sensing.* Springer-Verlag Berlin Heidelberg New York: Introductory Remarks.

2.3.2 Umwelt

The human sensome provides the scaffolding for the semiotic envelope constructed around an individual. In other words, every individual exists within a sphere of projected perceptions circumscribed by the limitations of their sensory apparatus. German Biologist Jakob van Uexküll's notion of the *umwelt* (translated as "self-centered world"[46]) can help elucidate the constructive intercorrelation between the concepts of sensing and perception.

The specific sensory filters that an organism carries result in a species-specific model of the world that this organism can perceive and experience.[47] And so, different animals have access to different signals within the same ecosystem. These signals are limited by the species' sensory envelope. As a result, each species inhabits a different "world." In this species-specific perceptual world, termed by Uexküll as the organism's individual *umwelt*, the organism —either human or non-human— exists and acts. And so, in an environment, there is an ecology of *umwelten* interacting in space and time.

American philosopher and semiotician John Deely used the term "biological heritage"[48] to refer to the distinctive bodily constitution of our species, which differs from the sensory modalities of other biological life-forms. This biological heritage, or else our biological sensory apparatus, provides the input signals that form the basis for what we perceive and how we make meaning of the world. "Our type of eye is not the only type of eye, our type of smell is not the only type of smell," Deely describes.[49] Using the example of a meadow, he points out that the colors we see are a "shorthand for our limitations." "We see not all colors possible," he describes, "but only those that, under given conditions of light and shade, fall within the range of our type of eye."[50] The input of our senses ("elements of sensation") is therefore different from our cognitive constitution ("objects of experience.")

An organism's specific perception of the world is related to its survival and well-being. "For it is the interests of that organism, not the 'independent' nature of the source of the sensory stimuli, that is at issue in the perception as such that the organism finally acts upon and uses to orientate itself within the

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[46] Kull, Kalevi (2010). "Umwelt". In Cobley, Paul. *The Routledge Companion to Semiotics*. London: Routledge. pp. 348–349.

[47] Von Uexküll, J (1992). A Stroll Through the Worlds of Animals and Men: A Picture Book of Invisible Worlds, In *Instinctive Behavior: The Development of a Modern Concept*, ed. & trans. Claire H. Schiller, New York: International Universities Press, 1957, pp 5-80; repr. in Semiotica 89:4 (1992), pp 319-391

[48] Deely J., (2001) Umwelt. In *SEMIOTICA* 134(1/4): 125-135, special volume about Jakob von Uexküll (guest editor K. Kull)

[49] Ibid

[50] Deely 2001. Op. cit.

environment for the purposes of its life and well-being."[51] Finally, the difference between a human and a non-human animal is that the former is a semiotic animal, in the sense that it can model its own world.[52].

Before the science of Cybernetics had been developed, Uexküll drew little diagrams describing the feedback loops that happen during an organism's interaction in a natural environment. In these diagrams he graphically represented his findings on how perceived information from the environment is fed back into the afferent side of the organism's nervous system, generating a reaction.

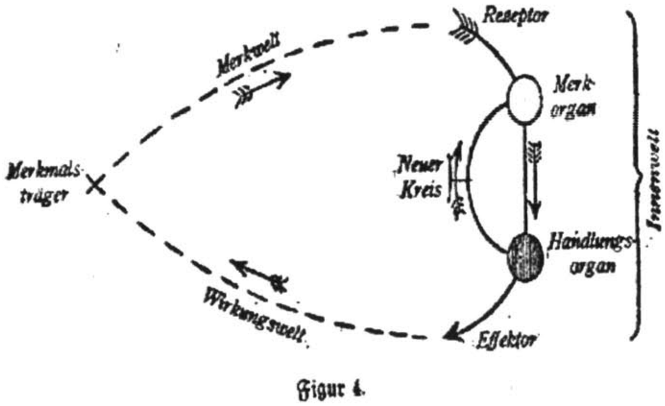
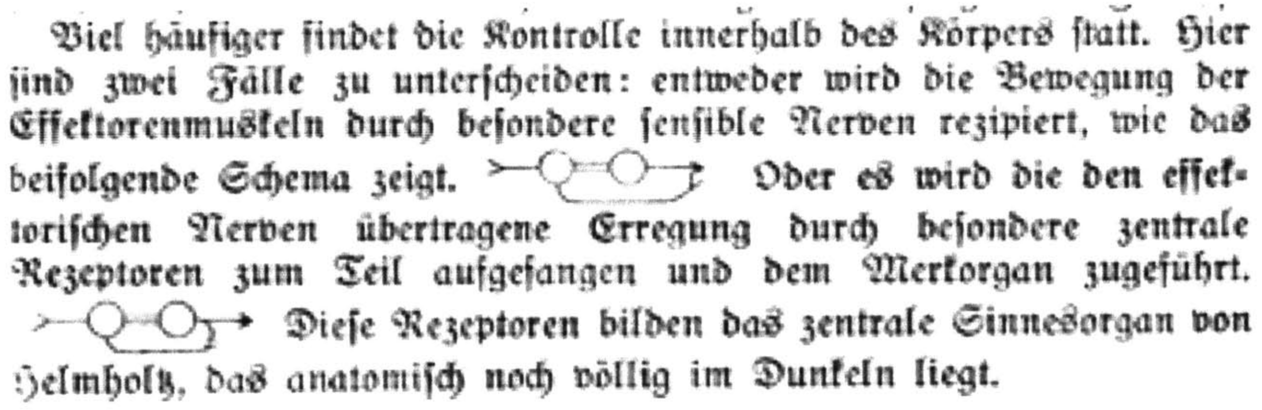


Figure 1. Functional cycle with reafferent cycle. (Uexküll 1920:117)



Viel häufiger findet die Kontrolle innerhalb des Körpers ſtatt. Hier ſind zwei Fälle zu unterſcheiden: entweder wird die Bewegung der Effektorenmuskeln durch beſondere ſenſible Nerven rezipiert, wie das beifolgende Schema zeigt. Oder es wird die den effektoriſchen Nerven übertragene Erregung durch beſondere zentrale Rezeptoren zum Teil aufgefangen und dem Merkorgan zugeführt. Dieſe Rezeptoren bilden das zentrale Sinnesorgan von Helmholtz, das anatomiſch noch völlig im Dunkeln liegt.

Figure 2. Little diagrams in the Uexküll illustrating a description of feedback and reafferent control (Uexküll 1920 : 201)

The notion of *sensory ecology* makes evident that human sensitivity is limited to the phenomena which our defined set of biological sensors can detect, whereas the notion of *umwelt* describes that part of the world that our senses allow us to perceive and model. In short, our experience of reality is constrained by our biology.

Human beings seem to be the only species that can participate in its own evolutionary process, to expand its own *umwelt*. Since the dawn of mankind, and through our technologies, we have been

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[51] Deely 2001. Op. cit.

[52] Deely therefore suggested the term *lebenswelt* to differentiate the human from other non-human *umwelten*. Deely 2001. Op. cit.

extending our ability to impact the world at scales that are not directly discernible through our senses and actively shape our perceptual worlds.

2.4 REVISITING THE THESIS CLAIM: MICROBIAL EXTENSIONS OF THE HUMAN SENSES

Our made material world is embedded with information carrying structures; it is sending an unprecedented quantity of signals. Data-generating digital technologies have afforded the opportunity to extend our senses and bring back information from scales and ranges that are not naturally perceptible by our senses. This is part of the extensible nature of all of our technology. However, this moment offers unique opportunities for distributing participatory processes and re-localizing sensitivity to our world through the design of the mediations between the individual and their hybridized environment.

The capacity of our technological impact has been increasing, but our sensitivity to that impact has lagged. The phenomena that occur in the hybridized world of the human and the natural, are either materially imperceptible to our senses —with no meaningful patterns of stimulus occurring within the temporal, physical, or geographical spectrum culled by our sensory apparatus and recognized as meaningful upstream— or performatively inaccessible to our participation, as they are mediated by an increasing number of policies and stakeholders, systemic in scale.

In his 2007 talk, Zizek claimed that we do not take the "ecological threat" seriously enough. Why do we do so little for example about the ozone hole, even though we know so well? Why do we not act? This is because the ozone hole does not belong in our "subjective space," we are not getting the signals. It is an abstracted problem, a scientific construction. We cannot sense it.

As we increase our capacity to influence our world at very small and very large scales, there becomes a rapidly growing set of phenomena which deserve our sensitivity. In the hybridized ecology of the natural and the made, I suggest the design of microbially-mediated cyber-biological systems as extensions of our sensory envelope, as an artificial nervous system, opening up new horizons of enhanced integration of humans and their environment. With this claim and concepts in mind, I propose to design technologies whose import will no longer be to debunk prematurely naturalized and objectified matters of facts but to allow us "to protect and to care," as Donna Haraway would put it, about what Bruno Latour calls "matters of concern"[53] — the *webby* realities that occur dynamically through the natural and the made, as opposed to cause-and effect abstractions of reality.

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[53] Latour, B. (2003). "Why Has Critique Run Out of Steam? From Matters of Fact to Matters of Concern." In *Critical Inquiry - Special issue on the Future of Critique*. Vol 30 n° 2 pp.25-248, Winter 2004.

Waterfly

ABSTRACT

Waterfly is an autonomous, multi-vehicle drone system imagined as "digital insects" monitoring, informing, and modulating the relationship between the natural and built environment. It is a network of aerial UAV vehicles designed to scan natural environments, in this case, bodies of water, in order to identify indicators of pollution with high precision and in real time, and map their expansion over time. It utilizes sensing technologies that tap into signals in the behavior of microbes in the water to read the state of the natural system. Using quadrotor vehicles working as a swarm, the vehicles share different sensing tasks, enabling the system to map vast areas, and gather multi-layered data on the health of aquatic ecologies, by scanning locations and collect spot-readings from the water itself. The case study for this system was the hazardous cyanobacteria toxins in the Charles River in Boston, which presents a central environmental challenge for the wider Boston area. The vision behind *Waterfly* was to design a platform that undertakes effective water monitoring, while enhancing community engagement to decrease non-point source water pollution. Juxtaposed with current approaches to the problem, the *Waterfly* technology brings realtime data about the levels of toxins in the Charles River to our senses, and thereby sensitizes us to the phenomenon instead of automating a problem-solving strategy that leaves out the human factor. Ultimately this allows people to better sense changes in aquatic ecology, correlate these changes with other geographical and temporal factors, and finally yield a potential change in behavior with regard to the relationship between the human actor with the aquatic ecosystem.

Image Credit: Chris Green

Waterfly

3.1 WATER POLLUTION

3.1.1 Introduction

Water pollution has widespread impact on the environment and human health. It is an especially acute challenge in the developing world, where 1.1 billion people are estimated to lack regular access to clean water.[1] In 2006, China's State Environmental Protection Administration (SEPA) estimated that 60 percent of the country's rivers are contaminated to the point where they cannot be used as sources for drinking water.[2] The World Health Organization assesses that water quality, sanitation, and hygiene interventions alone can reduce by up to a third the 2.2 million deaths caused by diarrhea every year, most of which are children under five.[3] Water pollution directly impairs the health of millions of individuals around the world, and is one of the leading causes of mortality globally.

In spite of the fact that developed countries tackle different water pollution impacts, but the problems remain pervasive. For example, in the most recent national report on water quality in the United States, 44 percent of assessed rivers, 64 percent of assessed lake acres, and 30 percent of assessed estuarine square miles were classified as impaired.[4] Recent and future extinction rates for North American freshwater fauna are five times higher than those for terrestrial fauna, which suggests that North America's temperate freshwater ecosystems are being depleted of species as rapidly as tropical forests.[5]

These issues reflect a detrimental impact on aquatic ecosystems, which in-turn affects other terrestrial ecosystems, which provide many benefits to humans. This concept has recently been framed as "ecosystem services."[6] The UN-initiated Millennium Ecosystem Assessment grouped ecosystem services into four broad categories, including: "*provisioning services* such as food, water, timber, and fiber; *regulating services* that affect climate, floods, disease, wastes, and water quality; *cultural services* that provide recreational, aesthetic, and spiritual benefits; and *supporting services* such as soil formation,

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[1] World Health Organization (WHO) Water Sanitation and Health (WSH) program, "Health through safe drinking water and basic sanitation" Available at: http://www.who.int/water_sanitation_health/mdg1/en/ (Section: Drinking Water, Sanitation, Health and Disease). Last accessed May 15, 2015

[2] "China pays water price for progress," *Water 21*, Magazine of the International Water Association, August 2007: 6

[3] World Health Organization (WHO) and Unicef. 2000. "Global Water Supply and Sanitation Assessment 2000 Report" Available at: http://www.who.int/water_sanitation_health/monitoring/jmp2000.pdf?ua=1. Last accessed May 15, 2015

[4] United States Environmental Protection Agency. 2004. "The *National Water Quality Inventory*: Report to Congress for the 2004 Reporting Cycle — A Profile" Available at: http://water.epa.gov/lawsregs/guidance/cwa/305b/upload/2009_01_22_305b_2004report_factsheet2004305b.pdf. Last accessed May 15, 2015

[5] Ricciardi, A. and Rasmussen, J. B. 1999. "Extinction Rates of North American Freshwater Fauna." *Conservation Biology*, 13: 1220–1222.

[6] Millennium Ecosystem Assessment (MA). 2005. "Ecosystems and Human Well-Being: Synthesis" <http://www.unep.org/maweb/documents/document.356.aspx.pdf>. Island Press, Washington. Preface: V

photosynthesis, and nutrient cycling."[7] Technology and culture buffer human society against environmental changes; however essentially we are completely dependent on the flow of ecosystem services.[8]

In short, water pollution not only has a direct effect on the health of individuals, but it threatens many "downstream" processes required to survive. And so it becomes evident that the challenge of monitoring and mitigating this phenomenon is a serious global issue that requires the continuous development of new methods, interventions, and policies.

3.1.2 Description of Causes

Water pollution can result from the presence of foreign contaminants that are washed or dumped into waterways, and discharges from industrial waste and sewage.[9] Alternatively, significantly altered concentrations of a naturally occurring substance can have equally negative effects on the aquatic ecosystem. For example, of the top causes of water pollution in the US, both organic enrichment due to sewage discharge, or a nutrient excess of nitrogen and phosphorus due to water runoff, can significantly affect levels of dissolved oxygen.[10] The agents causing water pollution can be classified into two broad categories: a) harmful microorganisms referred to as *pathogens,* which can cause disease, and b) chemical substances, which are divided into organic and inorganic substances. Below is a brief outline of these categories.

In their 2014 book *Quantitative Microbial Risk Assessment*, Charles N. Haas et al. observe that even though infectious diseases such as plagues, pestilence, and epidemic fevers existed in the human population since the beginning of human kind, "it was not until the advent of microbiological and epidemiological methods that pathogens were identified as the hazards and the environment (e.g. waterborne) was identified as the exposure routes."[11] While most bacteria are harmless or beneficial, there are a few microbes found in surface waters that can cause disease. Bacteria such as *Campylobacter, Salmonella, Cryptosporidium*, and *Giardia* and viruses such as rotavirus, norovirus, and enterovirus are some of the well-known pathogens capable of waterborne transmission, but parasitic, protozoan, and algal infections are also possible. Water-related illnesses can be transmitted through the

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[7] Ibid

[8] Ibid

[9] World Wildlife Foundation (WWF) Official Website. THREATS: POLLUTION. Available at: https://www.worldwildlife.org/threats/pollution. Last accessed May 15, 2015

[10] US EPA. 2004. "The *National Water Quality Inventory*" Op.cit.

[11] Haas, Charles N., Joan B. Rose, and Charles P. Gerba. (1999). *Quantitative Microbial Risk Assessment*. New York: Wiley

consumption of water (ingestion), through contact with skin (dermal contact), or through breathing aerosolized water particles (inhalation).[12] Schets et al. highlight the fact that contact with "microbiologically contaminated surface water may have adverse health effects and may result in gastroenteritis (GE); fever; skin, ear, and eye complaints; or more severe illnesses, such as hepatitis and meningitis."[13]

Though there are various transmission routes, water plays a central role in many cases. Figure 1 shows the possible routes of enteric microorganism transmission through the environment.[14] Dated or poorly maintained sewage infrastructure is a key source for this type of transmission. This can result from deficiencies in home septic tanks, municipal sewage pipes, or sewage plants themselves. As a bacterial indicator of the possibility of sewage-borne pathogens, testing for the presence of the non-pathogenic coliform or fecal coliform bacterias is common. The quantity of these bacteria in feces is stable, which makes them suitable as a proxy for identifying and measuring sewage pollution in the waterways.

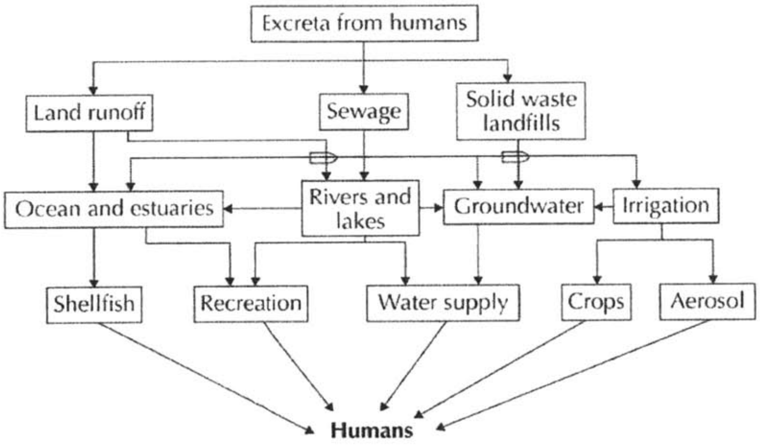

Figure 1. Possible routes of enteric microorganism transmission through the environment. (Haas et al. 1999)

Chemicals that are used to kill agricultural pests, such as pesticides, insecticides, herbicides, and fungicides can enter and contaminate water through direct application, urban run-off, atmospheric deposition. They can poison fish and wildlife, contaminate food sources, and destroy the habitat that animals use for protective cover. In "Organic Pollutants in the Water Cycle: Properties, Occurrence,

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[12] Centers for Disease Control and Prevention National Center for Environmental Health Vessel Sanitation Program, "Health Practices on Cruise Ships: Training for Employees Transcript: *Waterborne Illnesses*" Available at: http://www.cdc.gov/nceh/vsp/training/videos/transcripts/water.pdf. Last accessed May 15, 2015

[13] Schets, F. M. et al. (2008). "Monitoring of Waterborne Pathogens in Surface Waters in Amsterdam, The Netherlands, and the Potential Health Risk Associated with Exposure to *Cryptosporidium* and *Giardia* in These Waters ." *Applied and Environmental Microbiology* 74.7: 2069–2078.

[14] Haas, Charles N., Joan B. Rose, and Charles P. Gerba. (1999). *Quantitative Microbial Risk Assessment*. New York: Wiley.

Analysis and Environmental Relevance of Polar Compounds," Thorsten Reemtsma and Martin Jekel compiled a comprehensive reference on water contaminants, including residues of pharmaceuticals from human use, antibiotics for human use, veterinary pharmaceuticals, polar herbicides and metabolites, such as benzonitriles, aromatic acid herbicides, phosphorus compounds, nitrogenous compounds, surfactant metabolites, trihalomethane (THMs), Haloacetic Acids (HAAs) and Emerging Disinfection By-products that pollute drinking water.

Chemical contaminants may include organic and inorganic substances. Organic chemicals that cause water pollution include industrial solvents and other volatile organic compounds that are not properly stored, petroleum hydrocarbons such as gasoline, fuel, and motor oil that end up in streams, coastal waters, or other bodies of water due to stormwater runoff, disinfection by-products (DBPs) (e.g. chloroform), chlorinated organic molecules arresting from sewage or water treatment plants,[15] and many others.[16] Examples of inorganic chemical pollutants are heavy metals from motor vehicles,[17] ammonia from food processing waste, chemical waste from industrial by-products, and nutrients such as nitrates and phosphates included in fertilizers used in agriculture, or in commercial or residential uses.These chemicals end up in surface waters via storm water runoff, or runoff from construction sites and land clearing sites.[18] These lists are not exhaustive.

Besides these substances, there is also *macroscopic* pollution, which denotes larger-scale, visible objects, such as food waste, and other kinds of paper or plastic trash, debris from derelict ships, and other garbage, which are either intentionally or accidentally discarded and dumped into the water, or have been gradually washed into surface waters though urban stormwater runoff.

Another ecological imbalance considered as water pollution is *thermal pollution*, which describes the alterations in the temperature and the physical properties of a body of water caused by discharges that

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[15] Richardson, D., J. Plewa, D. Wagner, R. Schoeny and M. Demarini. (2007). *Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research.* Mutation research 636 (1-3): 178–242.

[16] Other organic water pollutants are food processing waste, (e.g. oxygen-demanding substances, fats and grease), organohalides and other chemical compounds which are used to kill insects (insecticides) or unwanted plants (herbicides), chlorinated solvents, such as polychlorinated biphenyl (PCBs) and trichloroethylene, chemical compounds from personal hygiene and cosmetic products, drug pollution involving pharmaceutical drugs and their metabolites.

[17] Burton explains that heavy metals end up in water bodies through urban storm water runoff.
Burton, G. Allen Jr., Robert Pitt. (2001). Stormwater Effects Handbook: A Toolbox for Watershed Managers, Scientists, and Engineers. New York: CRC/Lewis Publishers. Chapter 2, Available at: unix.eng.ua.edu/~rpitt/Publications/BooksandReports/Stormwater Effects Handbook by Burton and Pitt book/MainEDFS_Book.html. Last accessed May 15, 2015

[18] Ibid

typically affect "the metabolism of aquatic fauna in adverse ways."[19] Heated water results from the use of natural water as a coolant by power plants[20] and industrial manufacturers, such as pulp and paper, and steel mills,[21] from urban runoff, or from the release of cold water from the base of reservoirs into warmer bodies of water. For example, the Brayton Point Power Station in Massachusetts discharges heated water into Mount Hope Bay.

3.1.3 Point and Non-Point Sources

Pollutants are thought to reach surface waters from one of two types of sources. Sources that discharge pollutants directly into surface waters from a delivery system such as a pipe or ditch are called *point sources*, whereas sources that deliver pollutants to surface waters from diffuse origins such as runoff from streets or lawns are called *non-point sources* (NPS).[22] Examples of point sources include industrial facilities, municipal sewage treatment plants, combined sewer overflows, and storm sewers. Examples of non-point sources include urban runoff that is not captured in a storm sewer, agricultural runoff from cropland and grazing areas, leaking septic tanks, atmospheric deposition of contaminants from air pollution. To monitor for pollutants, the US Clean Water Act (CWA) regulates point sources, including municipal storm sewer systems and industrial storm water enforcement purposes. Its objective is "to restore and maintain the chemical, physical, and biological integrity of the nation's waters by preventing point and nonpoint pollution sources, providing assistance to publicly owned treatment works for the improvement of wastewater treatment, and maintaining the integrity of wetlands."[23] Non-point sources are significantly harder to monitor and understand, they are less accessible to regulation and enforcement, and represent one of the key challenges of intervention and mitigation.

3.1.4 Pollution of Urban Waters

Bodies of water that are situated in proximity to an urban environment represent a special case of the water pollution problem. Nearby bodies of water can be affected by a high contribution of both point and nonpoint source pollution. Urban environments sustain a high volume and diversity of human activities, and as such impose a significantly increased pollutant load on nearby surface waters. It is

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[19] Hogan, C. (2014). *Water pollution*. Retrieved from http://www.eoearth.org/view/article/156920. Last accessed May 15, 2015

[20] Laws, E.A. (2000). *Aquatic Pollution: An Introductory Text*. New York: John Wiley and Sons : 335

[21] United states Environmental Protection Agency (EPA) (2008) "Cooling Water Intake Structures - Basic Information," Washington, DC

[22] Hogan, Water Pollution, Op. cit.

[23] United States Environmental Protection Agency: Agriculture, Clean Water Act (CWA) available at: http://www.epa.gov/agriculture/lcwa.html, last updated August 29, 2014, last accessed May 15, 2015

estimated that impervious surfaces in urban settings, such as pavements and rooftops, result in five times more runoff than similar sized woodlands.[24] Runoff and overflow from streets contains oils, metals, rubber, road salts and other toxic contaminants deposited by motor vehicles. Air pollution can lead to acid rain, as well as to ammonium and nitrate deposition. If not properly treated, sewage from combined sewers, or in overflow situations, can be particularly problematic, introducing fecal coliform bacteria, shed pathogens, phosphorus, nitrates, various household and commercial detergents and chemicals, and other foreign contaminants.

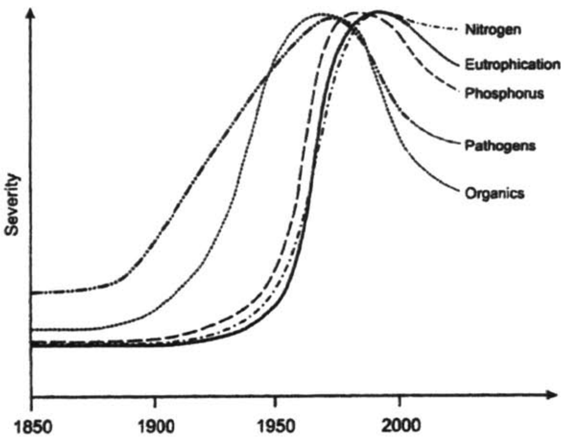

Figure 2. Schematic representation of the development of surface water pollution with pathogens, oxygen-consuming organic matter, phosphorus, and cyanobacteria in North-Western Europe and North America

Therefore, urban centers provide a significant challenge and opportunity for understanding the effects and potential interventions for a multitude of stressors to aquatic ecologies by a combination of point and nonpoint causes driven by human activity. A better understanding of these relationships also means that new interventions instantiated in cities can lead to a substantial improvement to water pollution management, which can be generalized to less complex contexts.

The increased number and diversity of nonpoint sources and an overall increase in pollutant load make for a more complex monitoring environment. Multiple human activities at both the individual and city scale aggregate over time and geography to contribute to the pollution and imbalance of urban water ecology.

Water pollution has risen with urbanization. Figure 2 shows a schematic representation of the development of surface water pollution with pathogens, oxygen-consuming organic matter, phosphorus

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[24] United States Environmental Protection Agency: Urban Nonpoint Source Fact Sheet (February 2003) "Clean Water is Everybody's Business" available at: http://water.epa.gov/polwaste/nps/urban_facts.cfm, last accessed May 15, 2015

and cyanobacteria in north-western Europe and in North America.[25] Bertram et al claim that: "Historically, the development of society has involved a change from rural and agricultural to urban and industrial water uses, which is reflected in both water demands and water pollution, as is illustrated in the figure. The general trend has been an increase in concentrations of pollutants and surface waters together with increases in urbanization."[26]

3.2 ALGAE AS INDICATORS AND FACTORS OF WATER POLLUTION

Two main issues of urban water pollution are high levels of bacteria and nutrient overabundance. An overabundance of the essential plant nutrients nitrogen and phosphorus can yield a rapid increase in the density of photosynthetic microorganisms that are naturally found in most habitats (algae), resulting in algae blooms. These are triggered by a combination of factors, including the concentration of nutrients, specific temperature, sunlight, and low flow.[27]

Karl Bruun from the Nostoca Algae Laboratory explains how water samples collection, and their microscopic analysis in order to determine the properties of the algal genera, such as their diversity and density, may provide useful information about the water conditions.[28] The relevance of algae for water quality assessment is based on their nutrient needs, rapid reproduction rate, and very short life cycle. Water conditions that involve changes in water chemistry cause the algae's rapid response both in species composition and densities. Examples that affect the composition of algae genera include acid-forming chemicals that increase the acidity of the waters, or heavy metals that are discharged from industrial areas.

Bruun also points to the importance of the Nitrogen:Phosphorus (N:P) ratio of the nutrients that flow into water bodies, and can result in eutrophic conditions (USEPA).[29] These elements, originating from various sources of pollution, such as household and commercial items (e.g. laundry detergents, lawn fertilizers), or sewage-related sources, (e.g. leaky septic tanks and livestock waste), end up in lakes and

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[25] Bartram J., Carmichael W.W., Chorus I., Jones G., Skulberg O.M. (1999). "Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management." In *World Health Organization (WHO)*. Available at: http://www.who.int/water_sanitation_health/resourcesquality/toxcyanchap1.pdf Last accessed 12 May 2015.

[26] Ibid

[27] St. Johns River Water Management District. (2014). "Understanding Algal Blooms." Available at: http://floridaswater.com/algae/ Last updated June 3, 2014. Last accessed May 15, 2015

[28] Bruun, K (Nostoca Algae Laboratory), "Algae can function as indicators of water pollution" available from the Washington State Lake Protection Association Official Website <http://www.walpa.org/waterline/june-2012/algae-can-function-as-indicators-of-water-pollution/> Last accessed May 15, 2015

[29] Ibid

reservoirs, which over time display "high densities of algae growth resulting in blooms of nuisance and/ or toxin-producing genera."[30]

Figure 3. Satellite image courtesy of NASA shows an algae bloom on the western side of Lake Erie. (Kate Abbey-Lambertz, Aug 6 2014, "These Disturbing Photos Show Why Algae Blooms Are A Growing Global Water Threat" *The Huffington Post*)

Eutrophic lakes occur due to thermally stratified water, intense sunlight, low N:P ratio, over periods of mild weather.[31] These most commonly shallow lakes are covered by "unsightly and potentially toxic surface scums"[32] formed by cyanobacteria. Bruun gives the example of Washington Lakes and reservoirs, where "blooms frequently involve a combination of relatively few cyanobacteria genera present at any particular time. Scum-forming blooms often consist of planktonic genera that contain cellular gas vacuoles and heterocytes. Gas vacuoles are responsible for cellular and colonial buoyancy that enables cyanobacteria to out-compete other algae for the available sunlight while heterocytes are responsible for nitrogen fixation. Benthic genera that form mats on the sediment and lack gas vacuoles

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[30] Ibid

[31] Bruun defines mild weather as "low turbulence and calm winds." Ibid

[32] Ibid

dislodge in large clumps, float to the top of the water column and also form unsightly, scum-like water conditions."[33]

In short, because of their vital role in aquatic ecosystems, algae can function as valuable indicators of ecosystem conditions, and thus water pollution. At the same time, high densities of cyanobacteria present concerns for ecological and public health, due to the hepatotoxins and neurotoxins that they produce. The presence of such toxins can result in food web disruption, recreation restriction, and drinking water contamination.[34]

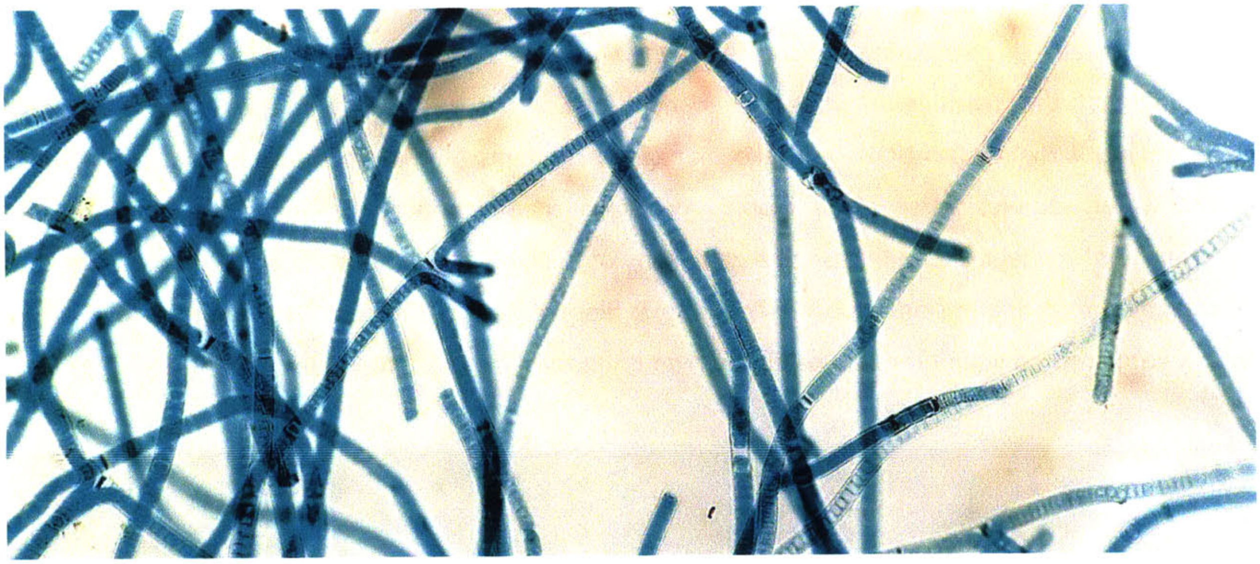

Figure 4. A form of cyanobacteria known as Tolypothrix. (Photomicrograph: Matthew Parker via Wikimedia Commons)

These observations highlight the unique property of cyanobacteria algae blooms where they in themselves represent a stressor response that is harmful to the aquatic ecology and public health, and they are also an indicator species for detecting and differentiating between other contaminants and the degree of pollution of surface waters. This duality makes these algae bloom of particular utility to monitoring programs, as it is necessary to directly track their toxins and they provide additional information about the state of the ecosystem.

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[33] Ibid

[34] Bruun explains that: "Toxin-producing blooms may disrupt lake food webs by killing fish, birds and zooplankton and can be responsible for hypoxia conditions that follow bloom die-offs. Toxic blooms can also restrict recreation like swimming, fishing and pet-related activities. Additionally, toxins produced from blooms can pose problems for households that get their drinking water from lakes and reservoirs." Ibid

3.2.1 Limitations of Current Methods of Monitoring Algae

The challenge of monitoring and mitigating water pollution has become a serious global issue that requires the continuous development of new methods, interventions, and policies. Very little of this allows for individual access and participation. We currently rely on institutional environmental agencies and federal regulation to mitigate these impacts, many of which are the result of aggregate daily actions of millions of people who are far removed by scales of time, space, size, from the issues they may be contributing to. More importantly, they are abstracted from the improvement their care may be able to create.

Current methods of monitoring include: a) *Manual sample collection for off site processing.* Bruun mentions that the Washington State Department of Ecology (WADOE) and the Washington State Department of Health (WADOH) ask lake managers, residents, and concerned citizens, to manually collect water samples when algae blooms occur so that identifications and toxin levels can be determined at the laboratory. In case of high density of toxins, warning signs are posted around the lake to prevent human health from exposure to the toxic blooms.[35] In the Charles River in Boston this is done by a devoted retired volunteer who pales the river in zig zags once very two weeks during the summer.

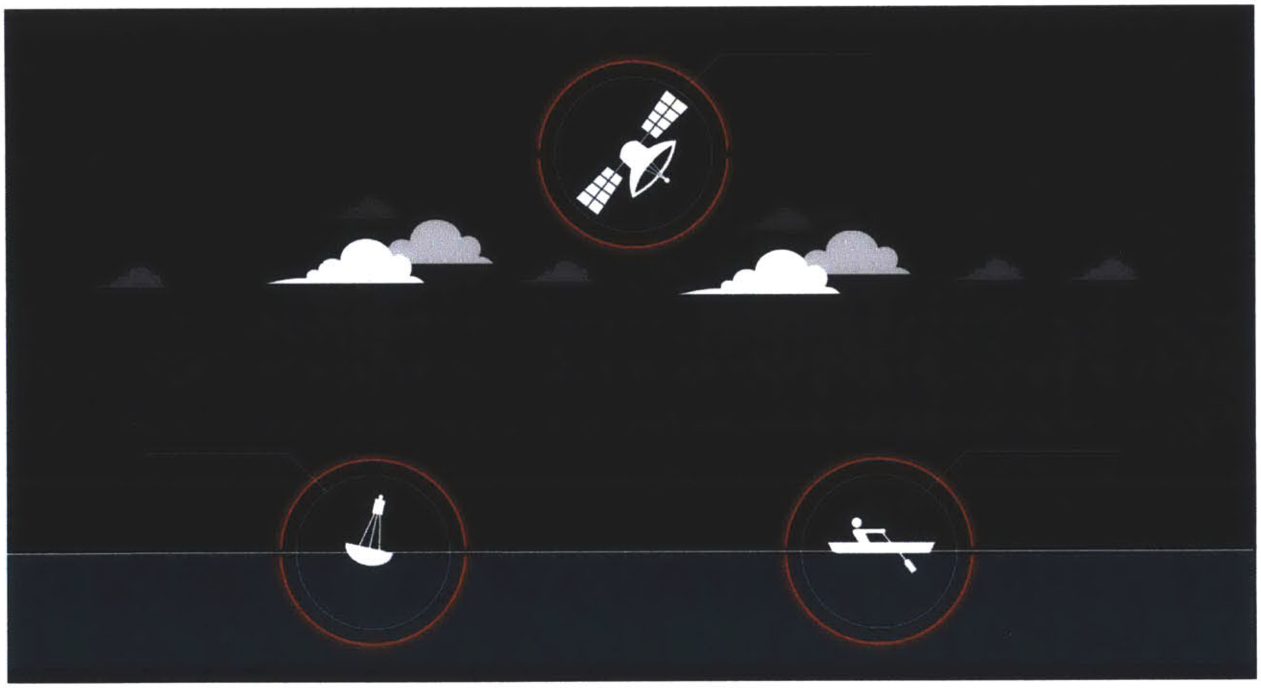

Figure 5. Current Methods for monitoring algae (Illustration Credit: Gabriel Kozlowski)

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[35] Ibid

b) *Monitoring buoys*,[36] which are a recent advance. However, their stationary nature means they collect information about a single point. As the challenge is to map non-point sources and capture changes in bloom size and location, this substantially limits their ability to generate an understanding of an issue.

c) *Satellite imaging* has also been utilized in recent years, and helps create a longitudinal time series covering large geographies. However, satellites can describe only pollution that has visual proxies, and only provide an approximation of what might be taking place on site. Additionally, the rate of capture is determined by the satellites orbit, and the resolution remains above 30 meters, both of which do not allow for an understanding of the necessary temporal and physical dynamics.

3.2.2 Limitations of Current Strategies for Intervention

Monitoring for pollution in urban waters focuses primarily a) on health risk assessment (HRA), defined by the Centers for Disease Control and Prevention as "a systematic approach to collecting information from individuals that identifies risk factors, provides individualized feedback, and links the person with at least one intervention to promote health, sustain function and/or prevent disease,"[37] and b) on ecological risk assessment (ERA), "the process for evaluating how likely it is that the environment may be impacted as a result of exposure to one or more environmental stressors such as chemicals, land change, disease, invasive species and climate change."[38] Regarding ecological risk assessment, Prosser et al. claim that "Understanding the ecology of microorganisms is critical to dealing with the challenges facing human society in the management of natural ecosystems and the mitigation of climate change."[39]

Using the data collected either manually, or via the river buoys, and satellite imaging, stormwater management practices are implemented top down in order to guarantee that the overall phosphorus discharges remain at a level that does not impair the quality of the waterbody. For example, based on the extensive data collected from the Charles River over a multitude of years, Ii October 2007, the EPA and the Massachusetts Department of Environmental Protection (MassDEP) established a target "Total

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[36] "Every 15 minutes, this self-contained solar powered buoy takes measurements for temperature, dissolved oxygen, pH, specific conductance, turbidity, chlorophyll, and phycocyanin. The sensors on the buoy are located 1 meter below the water's surface. The data are transmitted in realtime using telemetry for remote access. The data from this buoy is used to assess water quality conditions and to help track cyanobacteria (blue-green algae) blooms." From EPA's Official Website, "Basic Information about the Charles River Buoy," available at: http://www2.epa.gov/charlesriver/basic-information-about-charles-river-buoy. Last accessed May 15, 2015

[37] Centers for Disease Control and Prevention. (2010). "Health Risk Appraisals" Available at: http://www.cdc.gov/nccdphp/dnpao/hwi/programdesign/health_risk_appraisals.htm. Last updated January 6, 2010. Last accessed May 15, 2015.

[38] United States Environmental Protection Agency, "Ecological Risk Assessment" Available at: http://www.epa.gov/risk_assessment/ecological-risk.htm. Last Updated May 8, 2013. Last accessed May 15, 2015

[39] Prosser, J.I., et al. (2007). "The Role of Ecological Theory in Microbial Ecology.." *Nature (Reviews/Microbiology)* 5.5 (MAY): 384–392. Web. Available at: http://pages.uoregon.edu/bohannanlab/pubs/Prosseretal07.pdf. Last accessed May 15, 2015

Maximum Daily Load" (TMDL) for the lower Charles River. This number refers to the maximum amount of nutrient phosphorus that the river can receive before reaching harmful levels and failing water quality standards. The limitations of such strategies are related to their efficient application in the case of point sources of water pollution. More specifically, in the case of municipalities and other stormwater discharges that can reduce their contribution of phosphorus to the Charles River.

Alternatively, it has been suggested in other bottom up scenarios that stakeholders are actively participating in monitoring and improving the problem. The concept of local ecological knowledge (LEK) refers to a non-codified system of extensive ecological expertise of local communities, gradually developed through personal experience and interaction with a natural ecosystem. The National Marine Fisheries Service of the US Department of Commerce defines LEK as "knowledge acquired through experience and observation," which can "be acquired over a single lifetime or over many generations."[40] Many academics and activists have linked ecological and human social systems as a new way towards sustainability, arguing that local participation has the potential to enhance the sustainable development of communities and their natural environments, especially through the integration of the participants that are connected to a specific ecosystem.[41] In discussing the promise of LEK, Alexis Schulman describes a three-fold argument offered by proponents of integrating LEK into management's knowledge base: "They suggest that it can improve the understanding of local ecological and social conditions, producing management decisions and policies that are more responsive to these conditions, offer models of adaptive, sustainable resource use, and quell some of the conflict and mistrust that arises when local expertise is ignored and discredited as *anecdotal*."[42] However, LEK is rarely used in practice.[43]

In a 1993 *Science* magazine article, entitled "Uncertainty, Resource Exploitation, and Conservation: Lessors from History," Ludwig et al. claimed that the solution to environmental problems would not come from scientists, and the conventional scientific approach to sustainability was not working. I do not read this claim as a condemnation of the scientific enterprise but as an occasion for examining how

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[40] Local Fisheries Knowledge (LFK) Project: Definitions of Ethno-ecological Research Terms, by the NOAA Fisheries, available at: http://www.st.nmfs.noaa.gov/lfkproject/02_c.definitions.htm

[41] Yli-Pelkonen V. & Kohl J. 2005. The role of local ecological knowledge in sustainable urban planning: perspectives from Finland. Sustainability: Science, Practice, & Policy 1(1):3-14. Published online Mar 16, 2005. http://www.google.com/archives/vol1iss1/0410-007.yli-pelkonen.html

[42] Alexis Schulman, "Bridging the Divide: Incorporating Local Ecological Knowledge into US Natural Resource Management" Master in City Planning Thesis at the Massachusetts Institute of Technology, September 2007

[43] Huntington, H. P. 2000. Using traditional ecological knowledge in science: Methods and applications. Ecological Applications 10, (5) (OCT): 1270-1274.

expert-driven top-down application of science can be complemented with a participatory approach that allows the individual to participate in knowledge production, as well as ecological processes. Examples that help crystallize the different logics of LEK, compared to institutionalized hierarchies, include the "unscientific" expertise of primitive cultures. Based on sociologist Jean Douwe Van der Ploeg's study of the indigenous potato cultivation in the Andes, James Scott articulated the differences in the logics of the technocratic and the local model of knowledge.[44] On the one hand, the process of shaping technocratic knowledge begins with a model of the ideal plant type; "it is not realized on the ground," but involves the standardization of a farmer figure growing a standard seed variety "on similar soils and leveled fields and according to the instructions printed right on the seed packets, applying the same fertilizers, pesticides, and amounts of water."[45] Scott talks about a logic of an impossible homogenization, which is doomed to fail. On the other hand, the process of the local farmer is a *craft*: "The logic of actual farming is one of inventive, practiced response to a highly variable environment. The cultivator begins with an exceptional diverse local ecology and aims at adapting to it and gradually improving it." For Scott, the cultivation of such skills allow the Andean farmers to achieve results that are "extraordinary in terms of yields and sustainability."[46]

Even though the example of potato cultivation refers to an act of production, the same logics that support the incorporation of LEK into management science, can be extended to the mitigation of environmental problems. In the case of polluted bodies of water, a distributed platform of decision-making substituting hierarchical policy-making can involve more actors in the protection of the ecosystem, and can therefore significantly impact the sustainability of the hybridized system of the urban aquatic environment.

3.2.3 Conclusions regarding Urban Water Pollution Monitoring

Surface water pollution represents a highly complex challenge to environmental monitoring and intervention. Point sources are easier to monitor, and a portion of their contribution has been increasingly mitigated through regulation and enforcement. Non-point sources are substantially more difficult to monitor, understand, and mitigate, and remain a substantial contribution to surface water pollution. They are highly distributed contributions, which require monitoring at a high spatiotemporal

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[44] Scott, J. C. (1998). *Seeing Like a State : How Certain Schemes to Improve the Human Condition Have Failed.* Yale agrarian studies. New Haven Conn.: Yale University Press.

[45] Ibid

[46] Ibid

resolution and correlation with human activity and other environmental events so that mechanisms of contamination can be modeled and new interventions can be conceived.

Urban bodies of water are subject to particularly high contaminant load sourced from both point and nonpoint sources, including a wide array of substances and pathogens that change over time and location with human activity and environmental responses. A key issue is nutrient overabundance, especially of nitrogen and phosphorus, which contributes to eutrophication and the formation of harmful algal blooms. These algae are simultaneously suitable as indicators of water pollution, with different genera responding to specific nutrient or substance imbalances. This dual nature of the algae as both a harmful to the ecosystem — as a resource-burden to the aquatic environment and in releasing neurotoxins dangerous to humans and animals — and a proxy for poor ecological conditions, position it an important opportunity for urban water pollution monitoring. These algae respond very dynamically in space and time to introductions of contaminants, and monitoring requires a system capable of capturing this high spatiotemporal resolution information.

These data should be sufficiently interoperable with additional high-resolution physical information from the aquatic ecosystem (e.g., water temperature, flow, pH), the ambient environment (e.g., temperature, weather events, nitrogen and particulate matter in the air), and human activity (e.g., vehicle traffic, combined sewage overflow, aquatic recreational use).

Current methods are insufficient for this task, and monitoring would benefit from a system capable of high resolution distributed sensing that can locate subtle changes in stressors and responses, and provide insight to both point and nonpoint contributions. Moreover, as regulation and enforcement less effective in dealing with systemic nonpoint contamination, such a system would benefit from realtime visualization of the invisible pollutants and state of the ecosystem. This would bring to life otherwise intangible information for the benefit of both stakeholders making crucial decisions as well as individual citizens, whose ability to connect to an otherwise abstract issue is essential for the type of behavioral change needed to sustain our urban aquatic ecosystems.

3.3 THE WATERFLY PROJECT

3.3.1 Introduction

Waterfly is a method for distributed sensing for aquatic ecosystems. Using quadrotor vehicles working as a swarm, the vehicles share different sensing tasks, enabling the system to map vast areas, and gather multi-layered data on the health of aquatic ecologies, by scanning locations and collect spot-readings from the water itself. Specifically in the initial case study of the Lower Basin of the Charles River, the system is designed to exploit the natural properties of cyanobacteria to generate high-resolution, geolocated, spatiotemporal datasets describing potential harmful toxins as well as indications of other water contaminants.

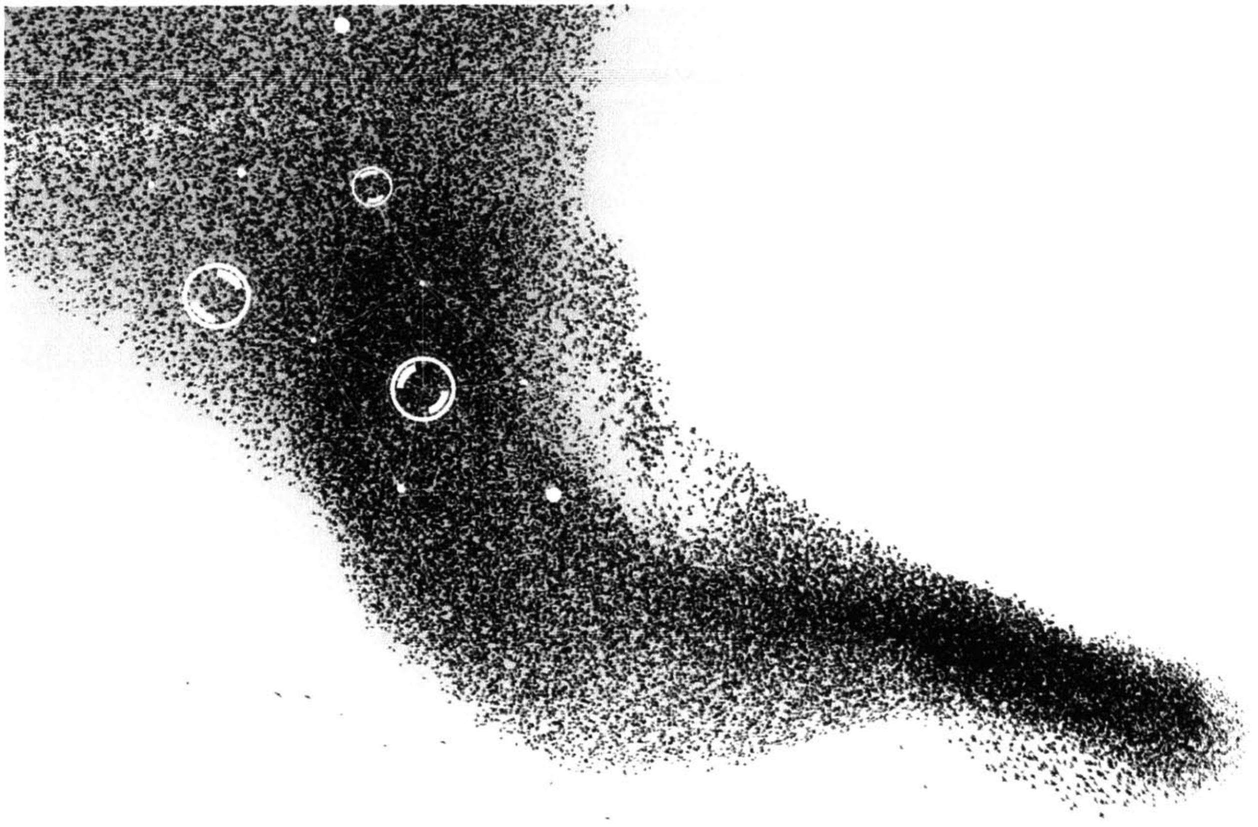

Figure 6. The Waterfly system gather spatiotemporal data on the health of aquatic ecosystems, and makes the data available to both citizens and researchers through an online visualization platform (Image Credit: Chris Green)

In June 2013, Mike Gineres, an Environmental Health Officer of the Cambridge Public Health Department, had first brought to my attention that the City of Cambridge had identified cyanobacteria blooms as a central environmental challenge, and sought solutions that would enable effective monitoring of the Charles River. He contacted our team, having seen the SkyCall project.[47] At this same time I was considering applications of UAV's in urban environments as a way of giving citizens individualized access to the invisible phenomena between the built and natural environments. The idea of "digital insects" — UAV's that would operate in a swarm and mediate processes between these environments — was one I had been considering for a while, and together with researcher Chris Green we began to imagine possible contexts for such an intervention. Gineres's suggestion that we focus on

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[47] For details about the SkyCall project, see: http://senseable.mit.edu/skycall/

cyanobacteria blooms in the Charles River lead me to the research on water pollution described above in this chapter, which supported the idea of monitoring these algae blooms to benefit aquatic environments. The scientific, engineering, and community engagement challenges presented by monitoring cyanobacteria blooms strongly supported the need for development of a distributed sensing and realtime information system for mitigating point and nonpoint pollution of urban waters, as described below.

3.3.2 Pollution of the Charles River in Boston

The Environmental Pollution Agency reports that as urbanization in Boston started in the early 1600s, the "associated degradation of the Charles River has occurred over a four hundred year period," starting from earlier industrial contamination.[48] The City of Cambridge has identified the Charles River Pollution as a central environmental challenge and has been searching for solutions that would enable effective monitoring of the river, and at the same time enhance community engagement to decrease non-point source water pollution.

The EPA notes that the extensive blue-green algae blooms that cover the Charles River during the summer are caused by nutrient overabundance, primarily phosphorus. Cyanophyta is a phylum of bacteria that obtain their energy through photosynthesis.[49] The name "cyanobacteria" comes from their color. They are often called "blue-green algae," but that is technically not correct as they are prokaryotic, i.e. single-celled. Cyanobacteria get their energy from photosynthesis, and the specific colors of their pigments can be detected through imaging from a distance. In the water, phycocyanin pigments can be measured and directly correlated with the abundance of the bacteria. Recent scientific work has also established cyanobacteria as an indicator species, making possible additional correlations about an aquatic ecosystem's state both in terms of contaminants and other wildlife.

The algae blooms are an undesirable component of the ecosystem due to the toxins that they release when they die, which are ecological and public health concerns. Contact with the toxins released by cyanobacteria blooms can cause dermal, eye, and throat irritations, while ingestion can lead to severe liver and nervous system damage. Additionally, the existence of the blue-green algae blooms affects the ecosystem as a natural fish habitat, as it reduces the clarity of the water, as well as the oxygen necessary for the survival of the fish that inhabit the ecosystem.

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[48] United States Environmental Protection Agency. (2005). "Clean Charles River Effort Celebrated For Ten Years of Environmental Progress - Water Quality Significantly Improved." (May 31, 2005) Available at: http://www2.epa.gov/charlesriver Last Updated May 21, 2015. Last accessed May 21, 2015.

[49] Ibid

Since 1995, the EPA and its partners have been working to reverse that degradation, by reducing reduce the amount of stormwater runoff entering the River. Their goal is "to reduce phosphorus discharges to the lower Charles by 54 percent to restore the river to a healthy state." According to the Agency's records, in 1995 the River met boating standards only 39 percent of the time and was safe for swimming just 19 percent of the time. Since then there has been notable improvement. The EPA records show that in 2013, "the river was safe for boating 96 percent of the time and safe for swimming 70 percent of the time, representing the highest safe swimming percentage in the past 19 years since the Charles grade was first issued."[50]

3.3.3 Properties of the Desired Monitoring System

Taking the case study of the Charles River Lower Basin in Boston, with its annual blooms of toxic cyanobacteria, we proposed to deploy a fleet of UAVs to autonomously monitor and sample bacteria levels in the water. Relaying information from the river in realtime facilitates a deeper dynamic insight into the microbial ecology and its behavior across the Charles. Together with the city, and in consultation with the Environmental Protection Agency (EPA) and the Charles River Watershed Association (CRWA), we defined the considerations for a monitoring system that would enable effective monitoring of the Charles River.

First, given the impact of both point and nonpoint contamination on cyanobacteria bloom development, and the fast development of blooms, we wanted a system that could measure with high spatial and temporal resolution. The ideal resolution for efficacious data could not be known in advance, and so the system should be able to provide flexibility in this respect, offering a monitoring strategy that would allow a temporal resolution on the order of hours, and a sub-meter spatial resolution. Second, we determined that the ideal measurements to characterize cyanobacterial growth would be a combination of visual imaging of photosynthesis to capture general bloom formation and identify "hotspots" of activity, coupled with direct readings of phycobilin pigments which correlate well with biomass and cell count, enabling quantification. In this way we would be able to both identify in realtime potential public health hazards of the cyanobacterial toxins, as well as potentially infer levels of additional contaminants affecting specific areas of the Charles Lower Basin, stressors to which a common consequence is the propagation of cyanobacteria. Third, to enhance LEK, the system would be designed with a human operator in mind, and would require both decisions from the system and user to

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[50] Ibid

operate intelligently. The interface should be aimed at informing the scientist and citizen alike on the state of the aquatic ecology, in order to make accessible the otherwise invisible issues of the river.

As such, the initial requirements description was formed as follows:

- The system should be able to operate autonomously, optimizing for information resolution, operation time, and environmental conditions. It should also offer realtime human intervention in the determining of targets for monitoring, resolution of monitoring, and operation time.
- The system should be able to cover the area of the Charles River Lower Basin, a man made intervention in the Charles River covering about 31 km of shoreline from the dam near Watertown Square to the New Dam at the Charlestown Bridge.
- The system should be able to take a complete survey of this area at a minimal rate of once per week, and all the data gathered in each full survey should have a temporal collection timestamp with a variation of less than two days.
- The system should be able to collect high resolution visual images of the Charles River Basin, and be able to identify planktonic algal blooms in these maps. These image maps should be able to achieve a 1-image-pixel-equals-1-real-world-cm resolution, and be able to repeatedly cover a bloom several times in a single day if required.
- The system should be able to collect information about the identified algal blooms and be able to distinguish toxin producing cyanobacteria from other groups of algae, *in vivo.* The system should be able to interpolate probed measurements to quantified metrics describing the development of algal blooms, to be used for building high-resolution dynamic models of blooms in the basin, for analysis in fusion with data about human activities and environmental changes.
- The system should have an interface for communicating autonomous flight information and controlling manual flight operations.
- The system should include an interface for viewing realtime information collected, to enable realtime decision making for future operations. This should be accessible to both the expert stakeholder and the individual citizen interested in understanding the basin's ecology. The system should also provide a database of historical data for further analysis and integration with other data sources.

3.3.4 UAVs may be a useful platform for water quality monitoring

We first had to determine if autonomous flight was an appropriate strategy for the surveying of the Lower Basin. As we had established that the imaging and probing would be effective techniques for describing the cyanobacterial blooms, it remained to be determined that aerial vehicles would be well suited to these tasks, and could provide advantages in their performance.

Methods for environmental monitoring by UAVs have been popularized in recent years. Anderson et al. highlight the benefits UAVs offer scientists for scale-appropriate measurement of ecological phenomena. UAVs can deliver high resolution spatial data at the temporal constraints defined by the end user. The authors also review several limitations in UAV technologies including platform, sensor, operating, and environmental constraints.[51] Bryson et al. have recently developed a UAV system equipped with an inertial measuring unit, a global positioning system, and a monocular camera capable of reconstructing digital representations of large-scale terrain and classifying different species of vegetation within the environment. The resulting system can be used for a range of monitoring missions, such as invasive plant detection and biomass mapping.[52] Goktogan et al. developed an autonomous rotary wing unmanned air vehicle for surveillance and management of aquatic weeds. The RUAV is able to fly at low attitude with a laser altimeter and spray aquatic weeds.[53]

In further evaluation of our requirements, a few references provided some precedent of UAV's monitoring aquatic environments. The paper from Ore et al. describes the design of a water sampling system that deployed a UAV to navigate to designated sampling GPS locations and hovered above the water using altitude approximation algorithm while capturing water samples.[54] Another commercial work has developed an Aquacopter UAV capable of landing on and taking off from calm water.[55]

Given the examples above which have taken steps towards utilizing UAVs to monitor natural environments, we felt there was a significant contribution to be made by developing a multi-vehicle system for distributed sensing, mimicking swarm-biology. Early studies of group behaviors showed that

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[51] Anderson, K., Gaston, K.J. (2013). *Lightweight unmanned aerial vehicles will revolutionize spatial ecology.* Frontiers in Ecology and the Environment 11 (3) 138–146

[52] Bryson, M., A. Reid, F. Ramos, S. Sukkarieh. (2010). *Airborne vision-based mapping and classification of large farmland environments.* Journal of Field Robotics 27 (5): 632–655.

[53] Goktogan, A.H., et al. (2010). *A rotary-wing unmanned air vehicle for aquatic weed surveillance and management.* Selected papers from the 2nd International Symposium on UAVs, Reno, Nevada, USA June 8–10, 2009, Springer, pp. 467–484.

[54] Ore, J.P., et al, *Autonomous aerial water sampling.* Ph.D. thesis, University of Nebraska (2014)

[55] Aquacopters <http://www.aquacopters.com.> Last accessed 21 May 2015

they arise from individual decisions that are transmitted to the collective,[56] a principle that inspired the design of our system. Relying on this principle, it seemed reasonable that different sensing functions could be assigned to specialized vehicles, each with a proprietary form and behavior optimized for its task. This strategy answered the need for dynamically distributing imaging and probing, with the ability to reconfigure the swarm and it's behavior to optimize for changing conditions or operational requirements.

3.4. SYSTEM DESIGN

3.4.1 System Overview

Waterfly was conceived as a cyber-physical system — a physical network of aerial vehicles, deployed as dynamic digital sensors. As an infrastructure for environmental sensing, the system is comprised of two key aspects: autonomous quadrotors performing sensing tasks, and a digital backbone for communication and data collection.

We defined a system that would behave in the following manner: (i) a UAV to fly and capture images of a designated area, (ii) the images will be fed to a computer vision system that will produce an aerial map of the area and detect algae area, (iii) a path generator will generate an arrangement of probing points and an optimized flight path in this area, (iv) a water probing UAV will fly to these probing points to take measurements of the water condition, (v) the probing data would be sent and processed at the base station in realtime, (vi) an interactive GUI interface enables user to view the aerial maps, deploy UAVs and read water contamination information interactively.

The system was designed with the following principle components:

- Web UI - A robust, user-friendly web-based interface, comprising of two principle modes:
  - Operation UI - A user interface that enables configuration and deployment of the system.
  - Data Visualization UI - A user interface that receives and visualizes data in realtime.
- Cloud Server - All communications are passed via the cloud, enabling the system to be deployed over vast areas in areas of good 4G coverage. Swarm-sensing between multiple vehicles is coordinated through the cloud backend, with scalable strategies to optimize the sharing of sensing tasks between quadrotors over large areas.

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[56] Kvajo, M. (2009). "The Wisdom of Crowds" In *Cell* 161.4: 697. Web. 12 May 2015.

- Imaging UAVs - Autonomous-flight-capable Imaging UAV leads the swarm of vehicles and relays images of the water back to the user interface in realtime.
- Probing UAVs - Autonomous-flight-capable Probing UAVs carry onboard probes, used to take readings from the water when the vehicle lands at spot locations.

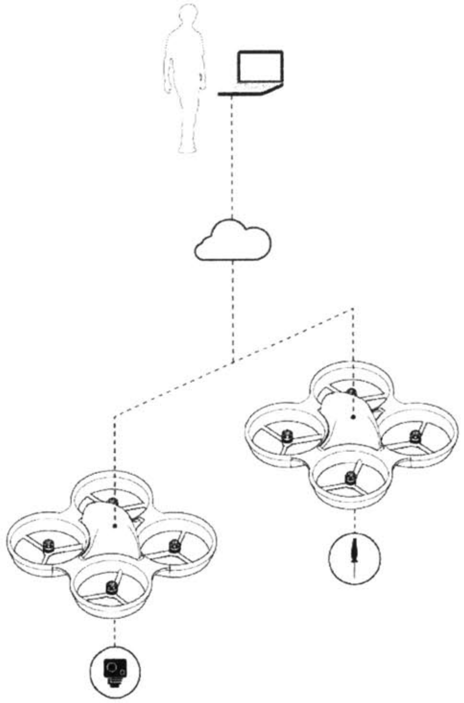

Figure 7. A typical flight operator is comprised by the following components: Operator, Cloud Server, Probing UAVs, Imaging UAV (Illustration Credit: Chris Green, Technical Submission to the UAE Drones For Good Competition. Dec 1, 2014)

The system's architecture is comprised of the following components: web user interface (Web UI), web server, flight path generator (FPG), Cloud server, image stitching algorithm (ISA), hotspot detection algorithm (HDA) and two types of UAVs — the imaging vehicles and the probing vehicles.

The operation of the system proceeds as follows:

1. The operator uses the web-based user-interface on a laptop or tablet device to launch the vehicles. The user begins by selecting an area of water to survey, identifying the outer boundary points of the area of interest.
2. Using the list of coordinates, the flight path generator (FPG) calculates the area information, and generates an optimized aerial flight way-path that enables the aerial UAV to capture multiple overlaying images of the water region specified. This path is a function of the area and the resolution of spatial information required from the resulting image maps.

3. Upon pressing launch from the user interface, the first Imaging UAV takes off from its starting position, and follows the set way-path over the body of water to be monitored. It collects images and relays them to the cloud server via onboard 4G transmission.
4. The image stitching algorithm (ISA) constructs a growing map of the monitored area in realtime during the flight, which appears on the user interface.
5. The images are processed on the fly by the hotspot detection algorithm (HDA) to detect potential areas of interest - in this case, classifying areas with high levels of chlorophyll a from hyper-spectral images. The result is an additional map representing 'hotspots' displayed on the user interface as geolocated regions of interest marked by their outer boundaries.

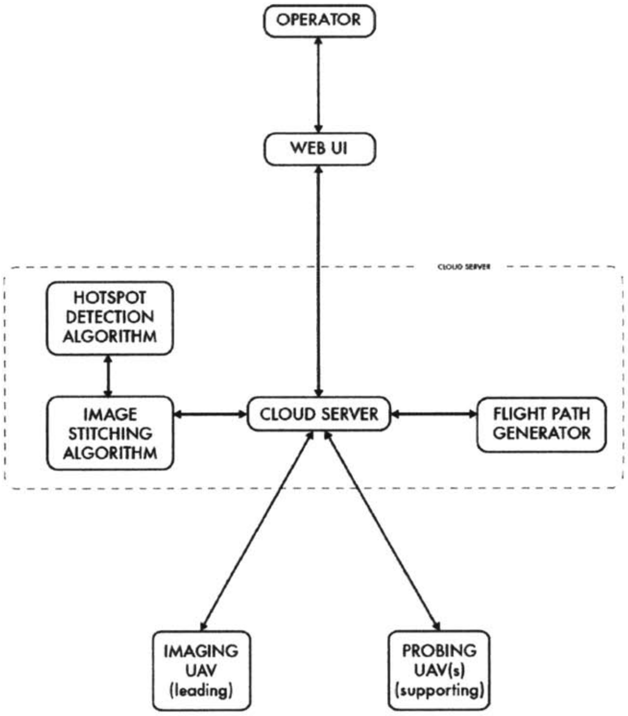

Figure 8. The System's Architecture comprised by the following components: web user interface (Web UI), web server, flight path generator (FPG), Cloud server, image stitching algorithm (ISA), hotspot detection algorithm (HDA) and the UAVs. (Illustration Credit: Chris Green, Technical Submission to the UAE Drones For Good Competition. Dec 1, 2014)

6. A human operator has the option to intervene and select specific hotspots of interest for the system to prioritize. Alternatively, the system will continue autonomously to collect information from all the areas of interest detected.

7. The FPG uses these geolocated boundaries to calculate a grid of probing points, and then creates way-paths for the Probing UAVs to investigate and take readings from these areas of interest. The number of sample points in an area depends on the resolution that the human operator inputs. As the system is able to support multiple vehicles flying as a swarm, the probing tasks can be shared amongst a number of vehicles. The waypoints are optimally distributed between the vehicles, enabling the system to probe many different areas simultaneously.
8. Once the flight path is generated, the human operator can deploy the water probing UAVs or the system can be configured to do so autonomously. At each point, the probing UAV lands on the water, takes a reading, and relays the information back to the cloud server via the 4G connection.
9. The data are then displayed on the web Ui in realtime, allowing the operator to take new decisions about continued monitoring. Alternatively, the system can continue to monitor a preconfigured area autonomously, collecting data for later analysis.

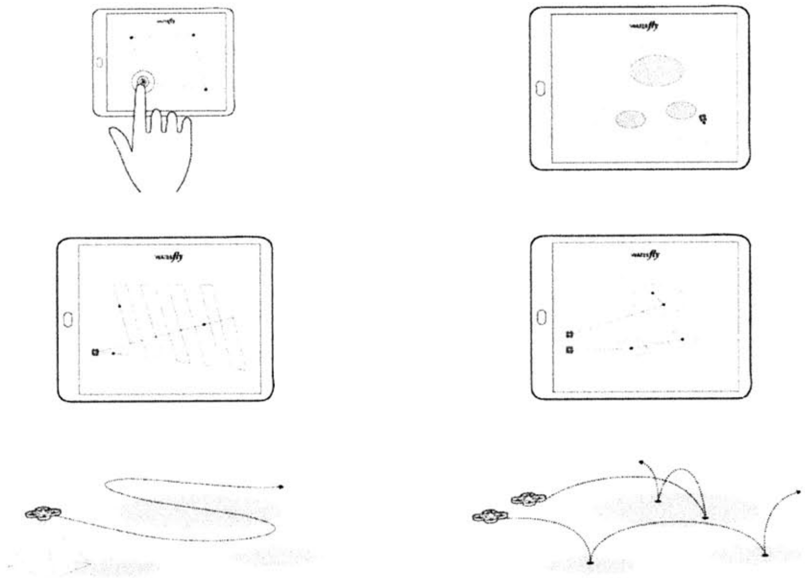

Figure 9. Steps of a typical flight operation. (Illustration Credit: Chris Green, Technical Submission to the UAE Drones For Good Competition. Dec 1, 2014)

3.4.2 Vehicles

During the course of the project development, we designed 3 primary vehicles, as follows:

1. Imaging UAV (Figure 10): For the initial prototypes of the Imaging UAV, we modified an off-the-shelf 3DR quadcopter, adding propeller guards to test the design for collision safety, and a modified GoPro camera capable of hyper-spectral NVDI imaging.

Figure 10. Early prototype of the Aerial Quadcopter built to perform hyper-spectral imaging (left). The S-shape route of the lead vehicle above the Charles River (right) (Illustration Credit: Chris Green, Technical Submission to the UAE Drones For Good Competition. Dec 1, 2014)

2. Probing UAV (Figure 11): For the initial prototypes of the Probing UAVs, we modified an off-the-shelf 3DR quadcopter, adding landing gear with foam floatation, and a Turner Designs Cyclops 7 fluorometer sensor hanging from the center of the vehicle.

Figure 11. Early prototype of the 3DR Aerial Sampling Quadcopter (left) Sketch of the type of route on which the probing vehicle s move (right) (Illustration Credit: Chris Green , Technical Submission to the UAE Drones for Good Competition. Dec 1, 2014)

3. For outdoor flight, we integrated to the system a **commercial prototype UAV chassis** (Figure 12) supplied to us for testing by BCB. The small, lightweight design enables the vehicles to be easily deployable and allows for longer flight times and larger survey areas. The carbon fibre frame also creates a robust layer of protection from propellers, ensuring the vehicles are safe and easy to handle. A completely waterproof design makes them suitable for operation across lakes and rivers. We modified the provided design to include additional floatation that would raise the vehicle from the water surface, and reduce the surface area exposed to adhesive forces from the water, facilitating takeoff.

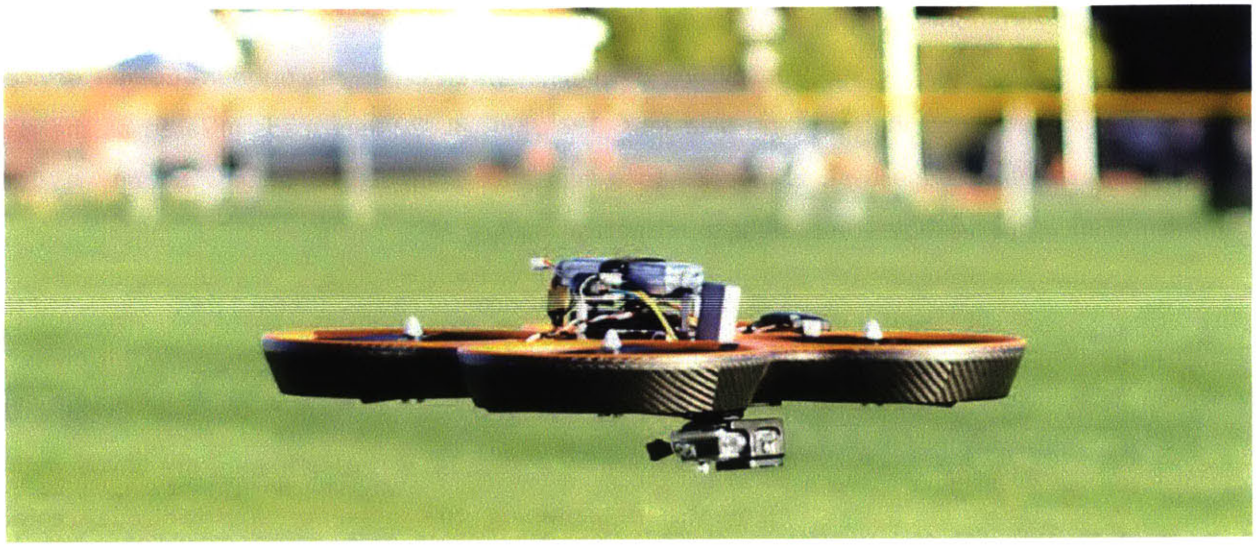

Figure 12. Waterfly development - Hyperspectral Imaging UAV (Photo Credit: Chris Green, Technical Submission to the UAE Drones For Good Competition. Dec 1, 2014)

3.4.3 Sensors

Two primary sensors were utilized to achieve the large scale imaging and point measurements required. One was a camera modified to image photosynthetic activity, and the other was an optical probe designed to detect the pigments released by cyanobacteria.[57]

Figure 13. GoPro camera with infra-blue lens fitted (left) Example of infrared images returned by camera (right) (Photo Credits: Chris Green, Technical Submission to the UAE Drones For Good Competition. Dec 1, 2014)

The aerial imaging quadcopter carries a downward-facing camera, the type selected and configuration modified according to the water quality indicators it is intended to detect. In this case, targeting cyanobacterial blooms, hyper-spectral imaging was chosen for detecting the photosynthesis carried out

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[57] When cyanobacteria blooms begin to die and disintegrate, the characteristic pigment they contain, and which gives them their blue-green coloration, colors the water with a distinctive blue color. Crayton M.A., *Toxic Cyanobacterial Blooms - A Field/ Laboratory Guide*. ed. Hardy J.F. Available at: http://nysfola.mylaketown.com/uploads/tinymce/nysfola/Toxic%20Cyanobacteria%20Blooms%20-%20A%20Field%20Guide%20(WA%20DOH).pdf Last accessed May 12, 2015

by planktonic algae blooms. For this purpose, we selected a GoPro Hero3 camera (Figure 13) capable of capturing 5-megapixel images, and transmitting the images wirelessly to the onboard CoPilot device. The camera was modified with an infra-blue lens. The NDVI BLU22 lens reads infrared as the red channel, and the visible spectrum is mapped to a light blue channel. This effectively converts the camera into an infrared/visible compositing multispectral imager.

Figure 14. Turner Designs Cyclops 7 probe. (Photo Credit: Chris Green, Technical Submission to the UAE Drones For Good Competition. Dec 1, 2014)

The water-probing quadcopter carries onboard probes selected to optically measure a different criteria pertaining to water quality. In our initial experiments, a Turner Designs Cyclops 7 fluorometer probe (Figure 14) was used to measure Phycocyanin pigments, determining the presence and abundance of cyanobacteria in the water. At 14.5 cm long, 2.3 cm wide and 142 grams, it is small, lightweight, and well suited to the quadcopter platform. When the vehicle lands on the water, the probe is submerged below the surface of the water. The probe emits light at the excitation wavelength of phycocyanin, and monitors the resulting fluorescence given off by any pigment particles in the water. The probe connects directly to the vehicle autopilot board, providing a voltage reading that, through pre-calibration of the sensor, is translated via the server into a phycocyanin reading in parts per billion (ppb). Rigorous measurements across and area can be correlated with total biomass of cyanobacteria.

3.4.4 Controllers

To fly indoor, we have developed an autonomous controller running from a laptop that sends roll, pitch, throttle and yaw PWM values to the quadcopter via a 915 MHz telemetry link.[58] The inner loop controller of the quadcopter is still intact and the same as the inner loop controller for outdoor flights. We use the Vicon positioning system as the indoor positioning system, and use the GPS module[59] to provide global coordinates for outdoor flights.

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[58] 3drobotics, Telemetry, Available at: https://store.3drobotics.com/products/3dr-radio-915_MHz. Last accessed 21 May 2015

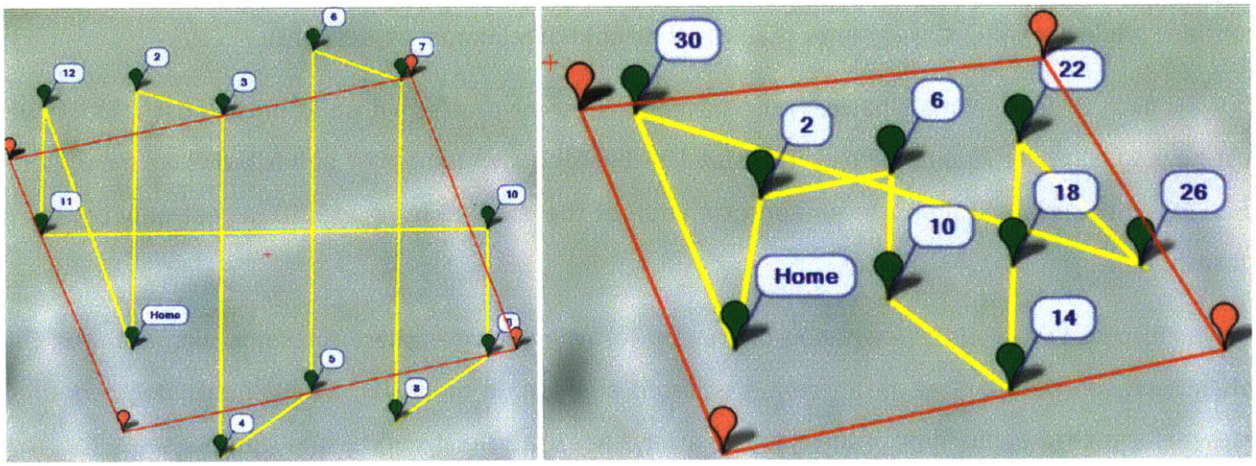
[59] 3drobotics, GPS Module, Available at: https://store.3drobotics.com/products/3dr-gps-ublox-with-compass. Last accessed 21 May 2015

3.4.5 Algorithms

We have developed two Flight Path Generator (FPG) algorithms, one designed for indoor flight and the other for outdoor flights. Additionally, we have developed algorithms for distributing probing tasks among a number of Probing Vehicles.

For indoor flights, we use Vicon as the positioning system and a flight 3D coordinate is defined in Vicon coordinates. Given the input of four coordinates of a rectangle in the Vicon area (Figure 18), the FPG will generate a flight path with appropriate velocity profiles at each time step. This will ensure the aerial UAV can follow the path more precisely.

For outdoor flights, we use the global positioning system (GPS) and a 3D coordinate waypoint is defined by its longitude and latitude coordinates, as well as its height in meters. Given a non-convex polygon as a list of longitude and latitude coordinates, the FPG algorithm generates an imaging flight path (Figure 15) that takes into account the camera field of views to ensure the stitching of overlaying captured images able to cover the desired area. The algae classification algorithm then identifies the potential algae areas. The FPG then generates a water probing flight path for each algae area, given a density area of each probing point (Figure 16).



Figures 15 & 16. GPS Aerial Flight Path (Left), GPS Water Sampling Flight Path (Right) (Image Credits: Univ. of Toronto Dynamic Systems Lab)

3.4.6 Formation Flight

We distinguish two types of formation operation: (a) when vehicles share the same airspace, and (b) when each vehicle operates in different, dedicated airspace. In mode (a), we plan path segments for each vehicle that at no time intersect and always keep a safe distance, and compensates for any unexpected delay of one of the vehicles. When each vehicle has reached their final waypoint, a new non-colliding path segment is assigned to each vehicle. In mode (b), safety of the vehicles is guaranteed by giving each of them their own space to operate in. Instead of 'synchronizing' their motions as done in (a), each vehicle is completely separately controlled.

3.4.7 Multi-Vehicle Sampling

A swarm of vehicles is dispatched to complete the monitoring task quickly. We use mode (a) at the beginning (and end) of a multi-vehicle operation. In real time as algae hotspots are detected by the imaging vehicle, sampling vehicles are assigned to it. Here, we use mode (b). The imaging vehicle is separated by altitude from the sampling vehicles. The sampling vehicles are each assigned a separate area and are responsible to probe any hotspot detected in their area. As a result, vehicles simultaneously and asynchronously perform water-probing at assigned locations.

3.4.8 Collision Avoidance

Swarm flight:

1) Probing Spot Distribution : We have a limit in the density of probing points given by the precision of localization. To select the best point, we take the maximum value of the object detection, then we take the second maximum if it's further than the minimum distance to all the probing points and we continue while the value stays greater than the threshold of minimum object density

2) Waypoints Ordering: This is basically the traveling salesman problem, if we want the best solution we just have to compare all possibilities. This stay feasible for a low number of points but if we have more than 10 points. We will then use an approximation of the solution using algorithm that only consider closest points.

We distinguish two types of formation operation: (a) when vehicles share the same airspace, and (b) when each vehicle operates in different, dedicated airspace. In mode (a), we plan path segments for each vehicle that at no time intersect and always keep a safe distance, and compensates for any unexpected delay of one of the vehicles. When each vehicle has reached their final waypoint, a new non-colliding path segment is assigned to each vehicle. In mode (b), safety of the vehicles is guaranteed by giving each of them their own space to operate in. Instead of 'synchronizing' their motions as done in (a), each vehicle is completely separately controlled.

A swarm of vehicles is dispatched to complete the monitoring task quickly. We use mode (a) at the beginning (and end) of a multi-vehicle operation. In real time as algae hotspots are detected by the imaging vehicle, sampling vehicles are assigned to it. Here, we use mode (b). The imaging vehicle is

separated by altitude from the sampling vehicles. The sampling vehicles are each assigned a separate area and are responsible to probe any hotspot detected in their area. As a result, vehicles simultaneously and asynchronously perform water-probing at assigned locations.

Nominally, mode (a) and (b) guarantee a collision-free operation. However, a simple collision avoidance strategy is implemented for any unexpected situation. All vehicles continuously report their position and velocity to the cloud. If two vehicles are detected that are close to each other and flying towards each other, hover commands are sent to all vehicles to hover at their current locations. The MFPG then sequentially re-routes each vehicle to a safe position to continue the mission.

3.4.9 Image Algorithms

To build image maps of the area survived, we are using several aerial imaging and image processing techniques. The GoPro Camera fixed on the imaging drone takes pictures at a fixed time interval. As we want to know the precise real-world location of every pixel seen on the image, we have to correct the lens distortion to achieve a "pin-hole" type image. To correct the images, we are use Adobe's LPC model to be able to directly use the coefficient calibrated in Adobe Labs for this camera. Following the lens correction, we correct any distortions due to the position of the UAV using the data supplied buy the drone's IMU (altitude, roll, pitch, and yaw). This compensates for the camera lens not being perfectly parallel to the water surface when the image was captured. With a fully corrected image, we use the attached GPS coordinates to position image with the others preceding it on the map.

For algae detection, we use infrared imaging to monitor the level of photosynthesis activity. The image classifier uses a threshold on the infrared level to determine an area of interest, and mark it with a polygon defined by GPS coordinates (Figure 17)

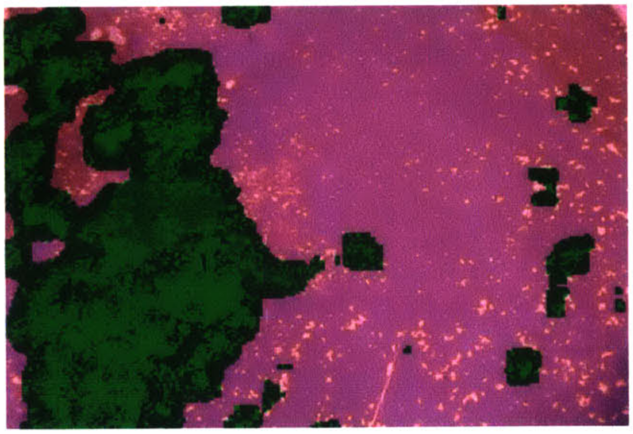


Figure 17. Image Analysis Algorithm using a threshold on the infrared level to detect photosynthesis activity. (Photo Credit: Antoine de Maleprade, Technical Submission to the UAE Drones For Good Competition. Dec 1, 2014)

3.4.10 Realtime Data

Using realtime data collection across cellular networks, a web-based platform has been developed that receives data directly from the UAVs out in the field, centralizing the data in the cloud, and making the data easily and instantaneously available to both researchers and the wider public through an easy-to-use UI. As data is collected over time, a rich repository of spatiotemporal data will be generated. Promoting both existing sensing techniques and future intermodal systems, the platform will also be able to receive data from separate sensor networks, creating a multi-layered archive of environmental data which can be easily combined, cross-correlated, visualized, and exported. This data will be made available with the intention of developing new spatiotemporal models for determining water quality, with the eventual goal of informing actionable change, through both data-driven policy-making and public awareness and engagement.

3.5 EXPERIMENT

3.5.1 Indoor Experiment

Many experiments were carried out to tune the indoor controller gains and achieve flight error averaging below 15 cm. We also implemented failsafe operations, including landing when the Vicon positions were lost or out of the predicted range for over a second, and not permitting take off when a quadcopter was unbalanced on the ground. The vehicle was able to follow curves at the end of straight paths as shown in Figure 18. Indoor flight of an Imaging UAV is shown in Figure 18, and the flight of a Probing UAV in Figure 19.

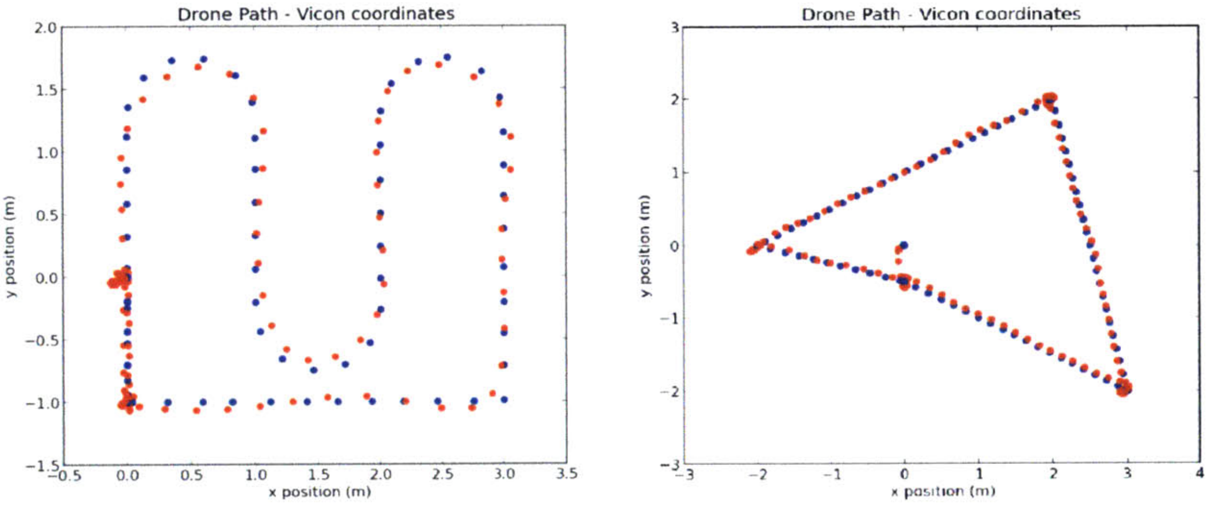


Figure 18 & 19. Indoor Flight Path (Left) Landing mission performance at 3 pools with cyanobateria readings in the Vicon room (Right) (Image Credits: University of Toronto Dynamic Systems Lab)

3.5.2 Outdoor Experiments

We changed the Arducopter firmware 3.1.5 so that the quadcopter does not wait after it arrives at a GPS waypoint. The PID values of the controllers are also tuned to fit our modified quadcopter. The aerial flight relative error on average was within 50cm in non-windy conditions. We also tested the landing accuracy by repeatedly landing the vehicle to a spot on a ground 15 times and measured the deviation of the actual landing position to the spot. During a non-windy condition, the vehicle was able to land within 1.2m radius. The quadcopter also successfully landed in a 1.2m radius pool during a 3 waypoint autonomous flight mission.

The GPS module was also tested by letting the vehicle stay on the ground for 10mins and hovering at one spot for 10mins. For each 10 min experiment, we get a list of current GPS coordinates of the quadcopter. We filtered out points for the first five mins to ensure GPS fix and points during take off. For staying on the ground experiment, the average of the absolute distance of the GPS coordinate list to its center point is about 1.2 m, while when hovering, the average absolute distance is about 20 cm.

3.5.3 Demonstration and Results

Indoor Demonstration Setup. We performed several aerial and water sampling flights in a rectangular area 3 m x 4 m in the MIT Holodeck room, equipped with the indoor Vicon positioning system. Three circular pools, each is 1m diameter in size, were filled with algae and randomly set around the area. The aerial vehicle was given an aerial flight path by the FPG software, flew about 2m height while capturing multiple overlay images to build the area map. The map was then shown to the user, highlighting the three pool locations. The water sampling vehicle sequentially flew to above pool locations.

Demo Results. During the 2 demo days, the aerial quadcopter successfully performed aerial flights all the time while the water sampling vehicle was able to land inside the pool 5 out of 6 times. The audience was able to deploy quadcopters, view discovered pools and flight paths from the web GUI interface.

3.5.4 Future Challenges

This project facilitates the water sampling procedure, compared to the costly traditional method of sending boats and manually measuring cyanobacteria reading at each river location. The up-to-date water condition information will quickly inform officials who are in charge of keeping the water safe for public use. The project aims to ward low costs and deployment ease with little preparation time. The future challenges involve deploying multiple vehicles at the same time, while further ensuring safety of human and vehicles.

For future water monitoring, a more diverse range of probes can be used to sense both indirectly for cyanobacterial blooms, and directly for other water quality issues, including: a) Phycoerythrin (marine), b) Chlorophyll in vivo, c) Turbidity, d) Wastewater Monitoring - Temperature, and e) Dissolved Oxygen - pH.

3.6 CONTRIBUTIONS

The Waterfly project is a novel, reliable and low-cost system that can quickly provide large amounts of realtime information about the water quality of aquatic ecosystems as requested. The system provides aerial maps of the areas of interest by stitching images captured from an aerial quadcopter, highlights potential algae areas, and deploys autonomous UAVs to land at these algae areas to provide realtime cyanobacteria reading.

The main contributions of developing a system like Waterfly are:

1. Through distributed sensing, Waterfly yields high resolution information about water conditions, significantly improving current ways of monitoring aquatic ecosystems, particularly those in high proximity to urban centers, where non-point source pollution is hard to be dealt with through top-down hierarchical policy-making strategies.

2. Waterfly monitors the quality of the water bodies by proxy, in the sense that it utilizes microbes as agents of distributed sensing. Future developments of the system could focus on also programming and actuating these agents.

3. Waterfly employs natural processes such as swarm sensing to monitor ecosystems that what in this thesis I call "hybridized ecologies," where the divide between the natural and the made is a no longer discernible or efficient as an ecological approach.

4. By bringing realtime information regarding the water levels of cyanobacteria blooms, Waterfly closes the loop between invisible processes that occur in the aquatic environments and our ability to sense them. By sensitizing the public, Waterfly can generate a change in the behavior of the individual, engagement and participation.

5. The development of the Waterfly system can be expanded beyond water quality monitoring, to provide information about other parameters such as the quality of air, and the levels of noise.

6. The idea of 'digital insects' and swarming is biomimetic in its implementation. However, these initial explorations are not mimicking a biological form but rather a process. The swarm behavior modeled

into the flight controls and operations of this system are an initial exploration of what promises to be a central feature of future robotic systems.

During the research, the Senseable City Lab team established Waterfly as a cyber-physical system. The use of cyanobacteria as indicators both of their own biomass as well as of additional pollution is an early step in interfacing with microbiological elements through cybernetic feedback. The system relies on optical measurements of physical properties of natural cyanobacteria. This allows the 'reading' of the abundance of algae blooms, and the inference of additional water pollution issues. However, the approach of this digital-biological interface is limited in the information it can collect, and can not suggest an extension to biological response or intervention. In essence, Waterfly is a traditional cyber-physical system looking at biological phenomena.

In continuing my research on microbial communities at the Alm Lab, at the Department of Biological Engineering, I believe there are further opportunities to develop digital-biological interfaces that would support harnessing the bacteria themselves to carry out the sensing, computation, actuation, and communication tasks. In the future, bacteria themselves can be deployed as biosensors, and can be programmed to remember the pollution they encounter, quantify their exposure to it, and possibly provide a useful response. There remains a compelling opportunity to leverage biological properties of the bacteria, and turn the Waterfly platform into a distributed system coupling digital computation and communication with similar biological capabilities, what I have described as a "cyber-biological system."

This initial exploration of a feedback system that includes a microbial component has raised further questions about the possibility of directly reading biological elements and code as a source of realtime information within built infrastructures and living beings. *Underworlds*, the next project described, aims to address this question.

3.7 ACKNOWLEDGMENTS

Figures and texts describing the project, development methodologies, experiments, demos, and results are taken from a draft publication kindly provided by Prof. Schoellig and researcher Thanh Pham of the Dynamic Systems Lab, as well as a technical brief co-authored by the project teams at Senseable City Lab and Dynamic Systems Lab for the UAE Drones for Good Competition. Full credits for the project are listed bellow.

3.7.1 Project Team

The project was a collaboration of teams which evolved during the development. At the Senseable City Lab, in my role as Research & Development Lead of the lab, I was supervised by Professor Carlo Ratti. Chris Green lead the team and the day-to-day development of the project under my direction. During the first year we established the research and developed with our collaborators the successful indoor demo and some outdoor tests. In Fall 2014, Antoine de Maleprade and Gabriel Kozlowski joined the team in preparation to scale up to a swarm of outdoor vehicles for the UAE Drones for Good Competition. In the final months leading up to the UAE competition, Professor Ratti took over the direction of the project.

At the outset of the project, I invited Professor Angela Schoellig, the Director of the Dynamic Systems Lab at the University of Toronto, and an expert in autonomous flight control, to collaborate with us. She and her team have been the main collaborators on all aspects of this project, and instrumental in developing the flight controls and carrying out the majority of the flight experiments. The work described above, as well as portions of the texts and images used in this document to describe the methods, experiments, and results of the project, are in joint authorship of the researchers of these two labs.

Professor Julie Shah, Director of the Interactive Robotics Lab, and her team were an invaluable support in providing resources for implementing the project as well as close consultation throughout the development of the first successful demo. Students from the MIT UAV Club comprised the initial development team at Senseable City Lab, and were generally helpful in the development of the UAV research agenda at Senseable City Lab. Turner Designs kindly supplied on two occasions of several weeks a loaner Cyclops 7 fluorometer probe carried by the water-probing vehicle. I formed the collaboration with DroneDeploy in order to integrate their front-end for flight operations, and on the back-end their API has enabled realtime integration of inflight image stitching, image processing and analysis, and automated way-path generation for the probing UAVs based on image analysis. The collaboration with BCB was formed in order to test out their UAV chassis designed to float on water, which were integrated for the UAE Drones for Good Competition.

The project went through three main phases. The initial phase culminated in a successful indoor demo at the CSAIL Holodeck for the Environmental Protection Agency (EPA), Charles River Watershed Association (CRWA), and the Museum of Science (MOS). The second phase involved successful outdoor testing of the system primarily at UT, and an attempt with the City of Cambridge to obtain an FAA public license to test the system over a body of water, which resulted in the required support of the

Massachusetts Attorney General, but dissolved in process with the FAA. The third phase was primarily aimed at scaling the project up to 5 vehicles and improving the system in preparation for the UAE Drones for Good Competition.

MIT
Senseable City Lab
DUSP
Carlo Ratti - Director
Yaniv J Turgeman - Research & Development Lead
Chris Green - Project Lead
Gabriel Kozlowski - Project Lead & Design
Antoine de Maleprade - Software & Engineering
Clara Cibrario Assereto - Legal Coordinator
Pierrick Thebault - Web Development
Carlos Greaves - Electrical Engineering
Dan Feldman - Computer Vision Advisor

Development Team:
Aashish Tripathee
Alex Huang
Ben Eysenbach
Camille Kerbaul
Diane Kim
Eric Tao
Evie Kyritsis
Faith Huynh
Fernando Yordan
Franco Montalvo
Janelle Mansfield
Marwan Sarieddine
Mike Xia
Nhat Cao
Ostin Zarse
Pedro Henrique da Silva Alves

Video Production

Creative Direction:
Carlo Ratti
Yaniv J Turgeman
Chris Green
Gabriel Kozlowski

Production & Post-Production:
Chris Green
Gabriel Kozlowski

University of Toronto
Dynamic Systems Lab
Institute for Aerospace Studies
Prof. Angela Schoellig - Director
Thanh Pham - Autonomous Flight
Kaizad Raimalwala - Autonomous Flight
Rikky Duivenvoorden - Flight Experiments & Safety Pilot

Development Team:
Chris McKinnon
Dorian Tsai

With support from

MIT
Interactive Robotics Group
Department of Aeronautics and Astronautics
Prof. Julie Shah - Director
Abhizna Butchibabu
Been Kim
Paolo Cesar

DroneDeploy
Mike Winn
Nick Pilkington
Manu Sharma
Jono Millin

Turner Designs

BCB

Underworlds

ABSTRACT

Public health is a notion that emerged with the city. In its early history, public health was enforced by the church through its community interventions that equated sickness with sin. Today, with the discovery of microorganisms and an advanced statistical capability, modern medicine has taken over the critical task of understanding and mitigating infectious diseases. More specifically, with the epidemiological transition towards chronic disease in the 20th century, new methods such as syndromic surveillance using patient data to identify and predict disease trends have begun to meet the rising challenge of these long term conditions. For example, recent molecular and computational biology methods have led to a vast body of research linking gut microbiome—detected in patients' stool--to a host of disease pathologies. On a macro scale, sewage systems themselves provide a yet untapped reservoir of health data, spatially and temporally connected to urban structure. *Underworlds* investigates the urban microbiome through the city's "collective gut." This project's aim is to develop the science and technology needed to build a proof-of-concept system to track microbial indicators of health and disease in realtime. Methods for developing a prototypical smart sewage platform include network analysis and modeling of the sewer system, the characterization and design of an automated physical and biochemical infrastructure, downstream computational tools and analytics necessary to interpret biological signals, and an open, cross-disciplinary data platform to inform policy makers, health practitioners and researchers. Early experiments and next steps are presented with the aim of developing initial assumptions about what meaningful signals can be interpreted and correlated to the population.

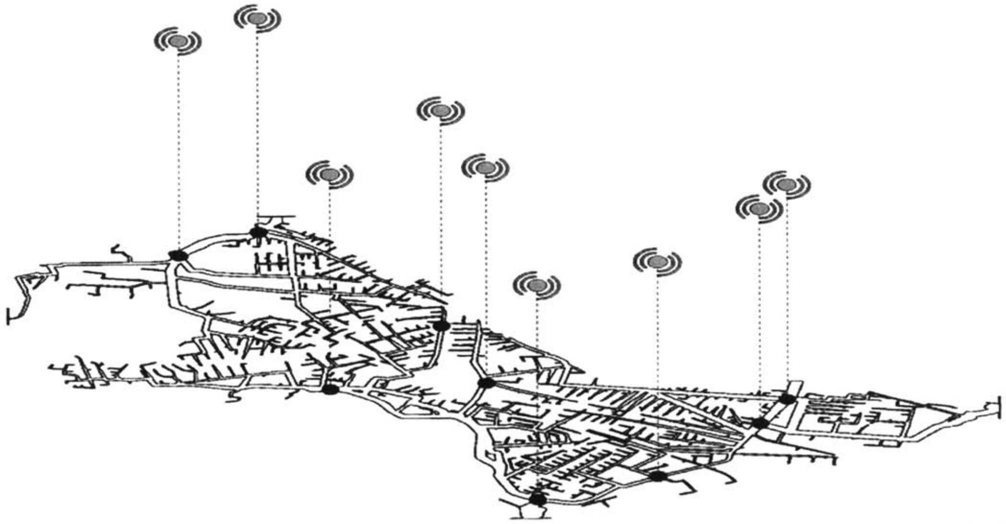


Image Credit: Chris Green

Underworlds

4.1 PUBLIC HEALTH

4.1.1 Introduction

The capacity of society to promote health and prevent the spread of disease is fundamentally tied to the ability to continuously and effectively survey the population. For example, in the context of communicable diseases, i.e. "diseases spread from one person to another or from an animal to a person [...] via airborne viruses or bacteria, but also through blood or other bodily fluid,"[1] the epidemiological practices of disease surveillance focus on prediction, early detection, minimizing the impact of disease occurrence, and increase knowledge about patterns of progression and aggravating factors that contribute to outbreak, epidemic, and pandemic situations, through case reporting.

In their 2012 report about Emerging Infectious Diseases,[2] Morens and Fauci used a number of example to outline the progress in understanding and controlling EIDs since the landmark 1992 Institute of Medicine report about emerging infections in the US,[3] which had brought the underestimated concept of EID to the epicenter of medical research. The emergence, spread, and epidemiological surveillance of HIV/AIDS — "one of history's deadliest pandemics"[4]— is a good example for discussing progress in monitoring disease outbreak. Since its emergence in the 1980s, the HIV virus has resulted in more than 35 million deaths. Today, there is still no cure to the viral infection. However, HIV/AIDS patients receiving antiretroviral therapy can expect to live normal life spans, while the risk of transmitting infection to others has also been significantly lowered.[5]

Progress has also been marked in the case of measles and polio. From at least a million annual children deaths of measles in 1992, fewer than 100,000 were expected to die in 2012, and an already-available and effective at the time vaccine was paving the path towards the disease eradication.[6] Finally, from childhood "lameness surveys" conducted to monitor polio circulation in developing countries back in

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[1] The most common and most severe communicable diseases according to the United States Department of Health and Human Services are HIV/AIDS, influenza, malaria, polio, tuberculosis, hepatitis. In "Communicable Diseases," GlobalHealth.gov. Available at: http://www.globalhealth.gov/global-health-topics/communicable-diseases/. Last accessed May 20, 2015.

[2] Morens DM and Fauci AS. (2012). Emerging infectious diseases in 2012: 20 years after the Institute of Medicine report. mBio 3(6):e00494-12. doi:10.1128/mBio.00494-12.

[3] Committee on Microbial Threats to Health, Institute of Medicine. (1992). Emerging infections: microbial threats to health in the United States. National Academies Press, Washington, DC.

[4] Fauci AS, Folkers GK. (2012). The world must build on three decades of scientific advances to enable a new generation to live free of HIV/AIDS. Health Aff. (Millwood) 31:1529–1536.

[5] Morens and Fauci, 2012. Op.cit.

[6] Ibid.

1992, Morens and Fauci reported that in 2012, most individuals infected by polio were adults "whose children were largely free of the threat of polio."[7]

The afore-mentioned facts demonstrate remarkable progress in EID surveillance. However, EIDs still result in 15 million deaths yearly, while at the same time new and deadly diseases continue to emerge and reemerge.[8]

4.1.2 Early Epidemics

In "Infectious Disease Epidemiology: Theory and Practice," Nelson and Williams collected some of the earliest examples of infectious disease epidemics documentation in history, including smallpox, leprosy, tuberculosis, meningococcal infections, and diphtheria.[9] These accounts demonstrate that from very early on, there was a profound need and desire to understand "the natural forces and risk factors affecting the patterns of illness and death in society."[10]

Watts reports that the first recorded epidemic of smallpox was in 1350 BCE, during the Egyptian–Hittite war.[11] Disseminated in the old world throughout the expansions of the early millennium, smallpox was thought to have been introduced to the Americans by the Spanish explorers. In 1520, engaged in a loosing struggle against the Aztecs, Cortez emerged victorious after a quarter of the opposing forces succumbed to these newly introduced pathogens.[12] Nelson and Williams report that the notion of disease transmission was understood reasonably well by that time:

> *At the least, it was appreciated that the skin lesions and scabs associated with smallpox could transmit the disease. It was known that survivors of the infection were immune to reinfection after further exposure. The practice of inoculation, or variolation, whereby people were intentionally exposed to smallpox was practiced in China, Africa, and India centuries before the practice would be adopted in Europe and the Americas.*[13]

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[7] Ibid

[8] Fauci AS, Morens DM. (2012). The perpetual challenge of infectious diseases. N Engl. J. Med. 366:454–461.

[9] Nelson, K.E., Williams, C. (2006). *Infectious Disease Epidemiology: Theory And Practice.* Jones & Bartlett Learning; 2 edition.

[10] Ibid: 3

[11] Watts, S. (1997). *Epidemics and History: Disease, Power and Imperialism.* New Haven, Conn: Yale University Press.

[12] Nelson, 2006. Op. cit: 4

[13] Ibid

However, means of intervention were not highly developed. In the middle ages, lepers were excommunicated, as leprosy was considered highly contagious. Intended to preserve public health, the church identified the disease as a sin, and carried out the formalities of social separation. Those afflicted were forbidden to visit public places, associate with others in close proximity, or touch objects of public use. Those suspected of leprosy had to carry a bell, in order to warn others of their arrival. However, these interventions of symptomatic cases were not an highly effective strategy for protecting public health and reducing epidemic spread, as leprosy develops significantly before symptoms appear (Figure 1).[14]

Figure 1. Image of a leper with bell (Image Citation: *The Medieval Leper and his Northern Heirs* by Peter Richards, August 2000)

4.1.3 Tracking Transmission

One of the first formal surveillance programs for public health was in the Republic of Venice in the 14th century. At that time, public health authorities boarded ships and prevented any persons suspected of being ill with bubonic plague from disembarking.[15] It was over the next several hundred years that clinical observation of individuals and exposed populations would transform the understanding of disease. Case surveillance involving individual or groups of patients were augmented with careful studies utilizing isolated geographies, demographic variability, and scrutiny of recorded hospital data led to breakthroughs in approaches to population health. Early statisticians applied probability and inductive reasoning to Bills of Mortality to inferring ratios of births and deaths by gender, geography, and season, as well as examining causes of death and constructing the first life tables.[16] These methods began the practice of statistical surveillance of population health, and established the use of public health data as a tool for shaping clinical practice and public health policy.

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[14] Ibid

[15] Thacker, SB. 2000. Historical development. Pp. 1-16 in: Teutsch, SM, and Churchill, RE, eds. *Principles and Practice of Public Health Surveillance, 2nd edition*. New York: Oxford University Press.

[16] Nelson, 2006. Op.cit.

In 1741, Rhode Island required tavern keepers to report patrons with smallpox, yellow fever, and cholera to local authorities.[17] With Antonie Van Leeuwenhoek's advent of the microscope and observation of the existence of microbes, followed by Louis Pasteur's germ theory, scientists embarked on a mission to understand the invisible causes of disease. In 1874, Statewide efforts began in Massachusetts with a voluntary, postcard reporting format that was used to provide weekly reports on prevalent diseases.[18] Two years later, Robert Koch showed that microorganisms could cause disease.

> *The identification of the causative microorganisms of specific infections allowed for a much better understanding of their epidemiology, which in turn informed prevention strategies and countermeasures such as passive immunotherapy, vaccines, and drugs against infective agents. The disciplines of microbiology, virology, and immunology paralleled and complemented the disciplines of epidemiology, statistics, and public health in the prevention of infectious diseases.*[19]

A testament to the collaboration of these disciplines over the next hundred years was the eradication of smallpox from the world. Logistical challenges thwarted early efforts to immunize nearly the entire population, but in the 1970s a new surveillance-vaccination approach proved successful. The "ring strategy" employed intensive surveillance to identify cases, isolation of all known cases, and immunization of individuals who may have come in contact with cases.[20] In 1980, smallpox was declared as the first eradicated infectious disease by the World Health Organization, after the last case was seen in Somalia in 1977.[21]

4.2 CHANGING ENVIRONMENTS, EMERGING INFECTIONS, AND CHRONIC CONDITIONS

4.2.1 Communicable Conditions in Cities

While there have been monumental advances in our understanding and treatment of disease, science and technology have also changed our behavior and rapidly reshaped environments for both humans and microbes alike. These changes present new challenges for health. In 1995, Morse wrote:

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[17] Thacker 2000. Op.cit.

[18] Ibid

[19] Nelson 2006. Op.cit: 12

[20] Institute of Medicine (IOM), (1988). The Future of Public Health. Washington: National Academy Press.

[21] "Smallpox" World Health Organization, Global Alert and Response (GAR). Availabe at: http://www.who.int/csr/disease/smallpox/en/. Last accessed May 21, 2015.

> *Most emerging infections appear to be caused by pathogens already present in the environment, brought out of obscurity or given a selective advantage by changing conditions and afforded an opportunity to infect new host populations (on rare occasions, a new variant may also evolve and cause a new disease) [...] A number of activities increase microbial traffic and as a result promote emergence and epidemics. [...] . Surprisingly often, disease emergence is caused by human actions, however inadvertently.*[22]

The 1992 IOM report that was mentioned in section 4.1.1 highlights the threat of emerging infectious diseases (EIDs), and breaks down the responsible factors into categories, which are listed by Morse as follows: "ecological changes, such as those due to agricultural or economic development or to anomalies in climate; human demographic changes and behavior; travel and commerce; technology and industry; microbial adaptation and change; and breakdown of public health measures."[23]

Spellman et al. claim that such ecological interactions can be complex, with several factors often working together or in sequence.[24] As an example, multiple processes converging in cities play a central role in disease emergence and epidemic spread. Increased migration from more isolated rural areas to urban centers may disseminate a once-localized infection. Overcrowded cities may strain the capacity of infrastructure and interfere with maintaining good public health practices. Urban centers also provide a gateway for transmission in population-dense environments with heterogenous immunologic fortitude.[25] This is further exacerbated by the fact that cities are hubs of national and international travel.

4.2.2 Case: AIDS/HIV

In the last three decades, humanity has been witness to the unanticipated emergence of one of history's most destructive pandemics. HIV/AIDS has been the EID with the most relentlessly devastating global impact, taking over 35 million lives.[26] Consider Morse's account of the environmental context in which the disease likely emerged:

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[22] Morse, S S. (1995) "Factors in the Emergence of Infectious Diseases.." Emerging infectious diseases 1.1: 7–15. Web. Available at: http://wwwnc.cdc.gov/eid/article/1/1/95-0102_article#r13 Last accessed May 21, 2015.

[23] Ibid

[24] Spellman, F.R. Stoudt, M.L. (2013). *Environmental Science: Principles and Practices*. Scarecrow Press, Inc. 244

[25] Morse 1995. Op.cit.

[26] Morens et al. 2012, Op.cit.

> *Although the precise ancestry of HIV-1 is still uncertain, it appears to have had a zoonotic origin. Ecological factors that would have allowed human exposure to a natural host carrying the virus that was the precursor to HIV-1 were, therefore, instrumental in the introduction of the virus into humans. This probably occurred in a rural area. A plausible scenario is suggested by the identification of an HIV-2-infected man in a rural area of Liberia whose virus strain resembled viruses isolated from the sooty mangabey monkey (an animal widely hunted for food in rural areas and the putative source of HIV-2) more closely than it did strains circulating in the city. Such findings suggest that zoonotic introductions of this sort may occur on occasion in isolated populations but may well go unnoticed so long as the recipients remain isolated.27*

Morse goes on to explain the role of urbanization, social behaviors, and even technologies of medical practice contribute to the pandemic spread:

> *But with increasing movement from rural areas to cities, such isolation is increasingly rare. After its likely first move from a rural area into a city, HIV-1 spread regionally along highways, then by long distance routes, including air travel, to more distant places. This last step was critical for HIV and facilitated today's global pandemic. Social changes that allowed the virus to reach a larger population and to be transmitted despite its relatively low natural transmissibility were instrumental in the success of the virus in its newfound human host. For HIV, the long duration of infectivity allowed this normally poorly transmissible virus many opportunities to be transmitted and to take advantage of such factors as human behavior (sexual transmission, intravenous drug use) and changing technology (early spread through blood transfusions and blood products).28*

At the same time, Morens et al. demonstrate how modern research tools have elucidated the history and pathogenesis of the emergence and spread of the HIV virus, while also made possible the monitoring and control of the HIV/AIDS pandemic through the development of life-saving treatment and prevention modalities.[29] In a 2003 historical review of the HIV/AIDS surveillance efforts in the US, Stoto stated that they are "among the most complex and carefully evaluated surveillance efforts in the United States."[30] Stoto explains that the different systems for monitoring and controlling HIV and AIDS,

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[27] Morse 1995. Op.cit.

[28] Ibid

[29] Morens et al. 2012, Op.cit.

[30] Stoto, M A. (2003). "Public Health Surveillance: a Historical Review with a Focus on HIV/AIDS." (2003): n. pag. Print.

which are mostly separate, are largely case based. However, many of the more important applications are statistical in nature.[31]

4.2.3 Epidemiological Transition to Chronic Conditions

In the 19th century the decline in many endemic infectious diseases was due both to the development of actual cures, but mainly to improvements in public health and nutrition.[32] With additional advances in public health and pharmacology,[33] communicable disease in developed cities saw significant decline through the 20th century, and management of epidemics and pandemic improved globally. While infectious diseases are still a significant challenge in many parts of the world, there has been a pronounced transition from infectious diseases to chronic non-communicable diseases (NCDs) as the major driver of morbidity and mortality globally. These diseases include high blood pressure, arthritis, respiratory diseases like emphysema, diabetes, Alzheimer's disease, mental illness and kidney diseases.

Impact of NCDs. The National Center for Health Statistics in the US reports that an annual percentage of 70% of deaths in the US are caused by chronic diseases. The World Health Organization reports that NDCs kill 38 million people each year.[34] In 2005 they were responsible for over 60% of all deaths.[35] In 2002 chronic conditions were listed as the 6th of the top ten causes of mortality in the general US population.[36] In 2014, the World Health Organization's *Global Status Report on Noncommunicable Diseases 2014* estimated that they were responsible for 68% of the world's deaths, more than 40% of them considered premature, i.e. under the age of 70 years. Almost three quarters of deaths linked to NCDs occur in developing countries. In the United States, nearly one in two people has at least one chronic condition, and one in four has multiple conditions.[37] Anderson reports that 90% of seniors in the US have at least one, whereas 77% have two or more chronic conditions.[38] These numbers have been steadily growing.

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[31] Ibid

[32] Ibid

[33] Murty K.G., (2015). *Case Studies in Operations Research: Applications of Optimal Decision Making*, Springer: New York

[34] World Health Organization (20150 "Noncommunicable diseases" Available at: http://www.who.int/mediacentre/factsheets/fs355/en/ Last accessed 12 May 2015.

[35] Chronic Diseases, World Health Organization.

[36] National Center for Health Statistics. " Health, United States" / 2004

[37] Ward, Brian W, Jeannine S Schiller, and Richard A Goodman. "Multiple Chronic Conditions Among US Adults: a 2012 Update." Prev Chronic Dis 11 (2014): 130389. Web.

[38] Anderson G. (May-june 2004). "The Growing Burden of Chronic Disease in American." *Public Health Reports* / Vol.119

The Centers for Disease Control and Prevention report that NCDs are responsible for 7 of 10 deaths annually, and they account for 86% of the national health care costs.[39] Gerteis et al. report that in 2010, 86% of health care spending was devoted to this population.[40]

These diseases require new approaches for monitoring, as they take palace over long time scales, and many driven by such factors as lifestyle and behavior, long term exposures to harmful substances, and genetic predisposition. New strategies for study and intervention have been developed to deal with chronic conditions as they associate with the modern lifestyle and environment. Early detection and continuous monitoring of these conditions, long term public health studies and policy, and dissemination of behavioral risks to the public have become important focus points in mitigating the rapid increase of chronic conditions.

4.2.4 Syndromic Surveillance

In addition to the continued improvement of traditional methods, information and computation technologies have enabled new approaches based on "pre-diagnostic data and statistical algorithms to detect epidemics earlier than traditional surveillance, including unusual diseases with nonspecific presentations."[41] These systems are known as outbreak detection systems, early warning systems, health indicator surveillance, and prodromal surveillance, but most common is the term syndromic surveillance systems. They use statistical algorithms and machine learning to process near-realtime indicator data, such as syndromes, medication sales, or patient chief complaints. The results are visualized and analyzed to infer early detection of outbreaks and improve situational awareness.[42]

The early efficacy of these systems shows the tremendous promise of harvesting massive health data streams across populations. The accessibility of multi-dimensional, high temporal and spatial resolution information is making possible realtime health sensing. This expansion of our sensory network is makes evident that useful data can provide actionable feedback, informing public health policy, clinical practice, medical research, and individual lifestyle choices at unprecedented scale.

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[39] Centers for Disease Control and Prevention, Chronic Disease Prevention and Health Promotion. Available at: http://www.cdc.gov/chronicdisease/ Last accessed 12 May 2015.

[40] Gerteis J, Izrael D, Deitz D, LeRoy L, Ricciardi R, Miller T, Basu J. Multiple Chronic Conditions Chartbook.[PDF - 10.62 MB] AHRQ Publications No, Q14-0038. Rockville, MD: Agency for Healthcare Research and Quality; 2014.

[41] Chretien, Jean-Paul et al. "Syndromic Surveillance: Adapting Innovations to Developing Settings.." PLoS medicine 5.3 (2008): e72. Web.

[42] Ibid

Such successful efforts lay the foundation for new methods of monitoring. These data currently represent only the portion of cases which are symptomatic, and where patients seek treatment. Few of the patients will undergo testing, and little can be studied about emerging pathogenic strains limiting both public health and medical benefits. This invites the identification of additional sources of pervasive spatio-temporal health data, particularly those which can provide not just symptomatic inference from compliant patients, but a passive surveillance representative of the entire population.

4.3 MONITORING THROUGH THE MICROBIOME

In the context of the afore-mentioned challenges of disease surveillance in the 21st century, identifying novel monitoring tools for continuously harvesting information in high temporal and spatial resolution can play a tremendously critical role in transforming public health.

4.3.1 Stool Surveillance

Stool are a old-new modality for monitoring human health and disease that corresponds to many of the above described characteristics of effective disease monitoring and understanding patterns of health and disease. Stool is produced on a daily basis and reflects key parameters of human physiology. It contains microbiota, shed human epithelial cells, as well as a host of organic and inorganic molecules that are the result of ingestion and processing in the body on a daily basis. In disease states, stool also contain pathogens and other biomarkers, such as blood cells, immune cells, inflammatory biomarkers, and tumor cells, that provide a wealth of information for early disease detection and disease activity monitoring to guide therapeutic intervention.

As such, stool provides unique opportunities for monitoring human health. On average, a human being excretes 100-200g, about 1-2 times per day. This is typically treated as taboo waste, but it is a noninvasive sample rich in health data. Normally, feces are made up of 75 percent water and 25 percent solid matter.[43] Dead bacteria, and indigestible food matter make up for about 30% of the solid matter each, whereas the rest (about 10 to 20% each) is fats such as cholesterol, and inorganic substances such as calcium phosphate and iron phosphate. Finally, 2 to 3% is protein.[44] Cell debris shed from the mucous membrane of the intestinal tract also passes in the waste material, as do bile pigments (bilirubin) and dead leukocytes (white blood cells).[45]

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[43] Noyd R., Krueger J., Hill K., Biology: Organisms and adaptation:253

[44] Encyclopaedia Britannica, "Feces." Available at: http://www.britannica.com/EBchecked/topic/203293/feces Last accessed 12 May 2015.

[45] Ibid

Collected non-invasively and submitted routinely, stool provides the ideal opportunity for monitoring and understanding infectious and chronic diseases. Disease can be tracked through identifying the bacteria and viruses that we shed in stool. Additionally endogenous metabolic products from substances we eat or drink such as caffeine, alcohol, or artificial sweeteners, and prescription drugs we take, can be monitored as indicators of behavior related factors as well as direct drivers of disease.

Utilization of excreta to monitor infectious diseases. Utilizing excreta to monitor infectious diseases was probably one of its earliest applications for disease monitoring. For example, the Polio Global Eradication Initiative[46] includes case investigation with a focus on isolation of virus from stool, as the gut is the host organ for the poliovirus pathogen (enterovirus). Stool samples are also the most frequently tested clinical materials in the investigation of gastroenteritis-related outbreaks, such as Salmonella,[47] diarrhea (rotavirus), and viral gastroenteritis (norovirus). Most of these agents are highly infectious, potentially epidemic, with increased public health burden.

Additionally, stool monitoring has the potential to identify non-symptomatic cases. Carriers of infectious disease such as salmonella or typhoid can infect the public over years without themselves showing signs of illness, making detection and intervention particularly difficult. Stool monitoring can also be used to identify the emergence of drug-resistant bacteria, as in the case of Vancomycin-Resistant Enterococci (VRE).

Utilization of excreta to monitor chronic diseases. Stool monitoring is an obvious surveillance and detection method of chronic diseases that affect the intestinal tract. For example, the flare-ups of inflammatory bowel disease results in a noticeable change in the consistency of the stool, and visible blood. More over, clinical experience has led to the understanding that early detection of inflammation can guide treatment and lead to improved outcomes. In recent years, new biomarkers have emerged to be able to track inflammation in stool for this purpose.

In recent years it has been also shown that monitoring stool can provide important insight to chronic illnesses affecting areas of the body outside of the gut. Specifically, the microbiome has been the focus of intense investigation. This is through the realization that 90% of the cells in our body are bacterial cells, and these microbiota contribute 150 times the number of genes than our own human genome. With advances in genomics and computational biology, there has been a recent proliferation of studies

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[46] Polio Global Eradication Initiative <http://www.polioeradication.org/Dataandmonitoring/Polioeradicationtargets.aspx> Last accessed 12 May 2015.

[47] Salmonella Detection and Identification Methods <http://www.rapidmicrobiology.com/test-method/salmonella-detection-and-identification-methods/> Last accessed 12 May 2015.

correlating microbiome composition and dynamics to human health. Stool offer the ideal manner to monitor these relationships.

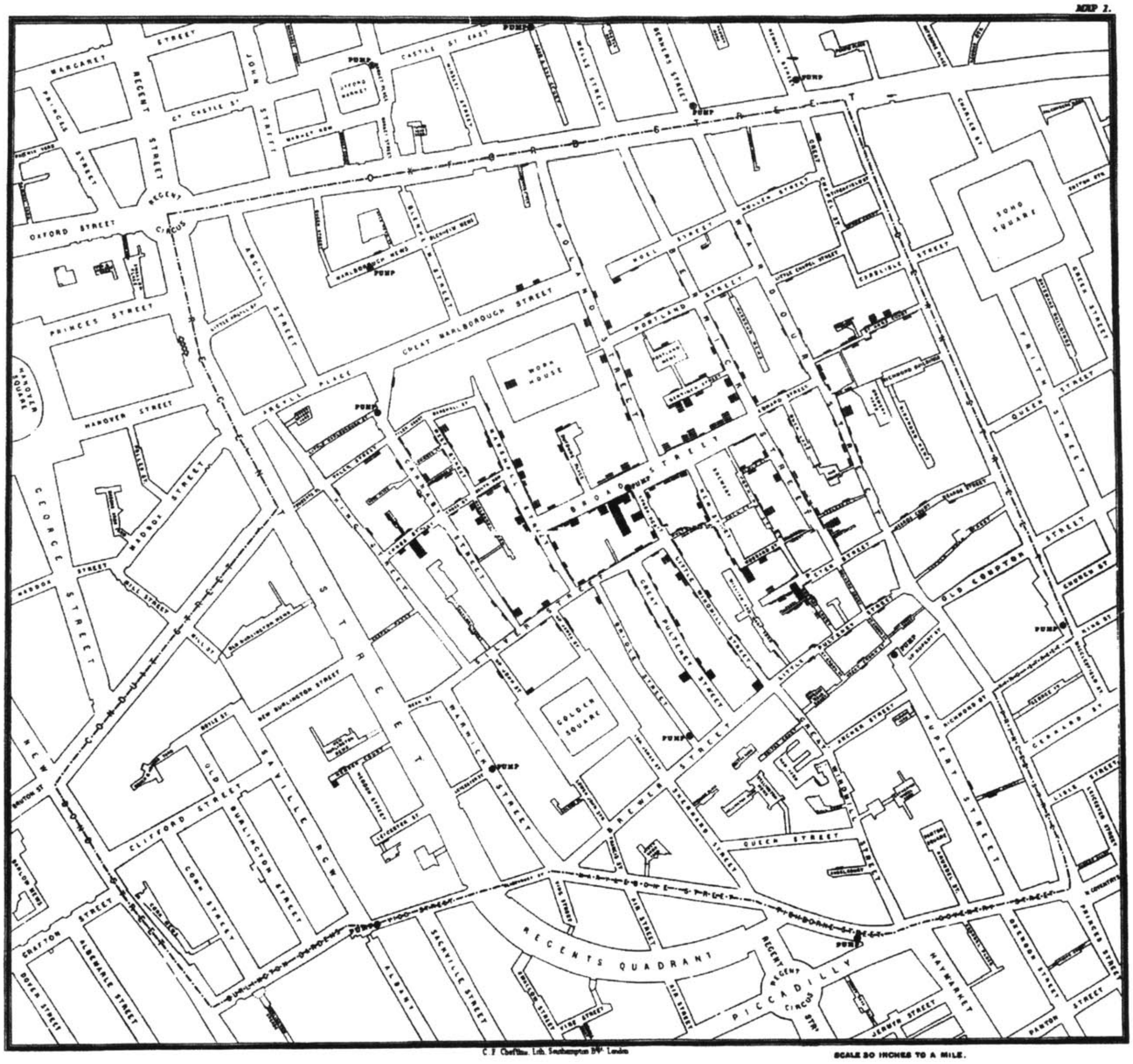

Figure 2. Original map by John Snow showing the clusters of cholera cases in the London epidemic of 1854, drawn and lithographed by Charles Cheffins. (Image Credit: Wikicommons)

4.3.2 Health and the City

Utilization of stool in monitoring disease at the population level. The birth of the city was spurred by the capabilities of water management and sanitation. One of the earliest acts of modern epidemiology involved an enteric pathogen. In the 1850s, John Snow used the structure of the city to trace the source of a cholera outbreak in London. Snow was not convinced by the miasma theory, according to which cholera was caused by a type of "foul air". To understand the nature of disease transmission, Snow mapped out all the cases of miasma, and identified a cluster of cases around a pump on Broad Street (Figure 2). This method lead him to identify a septic leak at the Broad Street pump, a public well for drinking water for the area. This is considered the first act of modern epidemiology, relating the structure of the city and the health of its population.

4.3.3 Monitoring the Urban Gut

A vast reservoir of information on human health and behavior lives in our sewage, but this vast resource remains untapped. Given the above, it becomes plausible to envision a future in which sewage is mined for realtime information. Sewage constitutes a natural aggregate of human activity and metabolism. As such, it has the potential to serve as a rich source of biomedical and behavioral information for understanding disease in local contexts. The following argument is formulated by Mariana Matus, one of the lead researchers on the Underworlds project:

> *There is precedence for the success of sewage surveillance systems in facilitating Public Health strategies in developing countries. State of the art disease surveillance systems rely heavily on historical accounts of syndromic datasets, which are reports of suspected or confirmed cases of a list of high profile communicable diseases (about 80 in Massachusetts). The inherent limitations of the current system has prompted development of more sophisticated analysis tools (e.g. forecasting models) as well as collection of new types of datasets. Sequencing urban environments to understand disease spread is gaining traction. Several groups are sequencing swabs from subway trains and stations, as well as other public transportation. New York University is launching a sewage surveillance platform as well.*
>
> *Polio is a highly contagious disease that mainly affects children under five years of age and that can lead to irreversible paralysis. Poliovirus surveillance has traditionally relied on the report of Acute Flaccid Paralysis (AFP) cases. In 2001, Egypt implemented a sewage surveillance program to identify and target the application of the oral poliovirus vaccine (OPV) to reservoir communities. The rationale for monitoring poliovirus in sewage was three-fold: i) Poliovirus is secreted in abundance in feces (up to 2.3x104 PFU/g stool) so it should be detectable in sewage; ii) Most infected subjects shed poliovirus in feces (up to 96.7%) but only about 1% develop paralysis, therefore sewage surveillance should be more sensitive than traditional AFP surveillance; and iii) Results can be translated into impact because Public Health authorities can target vaccination to the areas that need it most. Egypt was indeed very successful in detecting poliovirus in sewage and*

> *the country kept and expanded their sewage surveillance system even after they were declared polio-free in 2004. In late 2012, poliovirus was found again in Egypt's sewage. The finding prompted authorities to ramp up OPV vaccination and Egypt was able to contain the outbreak before there were any AFP cases.*

The poliovirus case study is a clear example of how we can translate sewage biochemical content into information that aids Public Health strategies. It also hints that sewage may be an untapped source of rich biomedical and behavioral information that can revolutionize our understanding of disease and human activity in local contexts.

4.3.4 Smart Sewage

The case for monitoring sewage is compelling, however scalability to achieve high spatio-temporal resolution and coordination with geographic and demographic features remains a challenge. However, implementing an ongoing sewage surveillance system is not trivial. Technically, very little is known as to how our biochemical signature fluctuates and decays through the sewage network, and it is necessary to ensure robust detection and quantification of the molecules of interest. Logistically, it requires the coordination of large teams that can collect and process liters of sewage safely and in a timely fashion, and automation technologies to be able to scale up such activities across a city. Politically, it requires cooperation from Water and Public Health commissions.

4.4 THE UNDERWORLDS PROJECT

4.4.1 Introduction

A vast reservoir of information on human health and behavior lives in our sewage, but this vast resource remains untapped. The vision of this project is to mine sewage for realtime information that can inform policy makers, health practitioners, and researchers alike. The *Underworlds Project* is an open, cross-disciplinary data platform for monitoring urban health patterns, shaping more inclusive public health strategies, and pushing the boundaries of urban epidemiology.

Brainstorming with Prof. Carlo Ratti in Fall 2013, he suggested we characterize at the urban microbiome through sewage outflow at Deer Island. Combining this with the relationship between the inhabitants of a city and the spatial structure and temporal dynamics of an urban sewer system, I suggested we utilize the entire sewage network to build system that would include the distributed sensing of biological activity across the entire city, to monitor these biological signals in realtime. Prof. Eric Alm provided the scientific insight to consider this idea seriously, and as such we set out together to establish the first proof-of-concept realtime epidemiology platform.

After initial experiments with the City of Boston, our team submitted a successful proposal to the Kuwait-MIT Center for Natural Resources and the Environment, directed by Dr. Murad Abu-Khalaf, and was awarded $4M to develop the project between MIT, Kuwait Institute for Scientific Research, and Kuwait University over the next three years (Detailed credits and acknowledgements and a full credit list at the end of the chapter).

4.4.2 Realtime Epidemiology

This project aims to establish the science and technologies to create a proof-of-concept system for realtime epidemiology. Its aim is to bring together novel methods in urban informatics together with those in molecular and computational biology to change the way that large-scale public health studies are carried out.

City Science Perspective. With recent advances in information technologies, today's urban infrastructure is increasingly integrating sensor technology, adaptive computation, and tightly coupled digital and physical processes. Bill Mitchell's vision of a "City of Bits"[48] has evolved, through the refinement of such technologies, into the reality of what Kitchin has referred to as "programmable urbanism"[49] (2011) – that is, our realtime ability to sense a city's activity, understand its changes and fluctuations, and deliver tailored responses to meet the needs of its urban environment. This project adds a new dimension to our current ability to capture the behavior and needs of urban populations. Its realtime geo-localized monitoring of a population's aggregate biological signature as present in the sewers offers new possibilities for the study of urban health and hygiene patterns. Other regions have already begun initiatives to "sequence the city,"[50] as well as to capture a community's collective *metabolism*. As it contributes to addressing immediate urban health challenges, this new infrastructure will be feeding into the larger picture of a growing urban information ecology.

Public health Perspective. The Underworlds Project aims at a proof of concept system for cities to make use of their waste water system to perform near realtime urban epidemiology and understand human health and behavior with a fine spatio-temporal resolution. Probably the most obvious first application of smart sewage technology is contagious disease surveillance, and the prediction of outbreaks of

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[48] cite mitchell's book

[49] cite... and remove the date from text

[50] new york jane carlton, pathomap, BioBE, MBTA swabing harvard

infectious disease before symptoms arise. Early warnings in relation to the presence of new flu strains in urban centers could significantly reduce a community's medical costs, save lives and help prevent pandemics. In addition, smart sewage will provide new ways of studying non-communicable diseases, because biomarkers for diseases such as obesity and diabetes can be measured at unprecedented scale and temporal resolution.

Beyond refining our present ability to detect and respond to pathologies, the technologies described here will give life to a data platform that aims to guide public health policy, municipal strategy, urban planning and epidemiological science in a larger sense. The system offers the possibility to build rich databases that document, integrate and analyze fluctuations in urban health parameters over time. Furthermore, it aims to provide a fine spatial and temporal contextualization of health-related data by correlating biometrics with static variables such as ethnicity, wealth distribution, urban density coefficients and other demographics.

The platform and the novel methods and technologies it establishes will be made available to public health stakeholders and researchers to use as a foundation for answering emerging questions and inform policy in ways that venture beyond those anticipated in the projects initial applications.

4.4.3 Project Overview

As no pre-existing model for establishing a smart sewage infrastructure exists, the aims of the project are to establish the scientific and technological foundations of such a system, and to better understand its possibilities and limitations. The project's activities are organized for this reason into three distinct parts: (i) network analysis and modeling, (ii) building the smart sewage infrastructure, and (ii) development and deployment of pilot applications. The first aim of Underworlds is to establish an understanding of how to sample over time and space, and how to interpret these samples meaningfully relative to population. The second is to assemble the automated infrastructure needed to mine sewage and gather chemical and biological data, interpret it, and communicate the results. Finally, the third aim is to use this infrastructure to implement a set of foundational applications. The first two aims are presented below in three methods sections, and followed by a section on the applications. The remainder of the chapter covers the initial experiments and conclusions on the current state of the work.

4.5 METHODS: NETWORK ANALYSIS

4.5.1 Making Sense of Sewage

The collection of biological signatures from the waste water stream requires a physical understanding of the sewage environment, a topological understanding of the sewer network, as well as the correlation to demographic data representing the population. A coherent network analysis of the sewer system is needed to interpret the chemical and biological signals into actionable public health insights.

The aim of modeling aspects of the physical, chemical, and biological dynamics of sewage is to test and validate it as a chemical and biological information platform, to be able to interpret the signals found in the signals, and to inform methods and best practices of sampling and processing sewage for surveillance, as well as characterize the technology that could automate and scale such a system. This provides the basis for meaningful sampling strategies to support for the research application is in part determined by both of these aspects.

The primary focus of the research has been to understand (i) how to model sewage flow and mixing, (ii) how to collect samples over space and time, (iii) how to link the biological and chemical information collected to demographic information describing the related population.

4.5.2 Physical Modeling of Water Flow Rates and Mixing

The modeling of flow rates and mixing, in conjunction with the modeling of the sewage network in the next section below, helps inform how best to sample the sewage, as well as how to interpret the biometrics collected in the initial applications. This model is currently being developed and will be packed into the algorithms that automate the partitioning of catchment areas/population samples and selection of sampling location access points in the sewage network.

With researchers Aldo Treville, Alexander Beyli, Stanislav Sobolevsky, we have created initial models which approximate flow and mixing based on sewer network morphology (Figure 3). The initial model accounts for the distance sewage travels, and a second iteration includes the slope angles of the pipes where the data is available. Sewage load s were estimated based on accepted conventions from built area and number of bedrooms (see more detailed description below). Currently more sophisticated models incorporating hydrodynamic mixing are being studied by researcher Peter Kang under the supervision of Prof. Eltahir.

In additional to theoretical modeling based on existing precedent, we combine an empirical approach to inform our model. This work has already started in the pilot studies carried out by Senseable City Lab and Alm Lab in Boston and Cambridge. In collaboration with the department of public works, we are analyzing flow meter data from 30 devices to develop an initial model of the flow and mixing of waste water through the sewage network over time.

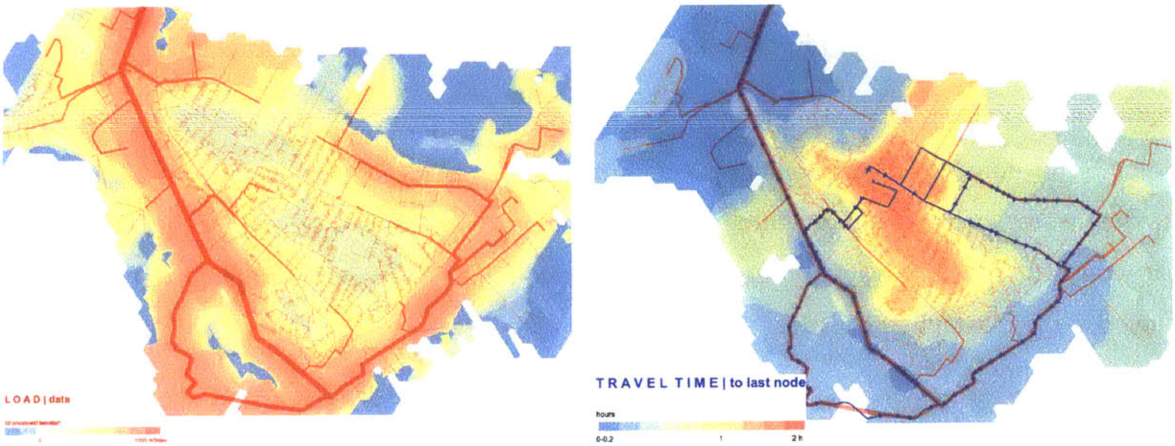

Figure 3. Travel time in sewage system of Charlestown, Boston. (Top Left) Travel time of waste water by path length, with the longest path delineated. (Top Right) Waste water load by population and built area modeling. (Image Credits: Aldo Treville, Alexander Beyli)

4.5.3 Water Balance Modeling

The majority of the daily movement of matter in and out of a city is the flux of water. Another area of interest for understanding and modeling the sewage system lays exploring the water balance of this flux of water within the water supply and waste water networks. Understanding and quantifying this balance should reveal valuable information for decision makers about:

- where and when water is consumed above the surface; and about
- where, when, and what quality of water is leaked from the system to the natural aquifer beneath the surface.

Information about water consumption will serve as input to models of water demand, which are valuable tools for water resources management. At the same time, information about leakage from the system will inform optimal aquifer management. The basic water balance of the underworld will help provide other collaborators in the project with valuable information about the underlying physical transport processes that impact the biological systems being studied. This research is being carried out by the Eltahir Group.

4.5.4 Sampling over Space, Time, and Population

Geographic Modeling of the Sewage Network. To determine the optimal sampling locations and frequency, the Underworlds team is studying the geographic morphology, network topology, as well as demographic distribution, in conjunction with waste water loads over time, to propose and validate a model which informs waste water sampling and correlates to target population samples.

Algorithms will pack this model into an automated sample access point selector, capable of outputting access points for sampling catchment areas determined by optimizing for population sample size, overall waste water load, area, and demographic parameters of age, ethnicity, and income. This allows us to carry out studies which will facilitate the selection and validation of each sampling strategy later to be deployed in the research applications.

This initial work on the geographic modeling and network analysis has been carried out at Senseable City Lab by Aldo Treville, Alexander Beyli, Stanislav Sobolevsky, under my direction. GIS maps for the sewer systems of Charlestown (~17,000 residents), Brookline (~60,000 residents), and Cambridge (~100,000 residents) were provided by the respective departments of public works. We have corrected anomalies in the data, and analyzed the geographic and topological character of the sewage tributary network.

We analyzed the network topology to look for unique features that could be exploited by our model and sampling strategy. The figures below show a comparison of the sewer networks of Charleston and Cambridge, looking at the distribution of incoming and outgoing nodes (Figure 4), estimated load distribution by hierarchical level in the network (Figure 5), the distribution of nodes per hierarchical level (from the end) (Figure 6), and some properties similar to self-similarity in the lower half of the networks (Figure 7).

These analyses were considered in the formulation an algorithm for the optimal selection of sampling points relative to demographic parameters and sampling resolution assumptions. We also integrated demographic data from a number of sources, in part provided by the cities. Having divided our model into a number of node types, we analyzed various corrections between demographic features and the sewer morphology and topology.

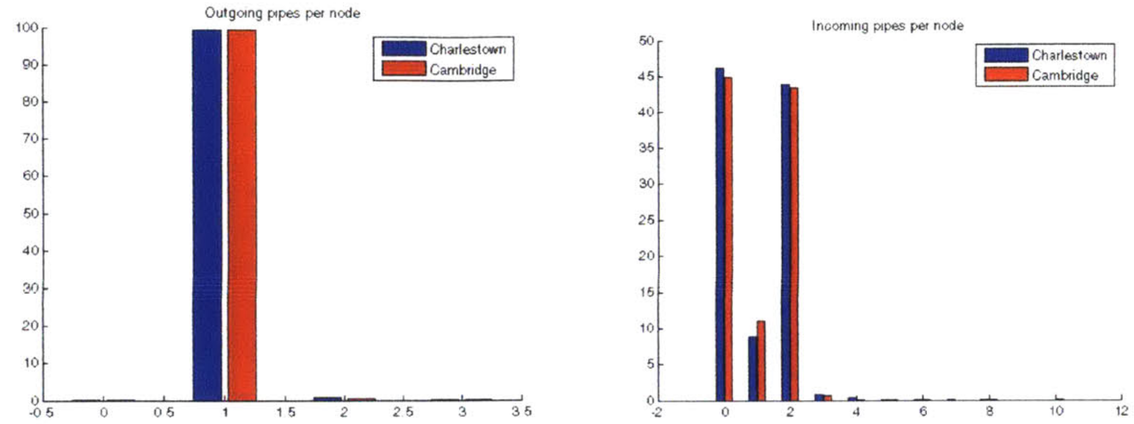

Figure 4. Distribution of the number of incoming (left) and outgoing (right) pipe per node (%%). (Image Credits: Alexander Beyli, Stanislav Sobolevsky)

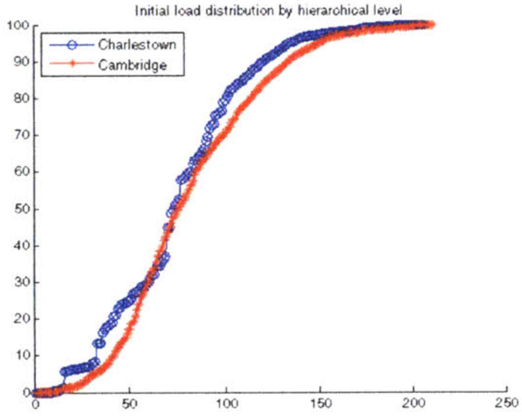

Figure 5. Initial load distribution by hierarchical level in the network (Image Credits: Alexander Beyli, Stanislav Sobolevsky)

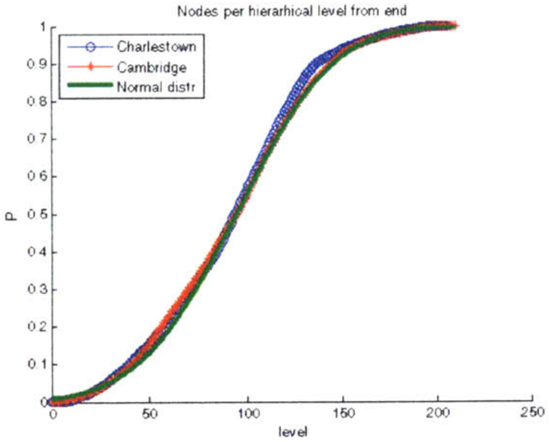

Figure 6. Counting from exit points, levels are distributed normally (Image Credits: Alexander Beyli, Stanislav Sobolevsky)

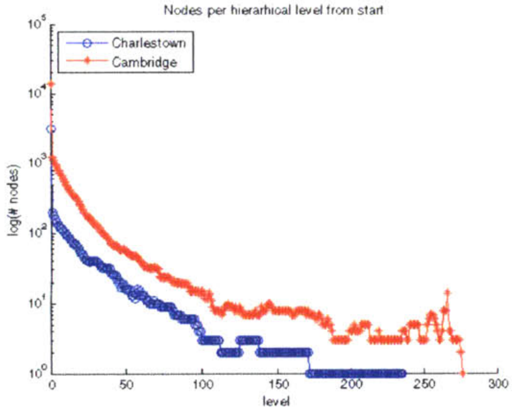

Figure 7. Here we can see something similar to self-similarity at the beginning of network (from second level to about level 100), and after that again linear structure (like one main pipe collecting all load) (Image Credits: Alexander Beyli, Stanislav Sobolevsky)

We also built a sewage load estimation model based on accepted estimation calculations. Residential sewage loads are commonly based on a "gallons per person per day" or "gallons per bedroom per day" rate. Residential flows vary and have been shown to be proportional to (1) family size, (2) socio-economic status, and (3) source of water supply. Secondary considerations include, (1) type of dwelling unit, (2) geographic location, and (3) methods utilized for wastewater disposal.[51] Using these methods of estimation, we integrated sewage load to our network analysis model. Figure 8 shows a sample analysis for Charlestown, used to develop a sampling strategy for maximum coverage and optimal population sample for our preliminary studies this year.

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[51] Siegrist, R, Woltanski, T, Waldorf, L. Water Conservation and Wastewater Disposal, Home Sewage Treatment, Proceedings of the Second National Home Sewage Treatment Symposium, American Society of Agricultural Engineers, Pages 121-136, 1977.

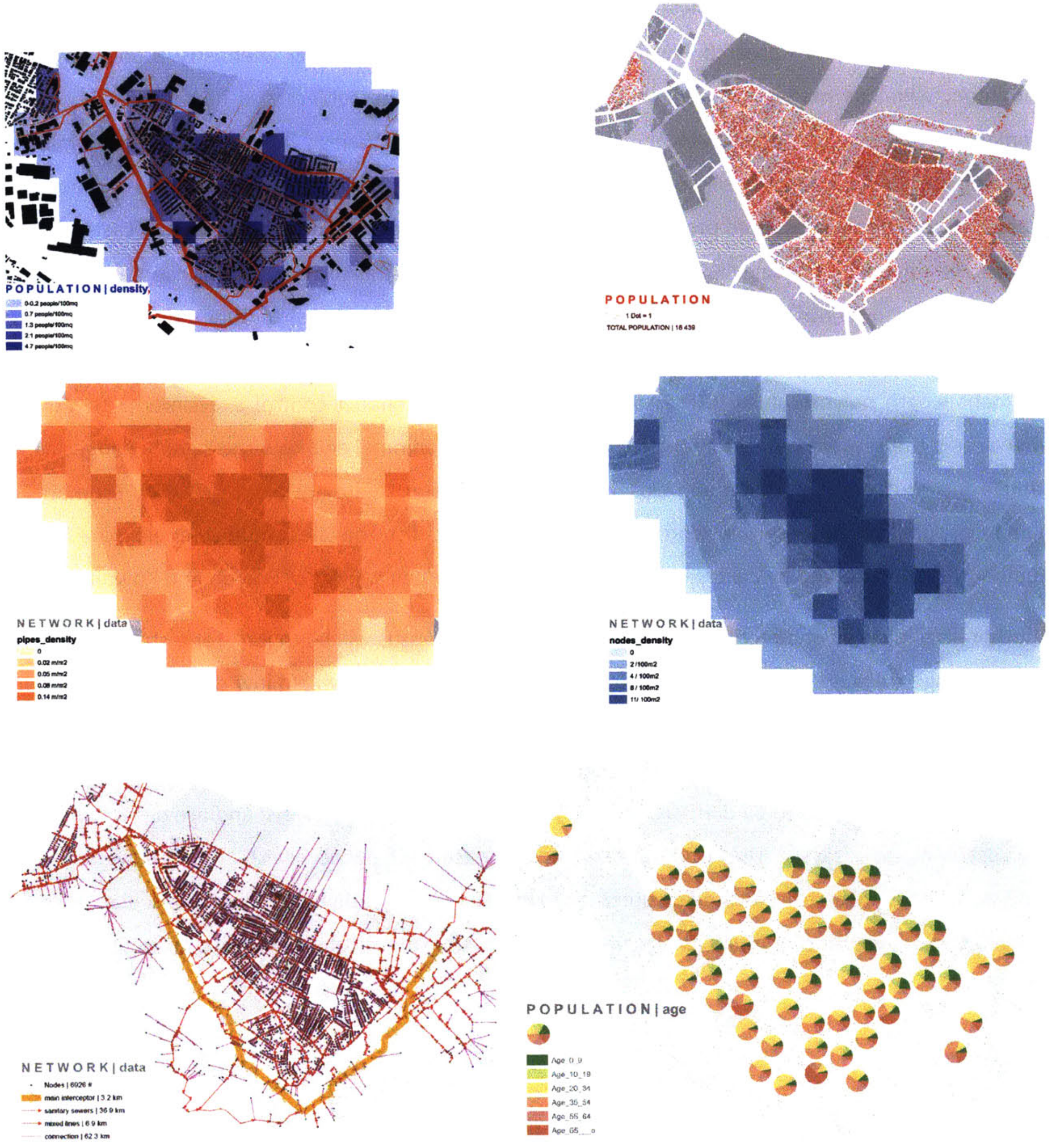

Figure 8. Tributary sewage map Charlestown, Boston. (Top Left) The tributary sewage map in Charlestown (Boston). (Bottom Left) Population distribution across sampling landscape. (Bottom Right) Demographic information aggregated by sewage catchment. (Image Credits: Aldo Treville, Alexander Beyli)

4.5.5 Sourcing Census Data in Sewage

The Underworlds platform will also be a proof-of-concept for estimating human population size based on an endogenous human biomarker. In order to gauge measurements of risk (i.e. viral load and chemical exposures), realtime knowledge of population sizes will be necessary. We build on published ideas published by EPA Research Physical Scientist in the Environmental Chemistry Branch Christian Daughton, who has made an extensive literature review on the subject and has suggested various biomarkers that may correlate with human population sizes.[52] Various human-produced biomarkers including creatinine, coprostanol, cortisol and androstenedione,[53] will be tested; as well as chemicals present in commonly consumed products, such as caffeine, ibuprofen, and paracetamol.[54] Lastly, we will explore the possibility of using SNPs present in human DNA and strain diversity of microorganisms in the gut (as measured by nucleotide diversity in a universal set of core genes) to augment these chemical biomarkers. These measurements can be made using metagenomic sequencing and/or restriction fragment length polymorphism (RFLP) analyses. We plan to conduct control experiments to validate how closely the sewage microbiome matches a human microbiome, and how the signal changes as we sample further from the source and closer to the waste water treatment plant (WWTP). Estimates of human population size based on biomarkers will be compared to the latest municipal estimates of the number of people served by each sewage catchment in the urban centers of Boston and Kuwait City.

Another very interesting direct extension of the population census will be to look for a stress biomarker in the sewage to gauge the health status of a city. In this respect, we will follow the workflow outlined by Christian Daughton on using isoprostanes as a general biomarker of health.[55] Isoprostanes can be used to measure oxidative stress and are remarkable biomarkers since they are excreted in urine; are biostable; are not impacted by diet; are relatively stable on a day-to-day basis; and are detectable using low-cost immuno-assays.[56] They can be used as a measure of public health since a diverse set of conditions give rise to elevated isoprostane levels: obesity, diabetes, cardiovascular disease, neurodegenerative disorders such as Alzheimers disease, smoking, alcohol consumption, among others.

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[52] Daughton, C. G. (2012). Realtime estimation of small-area populations with human biomarkers in sewage. *The Science of the Total Environment*, 414, 6–21.

[53] Chen, C. et al. (2013). Towards finding a population biomarker for wastewater epidemiology studies. *Science of The Total Environment*, (0).

[54] O'Brien, J. W. et al. (2014). A model to estimate the population contributing to the wastewater using samples collected on census day. *Environmental Science & Technology*, 48(1), 517–25.

[55] Daughton, 2012. Op.cit.

[56] Montuschi, P., Barnes, P. J., & Roberts, L. J. (2004). Isoprostanes: markers and mediators of oxidative stress. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 18(15), 1791–800.

4.6 METHODS: CYBER-PHYSICAL INFRASTRUCTURE

4.6.1 Establishing the Smart Sewage Platform

Establishing the infrastructure to mine the city's sewage is the first step towards the larger goal of realtime health biomarker surveillance across urban areas. To exploit this untapped rich source of information, tailored physical, biochemical, and computational infrastructure must be put into place. The methods currently in development are described below.

4.6.2 Collecting & Processing Samples

The project is implementing the foundational physical components necessary for enabling the collection and understanding of spatio-temporal biological signatures from the city sewage. This includes sensing the environment within the sewage system and monitoring its dynamics, the automation of sewage sampling processes, and the exploration of opportunities for in-situ processing of the samples.

MIT's Senseable City Lab has established research relating to sensor development and deployment, optimization of sensor networks, sensor fusion and realtime data integration.[57] Previous research projects including Real Time Rome, Live Singapore, Data Collider, Local Warming, and the Copenhagen Wheel serve as reference systems for the collection, analysis and distribution of realtime data relating to the urban environment. MIT's Distributed Robotics Laboratory at CSAIL has pioneered research in underwater robotics and sensor networks[58] and decentralized adaptive control systems, as well as realtime processing technologies for sensor data.[59] Together, we have been collaborating on developing unique networked sensors and robots specialized in automating the collection of data and samples from the sewer environment.

Sewage System Environment Sensing. Underworlds will deploy a prototype sewage sensor network comprised of digital sensors including pH, temperature, biological oxygen demand (BOD), water flow and level. Monitoring the sewer environment will inform the interpretation of biological signals, and monitoring the dynamics will inform the physical models needed to determine sampling relative to population and geography. To date, we have relied on traditional flow meters and data from existing municipal sensing infrastructures such as the networked flowmeters along Borston's Dorchester pipe or Cambridge's water consumption meter readings.

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57 Resch, B. (2012). On-the-fly Sensor Fusion for Realtime Data Integration. *Geoinformatics.*

[58] AMOUR (Autonomous Modular Optical Underwater Robot), http://groups.csail.mit.edu/drl/wiki/index.php?title=AMOUR

[59] Deployment and Optimization of Wireless ad-hoc Communication Networks, http://groups.csail.mit.edu/drl/wiki/index.php?title=Deployment_and_Optimization_of_Wireless_ad-hoc_Communication_Networks

Automation of Sampling. One of the primary needs for high resolution biological signals from the urban waste waters are high frequency samples collected from strategically selected access points relative to the sample population (see sewage network modeling, as covered below). characterize and deploy a prototype system for semi-automated sewage sampling to facilitate this labor intensive activity, and demonstrate a key automation necessary for the future scaling up of the smart sewage system.

Through our initial ad hoc pilot study collecting samples in Boston, our team has identified the main requirements of such automated sampling stations: a capacity for robust electrical and mechanical operation in the sewer environment, batteries operated with operational lifetime equivalent or greater than maintenance/sample collection intervals, programmable sampling strategy and metered collection volume, coarse filtration to ensure robust performance of pumping and filtering systems, weight and size commensurate with small team deployment, and ease of installation, maintenance and sample pick-up given the space restrictions of a typical sewer environment. The team has evaluated commercial automatic samplers to further identify the advantages of designing our customized system ex novo.

The team has worked over the course of this year to develop a prototype sampling robot designed to reside under the sewer manhole, and automate the collection of samples. The design is conceived in three isolated areas which work together including a mechanical area, pre-processing area, and a storage and collection area. The complete unit hangs from an expanding friction-fit brace that presses on the walls of the pipe just under the manhole. The hanging unit can adjust to water levels, and collect samples on a programed basis. The samples are pumped to a pre-processing area, where they are filtered an d concentrated as needed. Finally they are mixed with any necessary preservation agents, and stored for collection. A mobile phone app connected wirelessly controls the sampling robot, allowing the robot to push the manhole cover up, revealing the samples for easy and safe collection. An early iteration of the design lead by researcher Franco Montalvo of Senseable City Lab can be seen in Figure 9. The design has since been refined in collaboration with the Distributed Robotics Lab in CSAIL.

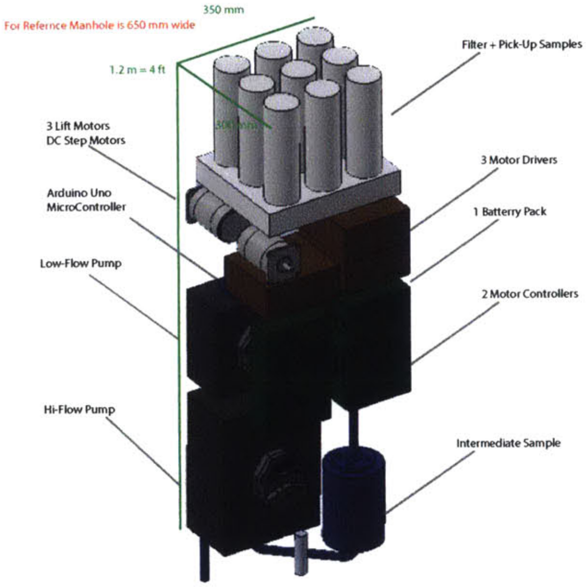

Figure 9. Early iteration of sewage-sampling robot being developed at the Senseable City Lab (Image Credit: Franco Montalvo)

Based on our Preliminary Studies in Boston (see end of Section) and Egypt's successful experience in monitoring poliovirus in Cairo's sewage at five sampling points,[60] we plan to deploy between five and eight automatic sampling robots by the end of the third year, as part of a prototype demo of the system.

4.6.3 Computational Infrastructure

In addition to the cyber-physical infrastructure above, we are developing key computational components for a smart sewage system. While the data collected by Underworld's sensing apparatus will constitute a valuable asset in and of itself, multiple additional possibilities will be enabled by performing advanced data analysis over time and space, thus shedding new light onto the factors that give rise to the underlying patterns and variations.

The Underworlds Data Platform will provide:

- Monitoring of Sensing and Sampling,
- Centralized Storage of Smart Grid Big Data, and
- Data Processing and Analytics, including bioinformatics and urban-informatics

This has not yet been developed, and is a future task of the project.

Monitoring of Sensing and Sampling. The Data Platform will centralize monitoring of the sensor network, and facilitate the coordination of automated and semi-automated sampling activity. This will enable Underworlds to focalize and regulate data-gathering operations easily across the prototype smart grid, intensifying for instance observation in a specific area or sampling point, or throughout specific time windows, in response to realtime environmental conditions.

Centralized Storage of Smart Grid Big Data. The information gathered through the grid's sensing devices and its semi-automated sampling apparatus will be centralized in server infrastructure that feeds the processing, analytics, and front-end interface of the Data Platform. The Underworlds Data Platform will provide processing power capable of storing, processing, and manipulating the continuous information stream of activity in the city's bowels over place and time. This will build an accessible archive of the city's sewage environment, layered with bio and urban informatics. These data will be made accessible through the server infrastructure to researchers and stakeholders to provide unique insights and scientific findings.

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[60] El Bassioni, L. (2003). Prolonged Detection of Indigenous Wild Polioviruses in Sewage from Communities in Egypt. *American Journal of Epidemiology,* 158(8), 807–815.

Data Processing and Analytics. A key capacity of the Underworlds Data Platform is its ability to process, fuse, and analyze the information collected through the grid's smart infrastructure with the outcomes of the lab assays of the bio platforms, as well as urban datasets to contextualize and correlate the bio-signatures to human behavior in the city. The Alm Lab specializes in bioinformatics, and the big data processing expertise of CSAIL as well as the work of the Senseable City Lab in urban-informatics will contribute to this aim.

Bio-informatics. The Alm Laboratory has developed new algorithms to handle the massive data sets that emerge from high-throughput biological techniques. In particular, we have established software packages to analyze 16S community survey data, metagenomic data, single-cell genomics analysis, and general sequence and genome annotation pipelines. The Alm Laboratory developed the widely used *MicrobesOnline* website and database, which provides annotation for thousands of microbial genomes and is in broad use by the microbiological community. Below is an overview of the bio-informatics tools developed by the Alm Lab that are of particular relevance to the Underworlds Project:

- **Algorithms for analyzing 16S community surveys.** The Alm Lab has established a suite of analytical tools to decipher these complex data sets, which preclude manual interpretation. As part of this project, the team at the Alm Lab will develop and validate a pipeline of these tools that uses principles from the field of machine learning to identify species significantly associated with environmental data such as biodegradation rates, geographic sites, or presence of dispersants. This software package will be customized for and made available to other stakeholders for future usage.

  The first tool included in the package is the Distribution Based Clustering (DBC) software package, which identifies ecologically meaningful operational taxonomic units (OTUs). This algorithm represents the first principled method to incorporate distribution of taxa across samples into defining taxon boundaries.[61]

  These tools operate by finding statistical associations between existing flora and target environmental factors such as geography, depth, and even complex traits, provided that a suitable training set is available. Often, these associations are weak, and difficult to detect by standard methods. Our approach uses statistical learning approaches to combine multiple weak signals into an accurate prediction.

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[61] Preheim, S. P., Perrotta, A. R., Martin-Platero, A. M., Gupta, A., & Alm, E. J. (2013). Distribution-based clustering: using ecology to refine the operational taxonomic unit. Applied and Environmental Microbiology, 79(21), 6593–603.

The basic approach has already been shown to work as part of a medical application.[62] We used our approach to diagnose inflammatory bowel disease, a complex human gastrointestinal disease, by sequencing bacterial DNA obtained from patients. No single bacteria formed a good indicator of disease, but our method successfully combined the whole community data to make an accurate prediction.

- **Algorithms for identifying species-species interactions.** The Alm Lab have developed the SparCC software package[63] to compute correlations among species from relative abundance data. SparCC relies on the realistic assumption that most species are not strongly with each other — true correlations between species are sparse. In practice, and in simulations, SparCC performs well even when the 'sparseness' assumption is violated (up to 35% of species-species strongly interact), and SparCC outperformed standard correlations in all conditions.

- **Analyzing bacterial population time series data.** Analysis of time series population data is non-trivial, but the Alm Lab have developed and validated approaches for analyzing bacterial populations. In the year-long time series from a human gut described below, and shown in Figure 10, we use a median-based approach to estimate relative overall bacterial concentrations in each sample, effectively converting relative counts to a common scale.

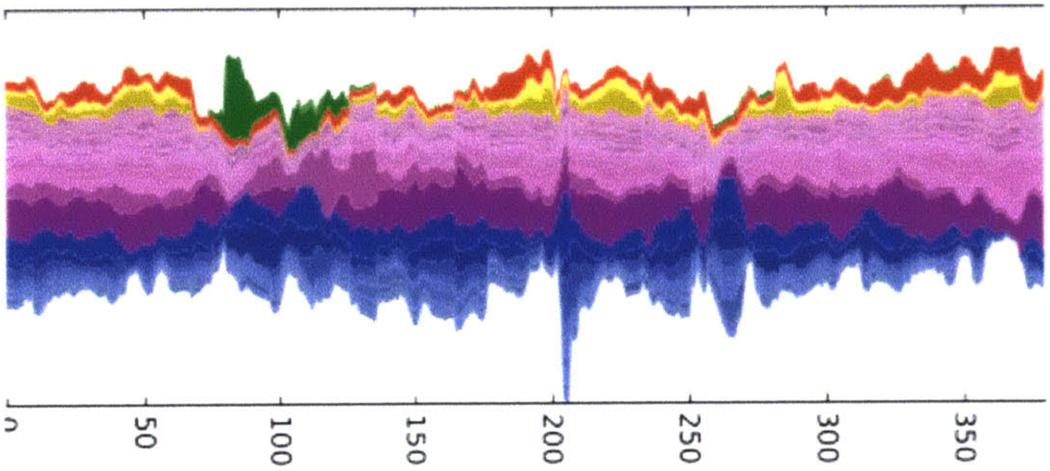

Figure 10. Normalized time series data. Relative abundances of microbial taxa are shown for a year long collection of daily samples from a human gut ecosystem. The x-axis indicates time in days, and the y-axis indicates abundance of each taxon. Phyla are shown by color (Green - Proteobacteria; Red - Tenericutes; Yellow - Actinobacteria; Purple - Firmicutes; Blue - Bacteroidetes). Color shades indicate distinct OTUs. (Image Credit: Alm Lab)

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[62] Papa, E., Docktor, M., Smillie, C., Weber, S., Preheim, S. P., Gevers, D., ... Alm, E. J. (2012). Non-invasive mapping of the gastrointestinal microbiota identifies children with inflammatory bowel disease. PloS One, 7(6), e39242.

[63] Friedman, J., & Alm, E. J. (2012). Inferring correlation networks from genomic survey data. PLoS Computational Biology, 8(9), e1002687.

They then computed a scaling factor for each time point by maximizing overlap between observed OTU abundances with expected abundances estimated from adjacent time points. They then computed the scaling factor that minimizes the deviations between expected and observed values, and fit OTU and environmental time series data to autoregressive moving average (ARIMA) models, which are common tools in statistics and econometrics for identifying trends in time series data. For binary or categorical data, they identify trends using a Markov-chain approach designed for categorical longitudinal data. This approach successfully identified a number of diet and other correlates with microbial populations.

Urban-informatics. The project addresses the opportunity to combine the spatio-temporally tagged bioinformatics data collected and analyzed onto other existing urban informatics infrastructures and the data that already populates the urban information ecology.

Demographics, spatial dispersion of urban population groups, area-specific health statistics, are some of the variables that can be mapped directly on top of the data collected by Underworlds, with a view to providing greater insight into each dataset's significance, and shedding light on the organic correlations that exist between them. Other such examples include: detailed call records and GPS readings for tracking human mobility, environmental data about air quality and pollution, outputs from health-related smartphone apps and search queries, and anonymized health records from local hospitals regarding, for instance, recent epidemics. The aim is to design a platform that will be open for future expansion and integration of new kinds of datasets, manipulation parameters and software implementations, as necessary. Senseable City Lab has made one such platform available for the fusion of spatiotemporal data with other data types, called the DataCollider.[64]

The Data Platform envisioned by this proposal will allow for Underworlds' general urban health findings to be monitored, observed through time and space, and utilized for generating novel research and policy insights. As a concrete illustration of this approach, mobility data can be used to visualize how epidemics spread across neighborhoods in the city over time, and to inform strategies for deploying medications and quarantine. This aspect of the project has not yet been implemented.

4.6.4 Communicating Results to Stakeholders

One of the important aspects of this type of system is to be able to interpolate the vast high resolution data to intuitive and meaningful insights for people. This includes stakeholders in public health, medical researchers, and the public directly. The notion of including the public and allowing more direct

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[64] DataCollider <http://datacollider.io> Last accessed 21 May 2015.

mediation between citizens, their environments and their health is a great opportunity of the technological moment, and can be of tremendous benefit to public health.

For example, the Public Health 2.0 initiative that aims to share public health data with the general public. Public Health 2.0 main activities are to present how public health officials use social media in order to approach the public, and to illustrate data gathered from different online technologies, such as social network sites and search engines queries. The initiative especially puts emphasis on describing activities that are completely user-driven. It uses Web 2.0 tools and concepts such as crowdsourcing, information sharing, and user-centered design.

These ideas inform the design of the Underworlds data platform, described below.

Data Browser. The Underworlds team will develop a Data Platform front-end that will facilitate exploration, manipulation, and communication of the spatio-temporal biological and geographic insights of the Underworlds research. The platform will be designed with appropriate data storage capacities and an overall computational power sufficient for running all data fusion and subsequent data processing activities, and coded to be accessible through web technologies to provide dynamic visual rendering of the data. Recent projects of the MIT Senseable City Lab demonstrating this kind of activity include: Live Singapore, Data Collider, Real-Time Rome,[65] and Signature of Humanity (for more information see the Senseable City Lab website, 2013).

Data Visualization and Live Data Interfaces. Effective and intuitive visual renderings of urban information coupled with dynamic live data interfaces will enable the exploration of multidimensional datasets and the conversion of data into knowledge. These can reveal insights into statistical relationships between exposure and health that would not be seen otherwise. Sophisticated graphic representations of the Underworlds data will help ensure a quick, accurate and engaging relay of information to researchers, local stakeholders and other audiences.

As a health domain specific example of interactive visualization, Health InfoScape is a project carried out by Senseable City Lab in collaboration with General Electric. Here, 7.2 million anonymous electronic medical records from across the US were analyzed to uncover statistical relationships between geography and health. The project combined network analysis and graphic visualization, and presented a succinct picture of the United States human health network.[66]

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[65] Offenhuber, D., et al.(2012). Putting Matter in Place. Journal of the American Planning Association, 78(2), 173–196; Calabrese, F., & Ratti, C. (2006). Real Time Rome. *Networks and Communication Studies*, 20(3-4)

[66] Senseable-City-Lab. (2011). Health Infoscape Project. Retrieved from senseable.mit.edu/healthinfoscape

Data Fusion & Browsing Tools. In analogy to the Lab's extensive past work portfolio on data visualization and browsing tools, the Data Platform delivered by Underworlds will allow:

- the potential sharing of data with multiple agents, as well as the opening of the platform, or sections thereof, to the public;
- the integration of such information with diverse kinds of data, such as human mobility and transportation, climate, energy, etc.; and
- the comparison between the local signature connected to different land uses (commercial, industrial, residential, recreational, commuting connections, etc.) and their respective variations throughout the day, week, and time of the year.

To illustrate the processing of transforming raw data to a meaningful signal, and then to an interface which allows exploration, combination, and interpretation of these signals, an example of a Data Browser created by Senseable City Lab in collaboration Ericsson is shown in Figure 11. This extracts signatures of human activity in millions call data records, interprets distinct patterns into clusters of discernible activity, and visualizes these spatiotemporal insights in an intuitive manner for further research, decision-making, and communication.

Figure 11. Visualization and Interactive Interfaces, The Health InfoScape Project. Visualization and interface exploring relationships between 7.2 million electronic medical records. (Image Credit: Senseable City Lab)

In addition to facilitating research leading to new scientific knowledge and stakeholder insight, the Underworlds Data Browser will potentially open the way for additional audiences to engage with the data, potentially allowing for more the participation of policy-makers, medical practitioners, public health emergency responders, environmental stewards, as well as the public to explore, utilize, and benefit from open access to our platform. The availability of intuitively comprehensible realtime data made available via open web platforms and phone apps is particularly effective in educating citizens in relation to pressing issues such as urban environmental pollution and its collateral wide-ranging effects. This, in turn, can trigger and support long-term distributed behavioral change.[67] The Underworlds project will work with stakeholders to develop the Data Browser in order to make it the most informative for local needs.

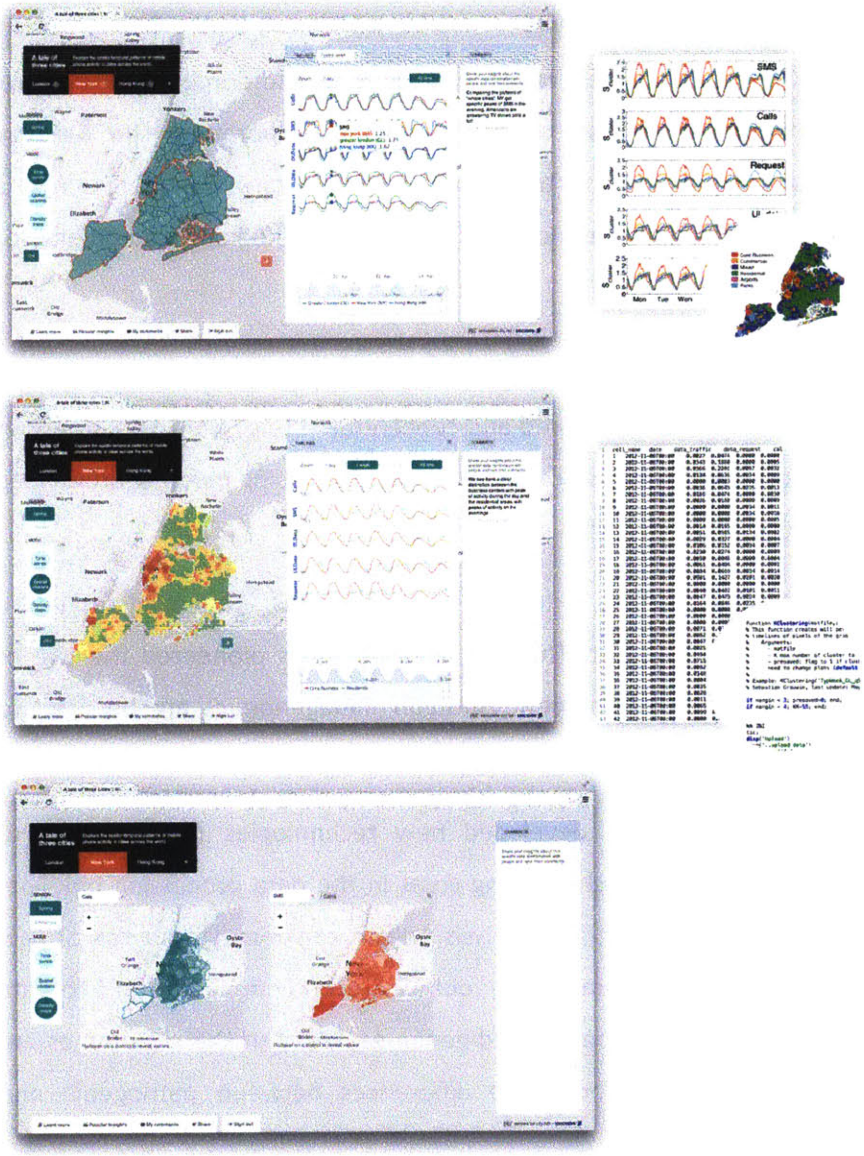

Figure 12. Example of data visualization tool. Screenshots of a data analytics and visualization platform created by Senseable City Lab in collaboration with Ericsson for investigating spatiotemporal signatures of mobile communication data. (Image Credit: Pierrick Thebault)

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[67] Devarakonda, S., et al. (2013). Realtime air quality monitoring through mobile sensing in metropolitan areas. *Proceedings of the 2nd ACM SIGKDD International Workshop on Urban Computing - UrbComp '13*, 1.

4.7 METHODS: BIOCHEMICAL INFRASTRUCTURE

Underworlds is assembling an infrastructure for carrying out sample processing and laboratory assays, which build upon the genomic expertise contributed by the Alm Lab. To follow is a description of the biological platform.

4.7.1 Detecting the Metabolic Signature of Human Activity

Metabolomics. Metabolite analysis is complex, and requires significant additional infrastructure. Therefore, we have outsourced metabolomic analysis to the Metabolon Corporation[68] for detailed analysis. We have for metabolic analysis that allow us to perform comprehensive sampling across a large number of samples. In future years, targeted and more scalable assays will be adapted on-site for specific applications.

4.7.2 Detecting Pathogens of Interest in the Collective Microbiome

Viral Detection Platform. Raw sewage must be first filtered and concentrated (e.g. from 10 L to about 10 mL), either *in situ* or in the laboratory. This viral fraction can be sequenced using metagenomics (see below) or amplified directly with qPCR to detect specific viruses of interest.

Metagenomic Platform. Total DNA (or viral fraction) extracted from sewage is sequenced to yield a comprehensive view of the genetic material present. The Alm laboratory has pioneered the use of metagenomics to make inferences about the environment. Shotgun metagenomics are be used to investigate the genomic repertoire of functions present in a sample.

Metagenomic Assembly. The Alm laboratory has developed new technologies for assembly of metagenomic data, one of the most important but challenging steps in the data processing pipeline. They have developed an algorithm called Latent Strain Analysis, which can mine terabytes of raw sequence data to assemble individual bacterial genomes. This tool was recently applied to a 6Tb data set of human gut samples resulting in ~5000 newly partitioned genomes. Many of these genomes are different strains of the same species, allowing for subtle differences between pathogenic and commensal strains to be identified.

16S Community Profiling Platform. The Alm lab has developed a high-throughput approach to characterizing the composition of microbial communities that leverages the next-generation Illumina DNA sequencing platform. For each biological sample, either from the natural environment or enriched from a laboratory culture, DNA is extracted and the regions corresponding to the 16S rRNA gene are

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[68] Metabolon Corporation, Research Triangle Park, NC, USA.

amplified and sequenced. Over 100,000 individual molecules are sequenced per sample providing exceptional resolution into ecological community structure. In addition, this approach allows for 1,000 samples to be processed in parallel enabling experimental designs that capture a wide array of conditions, replicates, and/or time points.

Targeted Single Cell Genomics. The Alm lab has established a pipeline for single cell genome sequencing that allows the team to collect genomic information on strains resistant to direct culturing. The approach to single cell genomics is based on sterile capture of individual cells in microwell plates followed by Multiple Displacement Amplification (MDA) of genomic DNA. This process results in ug quantities of DNA with coverage between 60-85%, and we have used this approach to successfully generate Illumina sequencing libraries. Cells of interest, emerging pathogens for example, can be captured for sequencing using FISH probes targeting variable regions of their 16S sequence followed by flow sorting.

4.8 APPLICATIONS

Underworlds is primarily imagined as a monitoring system for the urban microbiome. However as a platform, it will be capable of a variety of beneficial applications. We identified several key areas in which Underworlds might have the greatest impact in upcoming years, and these are described briefly below.

4.8.1 Application Overview

Disease Outbreak. Underworlds will follow influenza and gastroenteritis-related outbreaks (e.g. Salmonella, rotavirus, norovirus) as proof of concept of the impact of this new type of realtime epidemiology. Currently, health authorities monitor the emergence and spread of such diseases through samples of hospitalized patients, but this limits surveillance until long after the time of infection. Underworlds aims to extend the surveillance capacity by gathering information from all population in a city quickly and effectively.

Health Policy Monitoring. The Underworlds platform can be used to test whether policy changes have a positive impact on public health. For example, monitoring obesity/inflammation biomarkers and how they change upon interventions in dietary intake, and monitoring phthalate levels and how they change upon banning specific personal care products or plastics to reduce exposure.

Security. Many governments have become interested in having a long-standing continuous surveillance system for biological and chemical threats. The major advantage of having a platform that runs continuously, is to detect new threats early and enable authorities to respond quickly.

Water Management. Departments of public works in cities are interested in extending realtime monitoring to the sewers. Many already have plans in place to deploy flow meters through the city, and are interested to incorporate sensitive monitoring of biological and chemical agents. Another concern is the detection of illegal connections from industrial complexes to the residential sewage network.

4.8.2 Monitoring Infectious Diseases

Objective. Screen sewage for the prevalence and distribution of infectious pathogens (Influenza, Rotavirus, Norovirus and Typhoid Fever).

Background. Infectious diseases result in significant health burdens. In the most recent H1N1 influenza pandemic, many healthy Kuwaiti children contracted severe infections.[69] Current surveillance efforts for influenza rely on samples collected from hospitalized patients, which intrinsically biases our detection towards the most pathogenic influenza strains and limits our understanding of viral dynamics in the wider human population. Furthermore, current surveillance data by the CDC lacks enough granularity and it does not lend itself for modeling flu prevalence at local levels,[70] despite the need for local preventative actions. Finally, the delay between time of infection and emergence of the first hospitalizations may impact the effectiveness of public health officials to implement preventative measures. Thus, early detection of infectious agents like flu in sewage can have great public health impact.

Influenza poses some unique technical challenges to detection in sewage since the virus is not shed in large amounts in stool or urine. In addition, it is prone to decay outside the human body because it is an RNA virus. Nonetheless, the virus has been reported in feces[71] and we have successfully detected influenza virus from sewage collected in Boston this winter (see Preliminary Studies), so we are confident of our ability to study the virus through the sewage.

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[69] Husain, E. H., et al. (2012). Hospitalization patterns and outcomes of infants with Influenza A (H1N1) in Kuwait. *J Infect Dev Ctries*, 2–6.

[70] Lazer, D., et al. (2014). Big data. The parable of Google Flu: traps in big data analysis. *Science (New York, N.Y.)*, 343(6176), 1203–5.

[71] Chan, M. C. W., et al. (2011). Seasonal influenza A virus in feces of hospitalized adults. *Emerging Infectious Diseases*, 17(11), 2038–42.

Additional infectious disease burdens include GI-tract infections such as rotavirus, norovirus and typhoid fever. These pathogens are excreted in high amounts, thus facilitating their detection in stool. Typhoid fever is particularly very relevant as the disease can be carried and spread by asymptomatic carriers, and there are multi-drug resistant *Salmonella enterica* Typhi strains circulating. Sewage monitoring may assist in determining locations of sputtering outbreaks that would be difficult to accomplish otherwise.

Biosecurity. In addition to the threat of infectious disease, the Underworlds platform will also target key agents identified as biosecurity threats. We begin by targeting those identified by the CDC as likely threats: *Bacillus anthracis* (anthrax), *Clostridium botulinum* toxin (botulism), *Yersinia pestis* (plague), smallpox, tularemia and viral hemorrhagic fevers. Identification of these threats will be primarily through targeting specific genomic regions of manipulatable pathogens. Single-cell genomics could subsequently be performed to provide information on the genetic make-up of these organisms which could inform public health officials and investigators as to their origin and infectivity. Several of the pathogens listed as potential biological agents are more successfully treated prior to the onset of symptoms (*e.g.*, anthrax) or contained (*e.g.*, smallpox). In addition, the Underworlds metabolomics platform could be used to identify chemical threats, or even the presence of conventional threats (*e.g.*, home-made explosives), directly or via their degradation products. These include a wide range of chemical agents: nerve agents (including sarin, and mustard gas), vesicants, choking agents and blood agents (including hydrogen cyanide).[72]

Approach. This project will utilize several of the Underworlds platforms. First, the metagenomics and single cell genomics platforms will be used to obtain genomic sequences for potential pathogenic agents. Second, our viral concentration and sequencing platform will be used to study viral pathogens. Once a list of target pathogens has been made, we will monitor pathogen counts using qPCR assays for high spatial and temporal resolution.

The questions to address in the project are:

- How does the diversity of pathogens circulating in a city compare to those detected in hospitalized patients?
- Do viruses detected in sewage provide more realistic estimates of the prevalence of infectious diseases in a population?

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[72] Popiel, S., & Sankowska, M. (2011). Determination of chemical warfare agents and related compounds in environmental samples by solid-phase microextraction with gas chromatography. *Journal of Chromatography*. A, 1218(47), 8457–79.

- Can viruses be detected in sewage before they are reported in hospitals?

If pathogens can be detected early and reliably, these data can be used by public health stakeholders to improve public health and well-being. We will facilitate rapid access to these data by creating a visualization tool that updates as soon as new data comes online. The platform can thus help authorities act promptly by alerting the local population to look for specific symptoms and to adopt targeted precautionary measures; by alerting hospitals so they increase inventories of medication; and by helping to identify the source of disease outbreaks

Outbreak and human mobility. Once Underworlds has detected marked increases in pathogens, we use the Data Platform to fuse these data with other urban datasets to identify correlations between viral mobility and city-wide demographics. We also use data on human mobility (such as mobile phone networks and credit card transaction data), to project how an outbreak may spread.[73] Senseable City Lab has established work in understanding human mobility through these types of varied data sources.[74]

Sampling effort. The current assumptions about collection suggest that we will collect biweekly samples throughout the year and the frequency will be increased to weekly sampling during the peak of a disease, for example winter time for influenza. The number of sampling locations is TBD.

4.8.3 Monitoring Chronic Conditions: Obesity & Inflammation Biomarkers

Objective. Use sewage as a realtime data source to gauge the efficacy of public health policy

Background. Many developed cities face an obesity problem, with rates as high as 70%. Obesity is a condition that changes slowly, making it difficult to determine if specific dietary interventions are effective.

The microbiome may provide insight into whether an individual is likely to become obese.[75] In fact, we have previously shown that gut microbiota can be both predictive and causative of weight changes,[76] in a process that also likely involves inflammatory biomarkers. Underworlds can provide policy-makers

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[73] Bengtsson, L., et al. (2011). Improved response to disasters and outbreaks by tracking population movements with mobile phone network data: a post-earthquake geospatial study in Haiti. *PLoS Medicine*, 8(8), e1001083.

[74] Hoteit, S., et al. (2014). Estimating human trajectories and hotspots through mobile phone data. *Computer Networks*, 64, 296–307.

[75] Turnbaugh, P. J. et al. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444(7122), 1027–31; Turnbaugh, P. J.,et al. (2009). A core gut microbiome in obese and lean twins. *Nature*, 457(7228), 480–4

[76] Poutahidis, T. et al. (2013). Microbial reprogramming inhibits Western diet-associated obesity. *PloS One*, 8(7), e68596.

with realtime feedback on the levels of microorganisms and biomarkers associated with obesity, that may allow for realtime evaluation of public health policy long before changes in population are observed.

Approach. We hypothesize that obesity biomarkers can provide realtime information on the propensity of a population for obesity. We will monitor sewage using high-throughput 16S community surveys for key biomarkers of obesity focusing on bacterial groups predictive of obesity.[77] In particular, we predict that the ratio of *Firmicutes* to *Bacteroides* taxa will serve as a proxy for the obese microbiome, and that the relative abundance of *Firmicutes* will be highest in those regions of the city with the highest rates of obesity. Ley *et al.* have shown that diet can directly affect this ratio, thus providing a rapid readout of whether policy changes (*e.g.*, limits on sugar content of beverages, changes in school lunch nutritional content, *etc.*) are likely to have an effect on long-term obesity rates.

Sampling efforts. We will conduct sampling across the city biannually. Sampling will increase in frequency after policy decisions are made to examine their impacts.

4.8.4 Bacteriophage Diversity and Potential Use as Antimicrobial Therapy

Objective. To develop bioprospecting strategies in sewage to establish phage therapy as an alternative to antibiotics in combating infectious agents

Background. Antibiotic resistance among bacterial infectious agents has risen at alarming rates over the past decades. Today, it is common for life-threatening infections to be caused by multi-drug resistant (MDR) bacteria, which no longer respond to most antibiotics. The most recent and alarming development is the emergence of bacteria that are even resistant to carbapenem antibiotics (imipenem, meropenem, ertapenem), which have served as a last resort. Carbapenem-resistant Enterobacteriaceae (CRE) have become prevalent in many areas of India, Pakistan and other Asian countries and have been detected in hospital settings worldwide. It is therefore of utmost urgency to develop alternative strategies to combat bacterial infections.

A potentially viable strategy to combat bacterial infections is the use of bacterial viruses (bacteriophage) as therapeutic agents. Phages are the most abundant biological entities on the planet and through their reproduction in host cells exert fundamental influence on the growth and composition of microbial

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[77] Ley, Ruth E, et al. (2006). Human gut microbes associated with obesity. *Nature*, 444.

communities. For example, in the ocean, phages kill an estimated 20% of bacterial biomass per day.[78] Importantly, phages display many properties that make them ideal agents to combat bacterial infections:

- They are often highly host specific, killing only their specific target and leaving other, possibly beneficial microbes unharmed.
- They effectively kill rapidly growing cells but also infect resting cells, which they can kill once growth commences.
- Resistance to phages is less of a problem since there is a sheer limitless reservoir of different variants available in natural communities.
- Phages are easy to isolate, store and apply.

Underworlds is positioned to take advantage of the large diversity of phages present in the sewage[79] to produce a library that can be mined for targeting particular pathogens. Our early studies (see Preliminary Studies below) show that we can isolate a large and diverse viral fraction from sewage that will be an excellent source of novel phage.

Approach. We use sewage samples to establish a collection of bacteriophage that effectively kill the common pathogens. Sewage is an ideal environment to isolate phage from since it collects samples from a very large number of people so that it is highly likely that many varieties of phage will co-occur. Over the past 5 years, we have established highly efficient concentration and isolation methods that allow us to obtain phages that occur in samples at low frequencies. Our pipeline also includes testing of infectivity as well as establishment of genome sequence, host range testing and determination of infection mechanism. Our ultimate goal is to establish phage collections for specific pathogens that include many different phage using a variety of infection mechanisms so that resistance to all of the phage will be highly unlikely. This is because resistance to phage infection usually causes a fitness defect in the resistant bacteria so that their growth will be impaired even in absence of damaging agents.

Sampling effort: We require few samples to construct a bacteriophage library for these purposes. To increase the diversity of bacteriophage observed, we will take samples from very divergent areas, ecologically as determined from the geospatial data.

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[78] Brussaard et al., 2008; Suttle, 2007; Weinbauer, M. G., & Rassoulzadegan, F. (2003). Are viruses driving microbial diversification and diversity? Environmental Microbiology, 6(1), 1–11; Wommack, K. E., Colwell, R. R., & Colwell, R. R. (2000). Virioplankton : Viruses in Aquatic Ecosystems Virioplankton : Viruses in Aquatic Ecosystems †, 64(1).

[79] Cantalupo, P. G., et al. (2011). Raw Sewage Harbors Diverse Viral Populations.

4.8.5 Antibiotic Resistance Dynamics in the City

Objective. Track the emergence of antibiotic resistance genes in the urban microbiome

Background. Antibiotic resistance is a serious threat, and there is evidence that we may be entering a post-antibiotic era, in which new varieties of pathogens emerge that show resistance to all known antibiotics.[80] Recent studies have identified an enormous diversity of resistance genes within the human microbiome,[81] and these genes are likely to be transmitted between humans and the environment.[82] A recent international workshop on antibiotic resistance identified sewage as a key reservoir for antibiotic resistance, and point of control.[83]

Approach: We track the abundance and type of antibiotic resistance genes found in sewage as a proxy for resistance in human populations. While antibiotic resistance genes are ubiquitous,[84] we hypothesize that clinically antibiotic resistance genes will reflect environmental selection due to antibiotic use by humans. Thus, antibiotic resistance should correlate with the presence of the corresponding antibiotics. Key scientific questions to be answered are:

- Do resistance genes show seasonal patterns based on antibiotic usage?
- Do policy changes related the usage of antibiotics affect the spread of resistance?
- Does resistance emerge from distinct regions of the city?

We will use a hierarchical approach to follow resistance genes. First, we use metagenomic sequencing and assembly to identify classes of resistance genes in a limited number of samples. We then construct targeted PCR primers to follow the abundance of specific gene families with high spatial and temporal resolution. We use our metabolomics platform to identify the presence of specific antibiotics in the sewage, to identify whether antibiotic presence correlates with the emergence of resistance.

Sampling effort. To identify mobile elements containing antibiotic resistance genes, samples will be taken quarterly in several locations for deep metagenomic sequencing. Once mobile elements are

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[80] Laxminarayan, R., et al. (2013). Antibiotic resistance?the need for global solutions. *The Lancet Infectious Diseases*, 13(12), 1057–1098. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S1473309913703189

[81] Moore, A. M., et al. (2013). Pediatric fecal microbiota harbor diverse and novel antibiotic resistance genes. *PloS One*, 8(11), e78822; Sommer, M. O. a, Dantas, G., & Church, G. M. (2009). Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science (New York, N.Y.)*, 325(5944), 1128–31.

[82] Smillie et al., 2011; Forsberg, K. J., et al. (2012). The shared antibiotic resistome of soil bacteria and human pathogens. *Science (New York, N.Y.)*, 337(6098), 1107–11.

[83] Bush, K., et al. (2011). Tackling antibiotic resistance. *Nature Reviews Microbiology*, 9(12), 894–896.

[84] Sommer, M. O. a, 2009. Op.cit.

identified, quantitative PCR can be used to measure their abundance in a larger number of samples more widely distributed throughout the city.

4.8.6 Toxicology: Monitoring Phthalate

Objective. Measure human exposure to harmful toxins.

Background. Phthalates are chemicals used in a large variety of products including plastics and personal care products that can disrupt endocrine signaling, resulting in asthma in children[85] and pre-term child labor.[86] Airborne exposures of DEHP, one of the most common and more toxic phthalates, extend beyond allowable EPA limits for children in Kuwait,[87] and these are amongst the highest in Asia.[88] Since phthalates are excreted in urine, have a relatively short half-life of 5-24h, depending on the specific phthalate,[89] and can be easily measured by mass-spectrometry, sewage monitoring offers a unique means of passive surveillance and measuring responses to policy changes.

Approach. The distribution of phthalates is not always known, although we consider primarily sources that related to industrial applications. Therefore, we anticipate that phthalate levels will correlate with residential areas closest to industrial zones. We expect that, similar to the results of previous studies, that DEHP will be the most prevalence. Our metabolomic approach using liquid chromatography-mass spectrometry offers the ability to simultaneously observe various phthalate concentrations. Specific phthalates have different sources and therefore their differing patterns of abundance through the city may assist policymakers in determining appropriate responses. The lack of expected patterns may represent alternative exposure routes (i.e. food contaminants, beauty product use). Since phthalates have a short half-life, we also expect that we may see periodicity corresponding to periods when factories are active.

Sampling effort. Phthalate concentrations will be measured from a wide number of samples distributed throughout the city, and then measured monthly. We will do specific experiments once source locations have been identified to measure short-term dynamics. If regulations are put into effect, we will measure their impact by sampling more heavily before and after regulations go into effect.

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[85] Bamai, Y. A., et al. (2014). Exposure to house dust phthalates in relation to asthma and allergies in both children and adults. *Science of The Total Environment*, 485–486(0), 153–163.

[86] Ferguson, 2014. Op.cit.

[87] Gevao, B., et al. (2013). Phthalates in indoor dust in Kuwait: implications for non-dietary human exposure. *Indoor Air*, 23(2), 126–133.

[88] Ying, 2011.

[89] Wittassek, M., & Angerer, J. (2008). Phthalates: metabolism and exposure. *International Journal of Andrology*, 31(2), 131–8.

4.9 CURRENT EXPERIMENTS

4.9.1 Preliminary Studies in Boston

Modeling sewage network. MIT's Senseable City Lab and Alm Lab have formed a collaboration with the cities of Boston and Cambridge in conducting a pilot project modeling the sewage network to further understand the system and produce an appropriate sampling strategy. The collection of biological signatures from the waste water stream requires a physical understanding of the sewage environment as well as chemical and biological understanding of the signals themselves. The sampling strategy for future research applications through the waste water system is in part dependent on both of these aspects.

Geographic Modeling of the Sewage Network Pilot Study. To determine the optimal sampling locations and frequency, we have begun an analysis of the sewage network in Charlestown, Boston, including geographic morphology, network topology, as well as demographic distribution, in conjunction with waste water loads over time, to propose and validate a model which informs waste water sampling and correlates to target population samples. Additionally, we are analyzing flow meter data from 30 devices to develop an initial model of the flow and mixing of waste water through the sewage network over time. Finally, we will be carrying out a tracer study, tracing the path of tracking devices through the network to further validate our modeling. We have been testing algorithms that pack this model into an automated sample access point selector, capable of outputting access points for sampling catchment areas determined by optimizing for population sample size, overall waste water load, area, and demographic parameters of age, ethnicity, and income. (Figure 13)

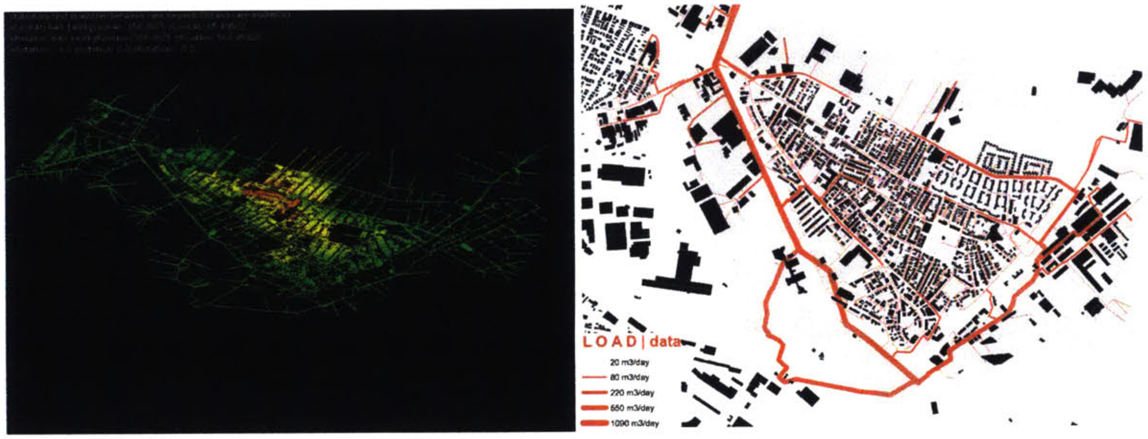

Figure 13. Geographic Modeling of Sewage System, Charlestown, Boston. (Top Left) Analysis of the tributary sewage map and (Bottom Right) load modeling of the waste water system in relation to built square meters. (Image Credits: Aldo Treville, Alexander Beyli)

Viral detection pilot study. The team collected 2L samples from October 2013 to March 2014 at a single point in downtown Boston. Every sample was collected on the same weekday and at the same time (9:00 am) and was processed the same way. Each sample was passed through 1.2 uM, 0.8 uM and 0.2 uM filters in tandem. Then the liquid is concentrated down from 2L to about 10 mL and stored at -80 degrees celsius. Nucleic acids (DNA and RNA) were extracted with the Qiagen blood and tissue extraction kit as reported.[90] Influenza was measured by qPCR and the overall viral diversity was assessed with a microarray chip.[91] One sewage sample was inoculated with influenza A to serve as a positive control for the detection methods and it indeed was detected with our methodology. Figure 14 depicts the number of sequence hits against the influenza genome in the control experiment. Most of the influenza genome was covered by high-confidence hits (purple triangles) showing that detection is robust. In addition to the positive control, two other samples were positive for influenza A: one collected on October 30th 2013, which was very early in the influenza season and another one on March 5th 2014 was corresponded to the peak of the season. The results show that the team is capable of detecting influenza with the implemented methodology.

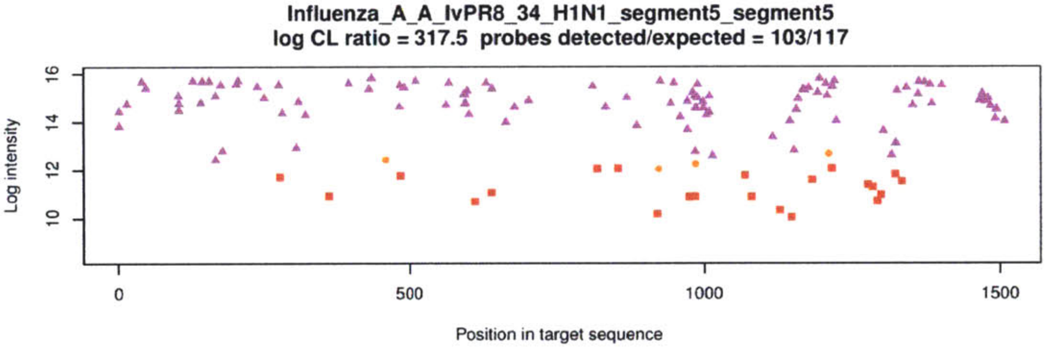

Figure 14. Detection of influenza A in control experiment. Intensity of sequence reads across the influenza A genome as measured by a DNA microarray. Purple triangles indicate high confidence hits; orange circles and red squares indicate low confidence hits. (Image Credit: Alm Lab)

The microarray also detected several human virus very consistently:

- JC polyomavirus is a DNA virus quite widespread in the population and cause illness in immunocompromised people. The result was confirmed by PCR.
- Human astrovirus is an RNA virus that causes gastroenteritis in children and adults.
- Human bocavirus is a DNA virus that infects gastrointestinal and respiratory tracts.
- Sapovirus is an RNA virus that causes acute viral gastroenteritis in adults.

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[90] Cantalupo et al., 2011. Op.cit.

[91] Gardner, S. N., Jaing, C. J., McLoughlin, K. S., & Slezak, T. R. (2010). A microbial detection array (MDA) for viral and bacterial detection. *BMC Genomics*, 11(1), 668.

- Human papillomavirus is a DNA virus mostly subclinical but can result in papillomas or cancers.
- Human adenovirus are DNA viruses have been found to cause a wide range of illnesses, from mild respiratory infections in young children to life-threatening multi-organ disease in people with a weakened immune system.
- Herpesvirus are DNA viruses that include Herpes simplex viruses 1 and 2, varicella-zoster virus, EBV (Epstein-Barr virus), human cytomegalovirus, human herpesvirus 6, human herpesvirus 7, and Kaposi's sarcoma-associated herpesvirus.

Finally, the microarray showed that the most commonly detected viruses are bacteriophages.

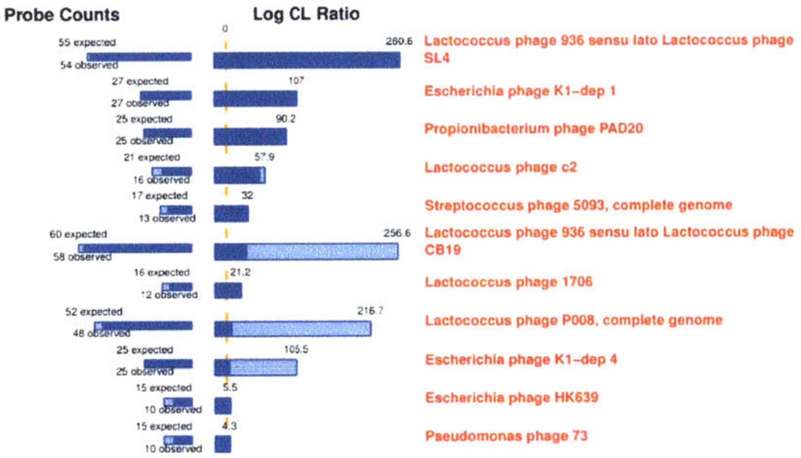

Figure 15. Bacteriophages detected in sewage. Top hits in sewage sample collected from Boston are a diverse set of bacteriophages, as measured by DNA microarray. (Image Credits: Alm Lab)

Sampling. The sewer at the intersection of Beacon and Dartmouth Streets was sampled 11 times between October 31st 2013 and March 19th 2014. All samples were taken on weekdays at 9 am sharp. Samples consisted of three liters of sewage water, which were immediately put on ice and transported back to lab.

Virus concentration: Two liters of sewage water were filtered through 1.2, 0.8 and 0.2 microns filters to remove debris and bacteria. The outflow was then concentrated down to less than 5 mL with a cross-flow filtration system. Concentrated samples were stored at -80 C.

Virus detection: Nucleic acids were extracted from 1 mL of concentrated sewage with the Qiagen Blood and Tissue DNA Extraction Kit without addition of RNAse. To test for influenza virus, RNA was extracted, reverse-transcribed and tested with qPCR and the LLND microarray. The positive control (sewage water spiked with influenza A, filtered and concentrated as the rest of the samples) was positive by both qPCR and microarray. Samples from Oct. 31st 2013 and March 5th 2014 were positive for influenza A by qPCR but the microarray did not pick up the signal. The microarray (preceded by reverse transcription) was successful in detecting dsDNA, ssDNA and (+) ssRNA viruses. The first round of Illumina sequencing (without reverse transcription step) detected only dsDNA viruses, as expected. Further sequencing and qPCR results are on the way. (Table 1)

Virus	**Replication site**	**Genome type**	**Envelope**	**RT-PCR + LLNL Microarray**	**Illumina DNA sequencing**
JC polyomavirus	Kidneys and urinary tract	dsDNA	No	Yes	Yes
Adenovirus	Multiple, some infect GI-tract	dsDNA	No	Yes	Yes
Papillomavirus	Body surface	dsDNA	No	Yes	Yes
Herpesvirus	Mucoepithelial	dsDNA	Yes	Yes	Yes
Astrovirus	GI-tract	(+) ssRNA	No	Yes	No
Bocavirus	Respiratory and GI-tract	ssDNA	No	Yes	No
Sapovirus	GI-tract	(+) ssRNA	No	Yes	No
Varicellovirus	Nervous system	dsDNA	Yes	No	Yes
Cytomegalovirus	Epithelial cells	dsDNA	Yes	No	Yes

Table 1. Human viruses detected by untargeted assays in the Boston pilot study. (Table Credit: Mariana Matus)

4.9.2 Current Studies in Cambridge

To establish the fundamental understanding needed to work with sewage, a few aspects need to be established, primarily (i) how signals in the sewage vary in time, (ii) how do signals in the sewage vary in space, and (iii) how to normalize the signals detected to interpret their relationship with the population. These are the primary aims of the next experiments we have designed.

Additionally, we look at various signals, including target pathogens of interest. We established a list of pathogens of interest with the Boston Public Health Commission and the Cambridge Public Health Department, which guide our targeted assays in the following experiments (Table 2). We also consider a broad sweep of metabolites in an effort to better understand what metabolism-related signals are present in the sewage, their limits of detection, and the potential usefulness of such signals to learn about human health.

Virus	Disease	Stool Shedding Time	Stool Shedding Quantity	Virus Envelope	Precedence for detection in sewage
Norovirus	Acute gastroenteritis, all ages	13 – 56 days post-infection [10]	Up to $1.64e10^{12}$ genomic copies/g stool [10]	No	Yes [15]
Rotavirus	Gastroenteritis, mainly in children	1 – 28 days post-infection [11]	$1.7e10^{9}$ genomic copies / g stool [11]	No	Yes [15]
Enterovirus D68	Severe respiratory illness, mainly in children	Unknown. Several weeks or months for other enteroviruses [12]	Unknown	No	No, but other enteroviruses have been observed [16]
Hepatitis A	Acute liver disease	1 to 7 weeks post-infection	10^{8} RFU/g stool	No	Yes [15]
Hepatitis E	Acute liver disease	10-121 days post-infection [13]	Up to $2.0e10^{7}$ genome copies/ml stool supernatant [13]	No	Yes [15]
Influenza	Mild to severe respiratory illness	1-6 days post-infection [14]	$2.5e10^{4}$ RNA copies / g stool	Yes	Yes but at very low concentrations [17]
Poliovirus**	Temporal or chronic paralysis in < 1% of cases, mostly children	3-40 days post-infection [4]	Up to $2.3e10^{4}$ PFU/g stool [4]	No	Yes [5]

Table 2. Viruses that have been previously detected in sewage, and that are of interest to the Boston Public Health Commission (personal communication). *BPHC is not interested in poliovirus surveillance. The virus is included in the table for comparison purposes since it has been successfully been measured in sewage for surveillance purposes. (Table Credit: Mariana Matus)

24 Hour Experiment, Cambridge. The aim of the first experiment in Cambridge is to learn about the variability of signals in the sewage over a 24 hour period, and establish assumptions about how these correlate with human behavior. To do this, we designed the experiment so that samples would be collected in the same location once an hour, for the duration of an entire day. Mariana Matus led the detailed design and implementation of this experiment, which involved over 30 researchers from the Alm Lab and Senseable City Lab. The team of researcher that volunteered was required to attend EHS training, as well as internal training designed by Mariana to become familiar with and practice the experiment protocol.

The location was selected based on the consideration that it would be as homogeneously residential in program as possible, the upstream sewage catchment area should represent over 1000 people, and that the location is safe and convenient for the manual collection (it should be conveniently close to the Alm Lab, and approved by the city in terms of traffic safety). The location selected is at the corner of

Portland and Washington in Cambridge (Figure 16), a catchment area that is 91% residential land use with a population of 4347 residents.

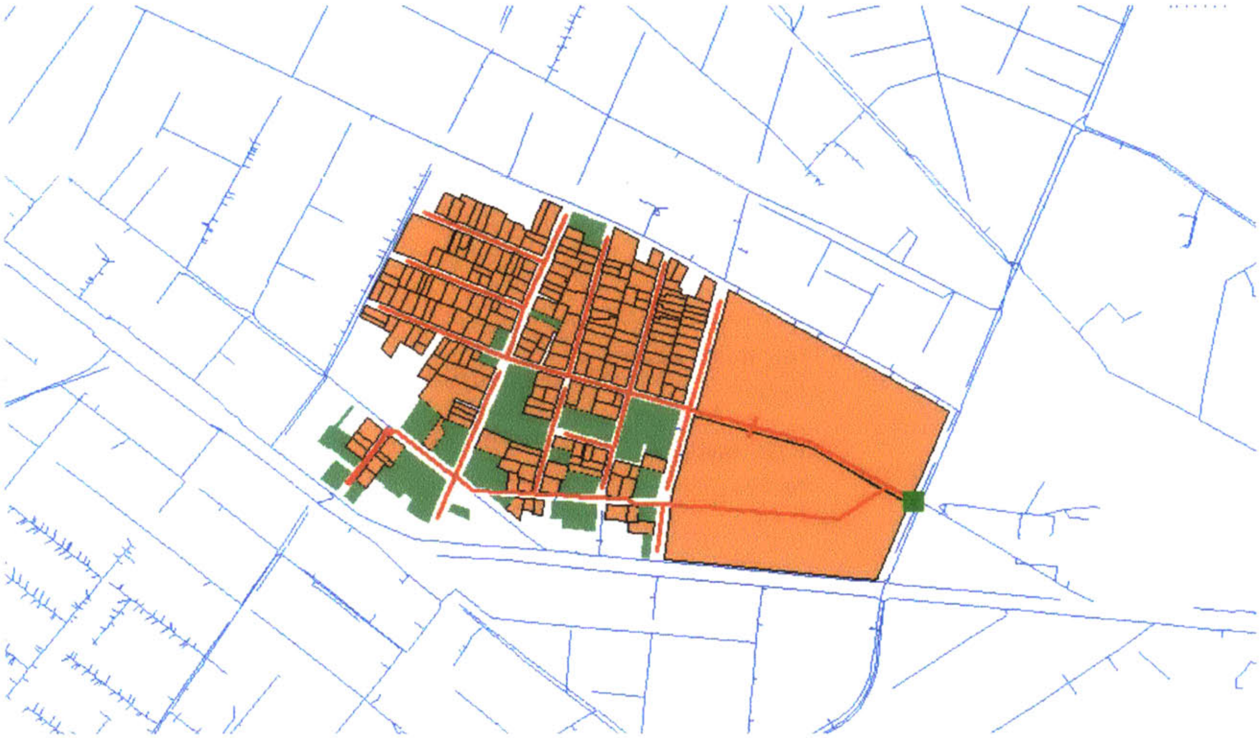

Figure 16. Sampling Location at the corner of Portland and Washington in Cambridge, and Upstream Catchment area (Image Credit: Newsha Ghaeli)

On April 8th, 2015, the team collected hourly samples over 24 hours from this site. Every hour, the sewage flow rate was measured and one liter of sewage water was collected and immediately transported on ice to lab. Sewage water was be filtered through a 0.22-micron polycarbonate filter. The filter was frozen at -20 C in RNAlater to preserve bacterial DNA. 100 mL of the filtered outflow was acidified to pH 2 with 1 M HCl and frozen at -20 C in polycarbonate tubes to preserve metabolites. Fecal content was determined by the amount of *Bacteroidales* as measured by qPCR. *Bacteroidales* quantification is the state of the art fecal bacterial indicator in water bodies: the genus is abundant in animal gut microbiomes and it is strictly anaerobic, which means it cannot reproduce outside the human body.[92] The time of the day at which *Bacteroidales* levels peak will help determine the hour at which we sample in future experiments. Additionally, samples are now being analyzed by untargeted assays to characterize how the bacterial and metabolic profiles change throughout the day. Our

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[92] S. Bae and S. Wuertz, "Survival of host-associated bacteroidales cells and their relationship with Enterococcus spp., Campylobacter jejuni, Salmonella enterica serovar Typhimurium, and adenovirus in freshwater microcosms as measured by propidium monoazide-quantitative PCR.," *Appl. Environ. Microbiol.*, vol. 78, no. 4, pp. 922–32, Feb. 2012.

collaborators at the WHOI will carry out untargeted metabolomics, and researcher Mariana Matus will generate 16S rRNA sequencing data.

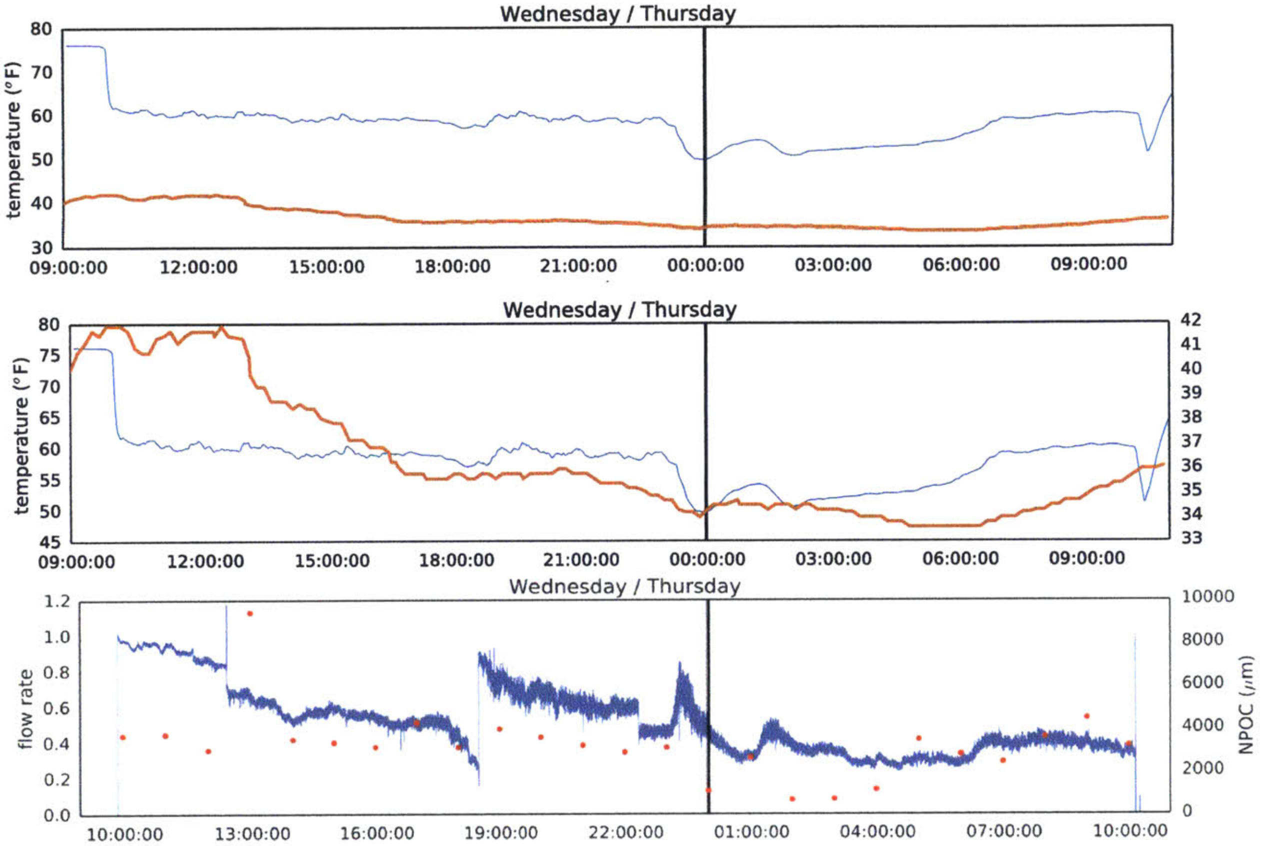

Figure 17. Physical data collected during the sampling experiments shows variability in temperature and flow rates. This will be used in conjunction with the biochemical assays to correlate signals in the sewage with trends in human behavior (Table Credits: Mariana Matus)

10 Location Geographic Variability Study. In order to understand the variability in signals over space, we designed an experiment that will take simultaneous samples at 10 locations across Cambridge. These locations will be grouped by similarity in size and land use so that they can serve as a basis for comparison of the signals of interest. The areas will include 6 residential, 2 biotechnology, and 2 education (MIT & Harvard) catchments. The time(s) of sampling will be selected based on the results of the above 24 hour experiment.

Further experiments. Further experiments have been suggested, and will be confirmed in response to the results from the above two experiments. These will include experiments to understand day-to-day stability of key biochemical indicators, as well as understanding the variability between locations directly upstream from one another. Generally, the expectation is that site-specific readouts are stable over a short period of time, that demographically similar regions exhibit similar signal readouts, and that differences in signals between demographically dissimilar regions exceed their temporal fluctuations.

Figures 18. Sewage-sampling station during the 24h experiment

Figures 19. Sewage samples collected at various time points show differences in coloration and consistency, likely as a reflection

4.10 CONTRIBUTIONS

Microbes mediate interactions between the human body, the made and the natural environment. Underworlds further explores this emerging continuity between the made and natural. In this case, at a unique intersection between the bacterial ecologies that reside within, and the bacterial ecologies that we have created in the vast underworlds of the modern sewage system. This extension of the collective gut contains a wealth of information that can facilitate public health and our understanding of disease.

Underworlds is a first of its kind proof-of-concept system aiming towards realtime epidemiology. The ability to tap in to the vast information present in our cities' bowels suggests a transformation in the methods available to public health. In addition to be able to track disease in realtime, Underworlds will provide information on public health and nutrition policy, biosecurity surveillance, and advanced sanitation methods.

The establishment of the scientific and technological methods to enable an urban scale system interfacing physical and digital components with the microbial ecology living in the collective urban gut is the primary contribution of Underworlds to date. The ability to track signals in the sewage at a high spatio-temporal resolution is an essential part of the system, much like Waterfly. Underworlds goes further to carefully consider a complex set of potentially useful signals, and understand their significance over time and space. This translation from microbiological phenomena to human scale events is an essential component to any similar future system. In pursuing this, Underworlds lays the methodological foundation for a large scale cyber-physical system.

With additional developments in the science and technology, realtime city-wide biosensing will provide transformative insights for public health, and open the possibility to consider many other applications, both infrastructural and personal. As a large scale multi-year project which has only recently started research activity, its main contributions have been in structuring the research agenda and galvanizing an interdisciplinary team of investigators to its ambitions. Over the next three years, as this research program is implemented, it will lay the early foundations for investigating cyber-biological infrastructures.

4.11 ACKNOWLEDGEMENTS

Figures and texts describing the project, development methodologies, experiments, demos, and results are taken from a proposal I co-authored for submission to the Kuwait-MIT CNRE, which has awarded the funding of the project, as well as from research done in collaboration at Senseable City Lab with Kevin Kung, Clara Cibrario, Aldo Treville, Alexander Beyli, Stanislav Sobolevsky, Newsha Ghaeli, Franco Montalvo and at the Alm Lab with Mariana Matus, Ilana Brito,

4.11.1 Project Team

Underworlds is a collaborative project between six labs at MIT, and two institutes in Kuwait, with a team of researchers that is evolving and growing as the project gains pace. From the conception of the idea in spring 2013, I have co-lead the research of the project, first under the supervision of Prof. Carlo Ratti in my role as Research and Development Lead at Senseable City Lab, and later under the supervision of Prof. Eric Alm as a Research Lead at the Alm lab. Throughout this entire process, my main co-conspiritor on the research was Mariana Matus, who has contributed substantially to my understanding of microbiology and provided many contributions documented above in this chapter. The key contributors to the project are listed below, however the team is in flux across many labs, and there are many names which deserve recognition which are omitted here.

This is a partial credit list of highlighting key researchers involved with the project

MIT Investigators

Carlo Ratti
Senseable City Lab

Eric Alm
Alm Lab

Daniela Rus
Distributed Robotics Lab

Elfatih Eltahir
Eltahir Research Group

Jon Runstadler
Runstadler Lab

Martin Polz
Polz Lab

MIT Researchers

Yaniv J. Turgeman
SCL + Alm Lab

Mariana Matus
Alm Lab

Newsha Ghaeli
SCL

Ilana Brito
Alm Lab

Franco Montalvo
SCL

Youjin Shin
SCL

Kuwaiti Investigators

Sameer Al-Zenki
KISR, Food and Nutrition

Kazi Jamil
KISR, Food and Nutrition

Tarek Al-Ati
KISR, Food and Nutrition

Ashraf Ahmad
KISR, Biotechnology

Entesar Husain
KU, Pediatrics

Rawa Aljarallah
KU, Civil Engineering

City of Cambridge

Sam Lipson
Jim Wilcox

City of Boston

John Sullivan
Patrick Greeley
Richard McKinnon

Conclusions

Technologic mediations — from the stone-axe and language, to computers and the internet — have extended our reach in the world, as well as our ability to understand it, act in it, and construct meaning within it. As such, the architectures that mediate 'what we are sensitive to' become in themselves rich domains for creative intervention, with the possibility to shape the environment we each live in. As humanity's sphere of influence has rapidly expanded through technology, and is extending beyond the made to the living, our impact on the world around us and our selves is increasing dramatically. At the same time, our made systems have scaled to such a degree that our technologic mediations have distanced us in both time, space, and size, away from many of the events that shape our world and our individual lives. We are abstracted from them by scale.

Simultaneously, natural and human-made systems are exchanging methods. A hybridizing ecology of made and natural processes opens new frontiers for intervention and mediation facilitated by flows of information across scales and substrates. The convergence in design strategies for generating interconnected objects and beings is bringing together evolution by design and evolution by selection. Such design by evolution and evolution by design is instantiated in novel applications in biological engineering, inviting the consideration of designers to seek new tools, methods, and materials.

In this thesis I have proposed a novel 'cyber-biological' system, one that utilizes the pervasive, endemic, and editable nature of microorganisms for generating feedback from events otherwise imperceptible to us in space and time. This system couples distributed physical and digital processes with networks of microbes which can serve as natural or programmed agents, with distributed sensing, computation, processing, and actuating abilities. The motivation is to extend the reach of our senses, and therefore our semiotic sphere, to access the phenomena that shape our hybridizing human-made and natural world.

The projects I have shared were conceived as cyber-physical systems. Their limited approach towards the incorporation of microorganisms as an active component of the system reflects the current limitations of the technology and methods available for real-world applications. However, each of these projects expands the frontiers of digital-biological interfaces, the scientific scaffolding needed to detect and interpret biological signals, and the ability to communicate these invisible events in realtime to a user. As such, they each have their yet unfolding contribution, as well as a more general contribution to speculation, initial exploration, and early application of cyber-biological systems.

The Waterfly and Underworlds projects help consider several cardinal features of the systems suggested by this thesis. Beyond their immediate applications, their central goal is to expand the human sensing across time and space, sensitizing to otherwise imperceptible events in our surroundings that affect our lives. Continuously collected, high-resolution, realtime, spatio-temporal data mediates a representation of the environment — natural or built, external or internal — that allows a performative process between human, digital, and biological agents.

In both cases, challenges arise due to the imperceptible scale of the processes involved. Taking water pollution as an example, even though temporally acute events are easier to identify, contamination can be the product of aggregate contributions over years. Spatially, non-point contributions are highly distributed across a broad geography. The physical material scale, whether minute chemical particles or massive city scale storm water overflows, defy conception. The processes leading to ecological imbalance are distributed, the aggregation of many individual behaviors across space and time.

Together these barriers of scale construct intangible processes, both in the creation of the 'problem,' and in the application of the 'solution'. The interaction of these out-of-human-scale dimensions not only challenges human perception, but also individual intervention. The strategy of Waterfly in this case is to mediate contamination events with distributed realtime feedback, creating a continuum of meaningful information between the substrates of the pollution process.

Such interpolation of environmental phenomena to human conception is shaped by the mediating architecture. The distributed realtime capabilities of cyber-physical technologies suggest the possibility of auto-regulation and adaptation. As can be seen in Waterfly, the intent is not a digital system that attempts to automate the regulation of a natural ecology according to a pre-assumed optimum. While assumptions are embedded into the design of the system itself, the aim is to generate the possibility of a performative process between a person and their environment. By distributing such affordances across our physical spaces, the interactions enacted between people and made sensing objects emerge as a self-organizing process.

The harmful overabundance of cyanobacteria on the Charles River is a product of human activity. This represents a hybrid ecology of interactions between human, made, and biological actors. Realtime information flows between the made and the grown shift these hybrid processes towards a self-organizing-like dynamic. The ability to 'read' the state of cyanobacteria positions them as mediators of events in the river. The same considerations apply for Underworlds. Realtime feedback of information sourced from living distributed agents in the form of the sewage microbiome inform about large scale processes of human health.

The design of a cyber-biological, in material and computation form, and in the processes it actuates, aims to embed a sensory process extending between the agents of the hybridized environment. In this manner they can be imagined as extensions of the human senses towards the world. By providing realtime signals form the biological ecosystem of the sewage, the Underworlds project essentially extends the human enteric nervous system into our collective urban gut. These mediations act as extensions of our sensitivity to events that shape the increasingly hybridized environments around and inside us. The ability to extend this sensitivity between to microbial communities provides access to phenomena across the built and natural environments. The ability to actuate these communities can provide new possibilities for human intervention.

Scientific methods for understanding the structure and behavior of microbial communities and the rapid development of breakthrough technological advances in sequencing and synthesizing DNA, as well as other biological elements, allow the imagining of full scale cyber-biological systems. The rhetoric proclaiming the 'Internet of Everything' is expanding to include not only objects and structures but also entire microorganismal ecologies. In the near future perhaps this will be thought of as the 'Internet of Living Things'. For my interests, the ultimate aim of these mediations is to allow individual access to the events in the hybridized world that shape our lives. The methods and technologies enabling microbial mediations outside of the lab are in development, and as such, these initial projects have served as a first step in a larger program of research.

5.2 FUTURE RESEARCH

I have been fortunate to have benefited from the vision and work of many pioneers at MIT in investigating my ideas, and hope they inspire others to explore the intersection of design, computation, and biological engineering. The projects I shared are a first step towards investigating design as a central force in the co-evolutionary process between made and grown things, and a step towards a greater vision of extending human sensitivity to hybridized environmental processes through microbes. Study and research in the Departments of Architecture (ARCH), Urban Studies and Planning (DUSP), Civil & Environmental Engineering (CEE), and Biological Engineering (BE) has allowed the development of such an integrative agenda.

My motivation to explore microbes as sensitizing mediators crystallized with the application proposal I submitted to CEE in the summer of 2014. Since that time I have been immersed in the study of microbiology under the guidance of Prof. Eric Alm. More than ever, I am inspired by the immense possibilities for harnessing microorganisms, and motivated to consider the hybridized processes they enable. The following projects represent research trajectories I am currently pursuing to further investigate the ideas presented in this thesis.

Everybiome

Mariana Matus, Manasi Vartak, Prof. Eric Alm

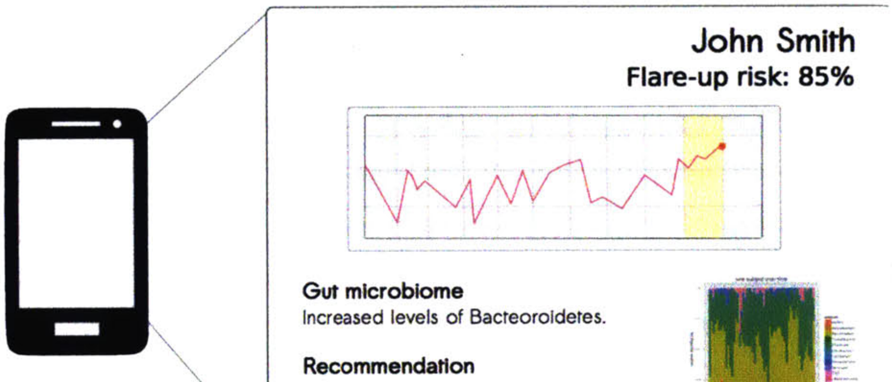

Figure 1. Continuous monitoring of personal biomarkers in the gut (Image Credit: Mariana Matus)

Everybiome aims to create tools for collecting and analyzing personalized health biomarkers in the home environment. As described in this thesis, microbes in stool are one of the most information dense sources of continuous, passive, non-invasive health data. Our technology provides sample collection that is passive and invisible for off-site testing. The device is paired with an analytics platform, which will enable precise and preventative therapies, personalized diet & baseline health monitoring, and real time epidemiology. We are initially targeting chronic conditions where continuous monitoring can significantly improve care management and patient outcomes to reduce the burden of disease. Our team will continue the scientific research at the Alm Lab, and further development through the MIT Martin Trust Global Skills Founder Accelerator over summer 2015.

Biovisualization

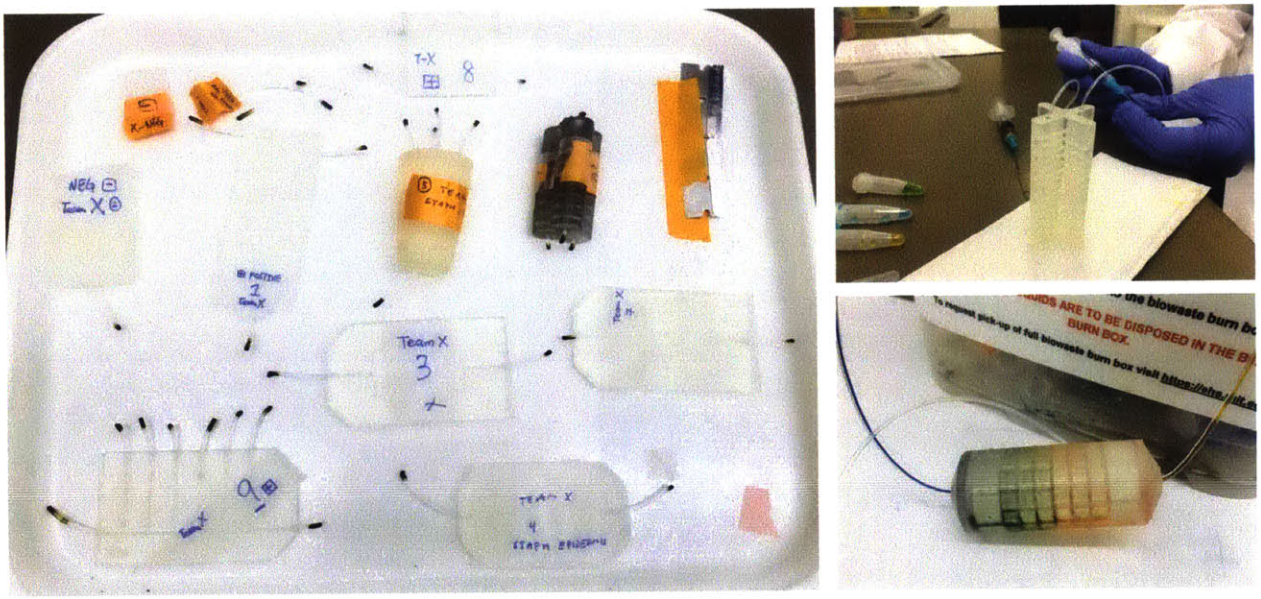

Figure 2. Prototype 3D printed bio-reactors made by Team X: Dan Chen, Noa Machover, Mariana Matus, and Sarvesh Varma (Image Credit: Dan Chen)

Microbial community structure is highly specific to its environment. Environmental changes are reflected in the response of these communities, and can be studied to infer events of interest to humans. Engineered bacteria can adopt additional functions that may be of use to detecting phenomena of interest to humans, and responding in ways that communicate for the human senses such as sight or smell. Considering these properties, the aim of this project is to utilize bacteria in specially designed microfluidic devices as living data visualizations of environmental phenomena. This is similar to methods of culturing bacteria in the lab. However, these microfluidic devices are designed specifically to exploit the desirable behaviors of a microorganism in response to environmental phenomena. The devices should be designed so that they allow intuitive interpretation, and are modular components that can be configured on a desk or mounted on a wall as a dynamic biovisualization. Initial candidates for these visualizations include air quality, temperature, or allergens, showing how they historically develop over time. These small bioreactors will reveal phenomena that are otherwise imperceptible to us, but that the microbes are sensitive to.

During the MIT's 2015 Independent Activity Period, I participated in Fluidics for Synthetic Biology taught by Dr. David Kong, and was able to work on an initial investigation of 3D printed devices. Our team included Dan Chen, Noa Machover, Mariana Matus, and Sarvesh Varma, and the images above reflect in great part their contributions.

Biological Homeostat

Figure 3. Illustration of the proposed Biological Homeostat

Some of the most intriguing emergent properties of natural systems include auto-regulation and homeostasis. These are fundamental properties of living things. In 1952, the cybernetician Ross Ashby built an electro-mechanical machine he called the homeostat, which was an investigation of these properties in a made machine. My proposal for a Biological Homeostat suggests a "toy" or model system for exploring the cyber-biological dynamics of feedback, auto-regulation, and sustainability. It is also an environment for testing features of microbes to process information and actuate their environment in dialog with human agents. This is imagined as a set of linked chemostats, with a digital-biological interface that will allow human interaction with the system.

To think about the architecture and dynamics of this system, Nezar Abdennur, Remi Trachet, and I modeled and simulated similar type of feedback structure in the form of genetic circuits in bacteria. This was done through the Principles of Synthetic Biology course taught by Prof. Ron Weiss and Prof Adam

Arkin in Fall 2014. The next steps involve the study of translating such a model faithfully to a real-world biological implementation. The full paper is provided in the appendix.

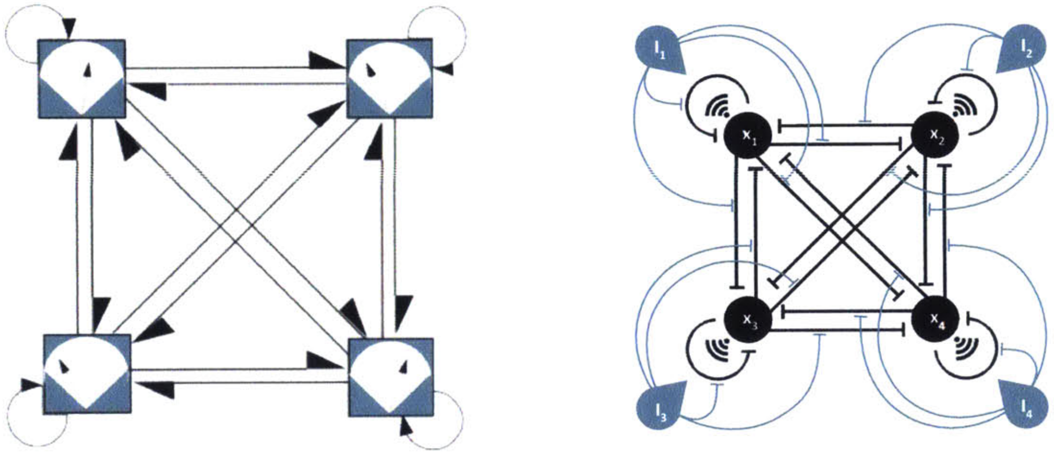

Figure 4. Schematic system diagrams illustrating regulation in Ashby's Homeostat (left) and the Biological Homeostat (right), as suggested in this proposal. (Left Image Credit: *Eldridge A. httpwww.ecila.org)*

5.3 EPILOGUE

Feedback from our environment offers an opportunity to tune our individual sensitivity into the phenomena that are of concern to us; to make them meaningful to our lives through individual access and shared participation in them. In a hybridized world, I believe agency can be afforded through the interactive processes between the individual and the living world around and inside ourselves. The motivation that lies beneath this claim looks at a sensitizing-oriented architecture that can complement the event-driven bottom-up service-oriented architecture that currently mediates the 'Internet of Things', and the design of digital technologies. In a hyper-technological context, I argue that as we continue to extend the scope of our technological intervention, we can also extend our immediate sensitivity to the important phenomena of our environments and our bodies. In this way we close the loop, and bring back information about the impact of our interventions across scales. This is not just to raise awareness about ecological processes and our contribution to them, but to allow individuated *performativity* within those processes, in contrast to the engineering approach of optimizing for system *performance.* I believe today's opportunity for sustainability is not to accurately identify the next optimum, but to create rich feedback between the actors participating in the processes of our hybridized grown and made ecology. For designers, there are new methods and potentials in the increasingly crossbred creative processes of evolution by design and design by evolution. Beyond creating automated conveniences, our opportunity lies in facilitating a shift in human intervention from sustainability through optimization to sustainability through sensitization.

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Appendix

A Biological Homeostat: exploring ultrastability in genetic circuits

Yaniv J. Turgeman[*]

[*]Departments: Civil & Environmental Engineering; Architecture; Urban Studies & Planning; Bioengineering | Massachusetts Institute of Technology

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Based on Ross Ashby's 1952 Homeostat machine, a cyber-biological system architecture is proposed with the intent of investigating its properties of stability and online self-optimization. Such behavior would reduce the reliance on precise upstream design, and may allow a biological circuit to better adapt to changes in its environment. Ashby's circuit is explained, and a biological interpretation of the homeostat model is proposed and simulated, here called the Represseostat. Analysis of the stability performance of the Represseostat is compared to that of a similar but uncoupled system. The Represseostat is found to have unique timing coordination and produce a tight protein concentration distribution, however is inferior in terms of time to achieving stability. Future designs are considered to improve the results of this proposal.

Cyber-Biological System | Homeostat | Self-Organization | Stability

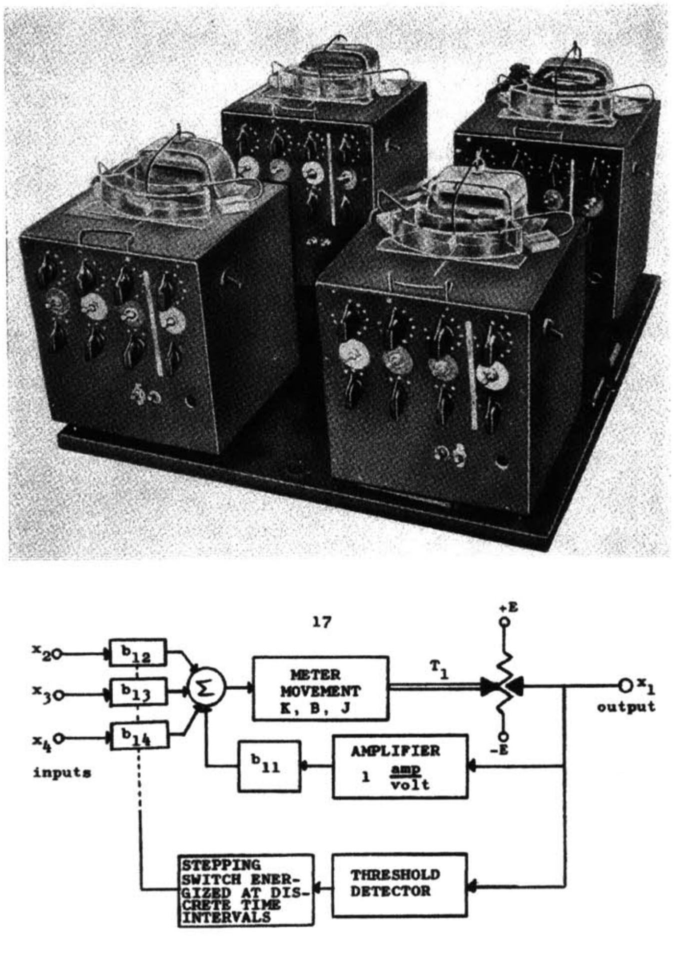

Figure 5

Block Diagram of one unit of the Homeostat.

Figure 1 - Ashby's Homeostat and circuit schematic.[2]

Introduction

Design inherently requires speculation. Much of the designers effort goes in to anticipating the outcome of a given design, especially if the objective of is to answer to a clear problem statement and the results are to be quantifiably measured, as is the case in most engineering applications. One approach to this is to describe and model the situation as coherently as possible, making careful choices in the representations selected, and the benefits of their constraints. "Top down" parametrizing and optimizing such a model to adapt to dynamic, and many times unclear, conditions can prove to be difficult.

This proposal for a cyber-biological system[i] attempts a regulatory architecture intended to enable a biological circuit to self-optimize and adapt, alleviating some of the limitations of very precise and fixed upstream design.

Background

In the mid-twentieth century, the rise of computation and information sciences enabled new speculation about open ended systems and ideas such as self-organization, adaptability, and learning were explored, setting the stage for much of todays work in adaptive systems and complexity. One such trajectory began with Cybernetics, or the science of communications and automatic control systems in both machines and living things.

In 1952, the Cybernetician Ross Ashby built an electro-mechanical machine he called the homeostat. Below is a

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[i] I am calling cyber-biological system (CBS) a system of collaborating computational and biological elements controlling physical and biological entities. This relates to the established cyber-physical system (CPS), which is a system of collaborating computational elements controlling physical entities. Today, a precursor generation of cyber-physical systems often referred to as embedded systems can be found in many applications. In embedded systems the emphasis tends to be more on the computational elements, and less on an intense link between the computational and physical elements.[1]

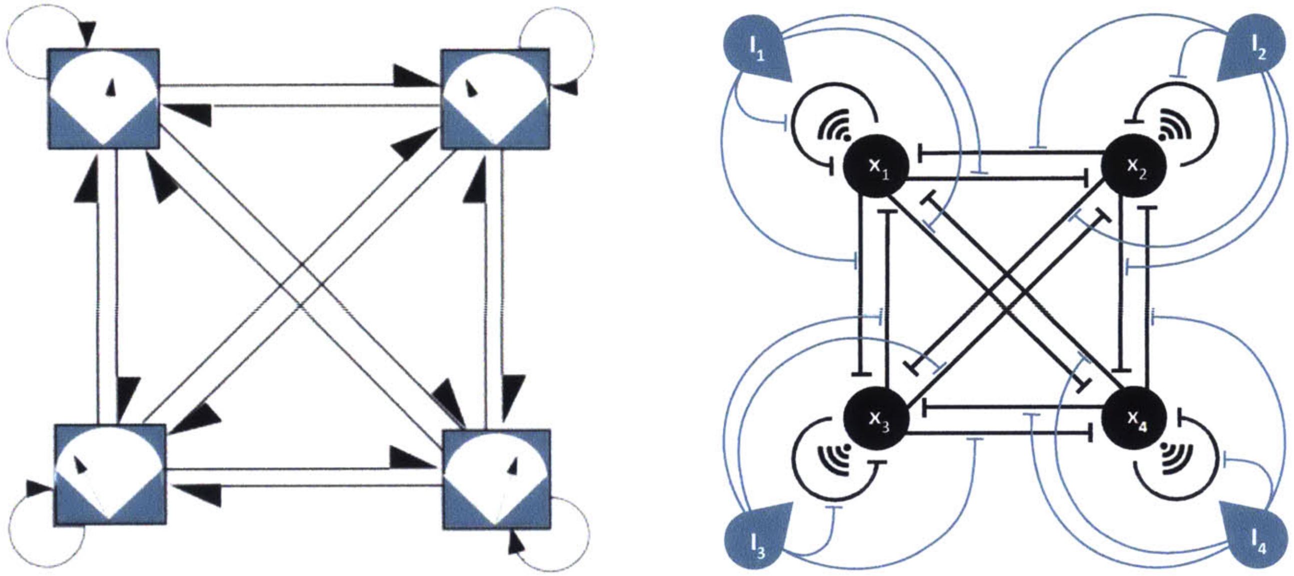

Figure 2 - *Schematic system diagrams illustrating regulation in Ashby's Homeostat (left)[2] and the Biological Homeostat (right), as suggested in this proposal. Four "functional units", each connected to every other in a regulation network of negative feedback. The output of each unit will consist of repressor levels and a fluorescent reporter. These outputs will be affected by the sum of the output of transcriptional repressors from all the other units. The fluorescence output will be measured online by a digital controller. If the output of any unit exceeds its pre-specified fluorescence control range, the corresponding inducer concentration in the system is randomized.*

description provided by Eldridge describing Ashby's motivations and the machine he built:[2]

One of the conundrums that preoccupied Ashby, was how a system (biological or mechanical) could be at once state-determined, and yet adapt to a changing environment and learn. Ashby (1952) proposed that one of the key mechanisms underlying adaptive behavior is homeostasis, and provided a concrete, physical device to demonstrate his theoretical notion of ultrastability.

Ashby's machine consisted of four units with a pivoted magnet on top of each. The angular deviation of each magnet's position representing the essential variables which were to be maintained within a certain angle. Each magnet had a wire hanging off it that trailed in a trough of liquid, creating a direct relation between the angle of deviation and the current running through it. These electrical connections model the primary feedback, where any one unit can be conceptualized (arbitrarily) as representing either the environment, or the sensorimotor system of an agent in that environment. Each unit also had a recurrent connection. The current on each was modified by passing it through a commutator and potentiometer which determined the polarity and proportion of each input which is passed. These act as parameters to the system, implementing a secondary feedback which was controlled by a uniselector on each unit. If the angle of deviation exceeds 45 degrees, new values are assigned to the commutator and potentiometer at each unit.

"When these parameters are given a definite set of values, the magnets show some definite pattern of behavior; for the parameters determine the field, and thus the lines of behavior. If the field is stable, the four magnets move to the central position, where they actively resist any attempt to displace them. If displaced, co-ordinated activity brings them back to the centre. Other parameter-settings may, however, give instability; in which case, a 'runaway' occurs and the magnets diverge from the central positions with increasing velocity - till they hit the ends of the trough" - Ashby (1952)

By a process of trial and error, the machine is able to maintain its essential variables within specified limits. Ashby also demonstrated that the machine could exhibit basic reinforcement learning, adapting to alternate environments and presented it as an example of basic self-organization.

Motivation

The generalized motivation for this is to explore in a biological system the possibility of implementing what Ashby called ultrastability. In this case to reliably control the expression of several proteins of interest, maintaining them simultaneously in a desired range. Rather than independently fine tuning each unit, such a system (comprising of the controlling device and the synthetic gene network) could self-optimize online rather then depend on precise upstream design. The behavior of such a system can not be easily anticipated upfront, and by simulating and investigating this model insights will be gained as to the capacity of this regulatory architecture to enhance the behaviors of a bioengineered system.

This can also be thought of more practically. For example, it may be useful in metabolic engineering where one might want to maintain constant concentrations of a metabolic enzymes in a pathway in the face of fluctuating and gradually changing cellular and environmental conditions.

Figure 3 - *A gene regulation schematic of one of the functional units (x1). Each unit is composed of four constructs (x1.1 - x1.4). Shown for each construct are the inducer input, and the transcriptional repressor (TetR) and reporter output (GFP), which are specific to this unit.*

System Description

The Homeostat model (Figure 1; Figure 2, Left) is explained by Eldridge as follows:

Ashby's machine can be modeled as a network of i units, each connected to j other units (Figure 2, left) where the output of each unit is updated according to the weighted sum of the output of all other nodes (these weights modeling the potentiometers and commutators described by Ashby). In this simulation if the output of any node exceeds a pre-specified value, weights connecting units in the network are re-randomized (by the uniselectors).[2]

To implement the Represseostat, a cyber-biological version of this architecture, the biological circuit will have four "functional units", each connected to every other in a regulation network of negative feedback. The output of each unit will consist of repressor levels and a fluorescent reporter. These outputs will be affected by the sum of the output of transcriptional repressors from all the other units. The fluorescence output will be measured online by a digital controller. If the output of any unit exceeds its pre-specified fluorescence control range, the corresponding inducer concentration in the system is randomized (Figure 2, Right).

There are four repressor proteins (x_1, x_2, x_3, x_4) controlled by four functional units. Each functional unit will be comprised of four constructs. Each construct is regulated by a promoter repressible by one of the four repressor proteins (x_1, x_2, x_3, x_4), yet all constructs of a given functional unit drive the expression of a common repressor and fluorescent reporter gene. Each of x_1, x_2, x_3, x_4 will be inducible by unique inducer molecules (Figure 3).

Each of the four repressors could be controlled by a different, orthogonal, inducer molecule, for example as follows:

TetR (pTet)	aTc
LacI (pLac)	IPTG
AraC (pBad)	arabinose
LuxR (pLux)	HSL

These promoters will also be driving four different fluorescence reporter proteins (amCyan, GFP, zsYellow, mCherry)[3] which will be the readouts of the system. For each repressor, the reporter gene will be down stream of the repressor gene, separated by a spacer and a RBS. We can imagine these as polycistronic genes in a bacterial system like *E. coli.*

The expression levels of these repressor proteins will be monitored (imagine an external digital device monitoring each of the four fluorescence wavelengths), with predefined target ranges for their output. Just as with the Homeostat, if any readout (as a proxy for the repressor protein) fluctuates out of its target range, the controller will randomly select a new inducer concentration to the offending repressor. This behavior will repeat in response to each functional unit until the system reaches simultaneous steady state levels of each of the read outs inside the desired ranges.

Therefore each functional unit is comprised of four constructs behaving as an OR gate. The output signal of each functional unit is the sum product of the four constructs.

Model

The system is represented by the following reactions:

$$P_{x_i} + x_j \overset{K_{rep_{ji}}}{\rightleftharpoons} P_{x_i} : x_j \tag{1}$$

$$x_i + I_i \overset{K_{ind_i}}{\rightleftharpoons} x_i : I_i \tag{2}$$

$$x_i \overset{k_{deg_i}}{\rightarrow} \emptyset \tag{3}$$

Assuming that the binding reactions are at equilibrium because they are faster than gene expression, and that mRNA levels are at steady state because mRNA dynamics are slower than protein dynamics (that is, mRNA is produced and degraded at a faster rate), these reactions can be described by the following rate equation:

$$\frac{dx_i}{dt} = \left[\sum_{j=1}^{4} \alpha_i \frac{K_{rep_{ji}}^{n_{rep_{ji}}}}{K_{rep_{ji}}^{n_{rep_{ji}}} + \left[x_j \frac{K_{ind_j}^{n_{ind_j}}}{K_{ind_j}^{n_{ind_j}} + I_j^{n_{ind_j}}} \right]^{n_{rep_{ji}}}} \right] - k_{deg_i} x_i$$

In this equation the x_1, x_2, x_3, x_4, represent the concentrations of the repressor proteins in a cell. I_1, I_2, I_3, I_4, are the concentrations of the corresponding inducers in the media.

For each ODE, the terms in the sum describe the inducible promoter kinetics. α_i is a constant representing the maximum contribution to the overall production rate. The outer Michaelis–Menten term describes promoter repression, and the Michaelis–Menten term nested in the denominator accounts for the inducer repression of the transcriptional repressor proteins.

As the inducer approaches the upper limit, the entire term approaches 1, and gene expression approaches it's maximal rate. As the inducer approaches 0, the maximal production rate is influenced by the total repressor protein.

Analysis

Parameterization & Assumptions

The behavior of this architecture is not easily predicted. A number of idealizations are adopted in order to isolate and investigate the nature of the architecture with the simplest parameters possible. First, a system of four independent repressors under the action of inducer randomization is simulated as a control for stability performance. Then the units are coupled as described above to investigate if the negative feedback network enhances the ability to constrain the concentration of the repressors within a desired control range.

We are primarily interested in assessing the feasibility of the architecture. Modeling a concrete implementation of the system, using a set of literature rate constants related to specific combination of parts, would be the right approach if we find evidence that the architecture is robust. We began by assessing performance in the case where the functional units had fully symmetric kinetics, and introduced shifts in the capture concentrations and attainable control ranges. However, it was empirically shown not to have a principle affect on the stability performance of the system.

However, care is taken to scale constants and select times representative of real world orders of magnitude. Assuming stable repressor and reporter proteins, decay rate is dominated by dilution due to cell division (e.g., *E. coli* doubling time ~20-30 min in rich media or ~200min in other media).[4] The overall process of transcription[4], translation[5], and protein production and maturation[6] can be on the order of several minutes.

To roughly reflect these differences, the α_i were set to 1 and k_{deg_i} were set to differ at most by two orders of magnitude less. The different promoters were assumed to have similar affinity (Hill constants kept in the same range) and mild or no cooperativity (n=1 or 2).

Performance metrics

The criteria to assess the performance of this system are those metrics which characterize the stability behaviors which are inferred from the proposed architecture. One meaningful metric describing such performance is time-to-capture, or the time required for the stabilization of all four repressor levels within the prescribed control ranges. As the process of searching for a common stabilization point is

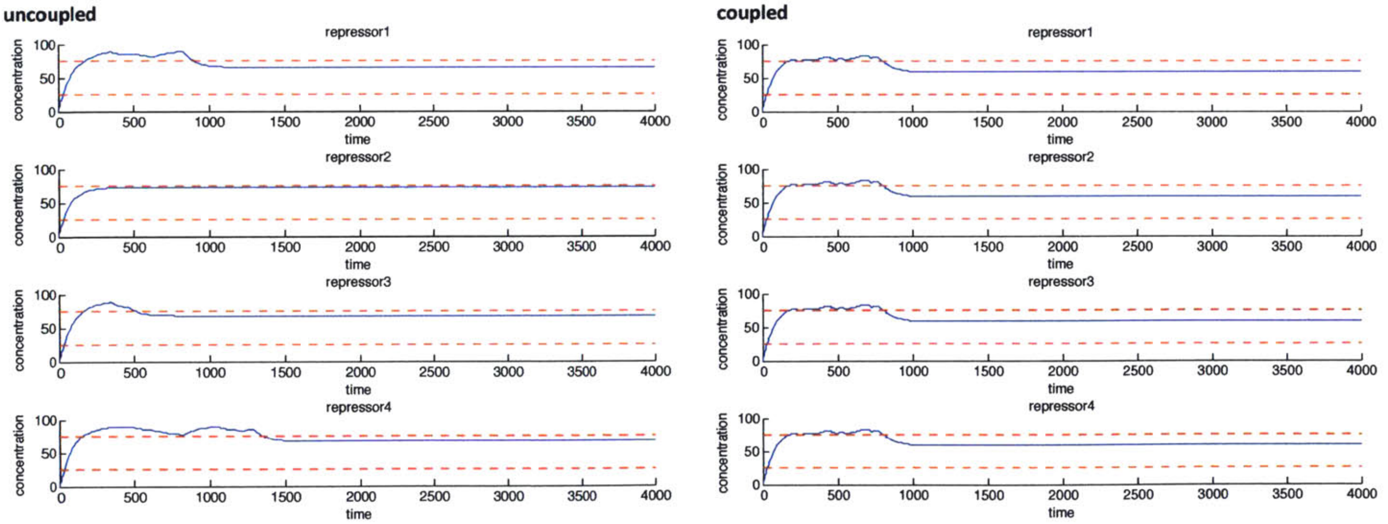

Figure 4 - *Time course plots of the uncoupled system (above) and the coupled system (below) showing time vs repressor concentration. Marked in red are the specified control ranges, defined as +- half of the x_c half-maximum full induction steady state. Note the coordinated time-to-capture in the coupled system.*

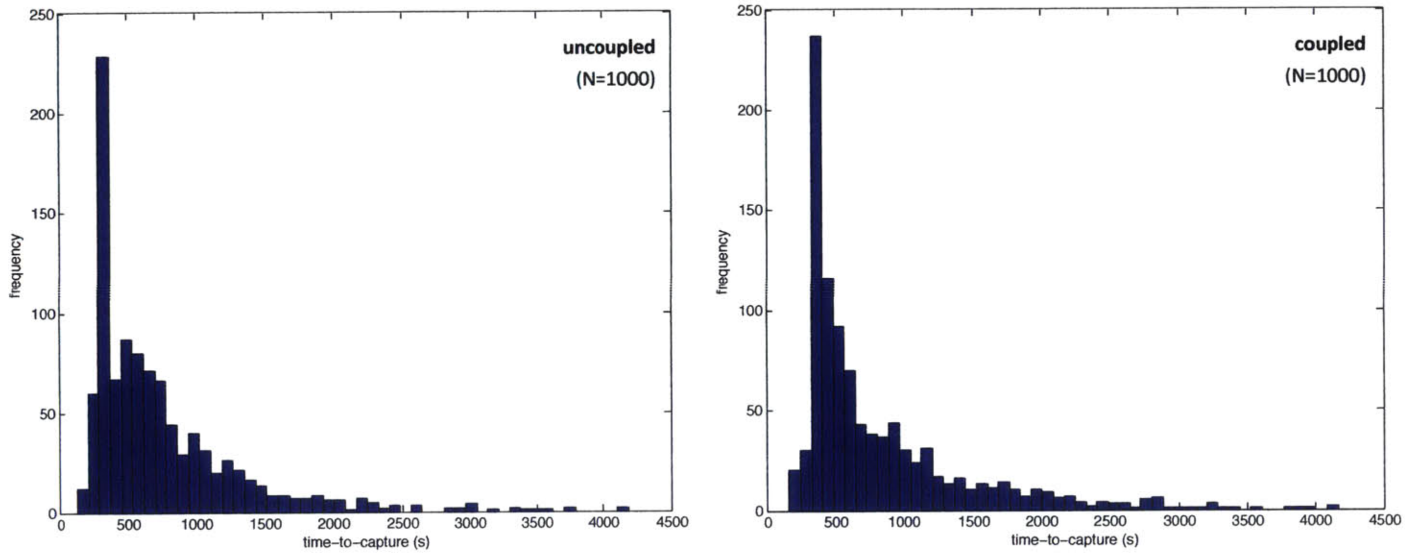

Figure 5 - *Distribution of time-to-capture (t_c) for the uncoupled system (left) and the coupled system (right) behave similarly, both with a peak followed exponential tail (baseline monitoring period defined as 60 s).*

driven by a random variable, it becomes meaningful to consider the distribution of time-to-capture (t_c) as well as the mean time-to-capture across a significant number of simulations. In addition to t_c, the distribution of and mean repressor concentrations at capture (x_c) are considered as well. Stability is defined as the fraction of trajectories that get captured in a "reasonable" time (here, defined as an hour and a half, 5400 s).

Analyzing the metrics

To analyze performance, first the steady state of the system under full induction (defined as having concentrations of inducer far greater then the dissociation constant for the inducer-repressor binding, K_i) is determined. The half-maximum steady state is used as the initial target value for repressor concentration at capture (x_c). An initial control range window is defined as +- half of the x_c half-maximum derived above. At this range, a period of 60 s and simulation time of time of 4000 s is selected, in which both the uncoupled and coupled systems are able to achieve stability for every simulation.

Given this parameterization, the systems (coupled and uncoupled) will be simulated (N=1000) to observe the distribution of t_c and x_c, as well as stability. This is followed by a simulation (N=100) which gradually constrains the control range window for all four repressors, and estimates t_c, x_c, and stability for each range.

Results

Given the parameterization described above for the system, time courses are run showing the baseline behavior of both the uncoupled and coupled systems (Figure 4)

For each such simulation, the uncoupled system arrives at stability at varied times (t_c) and concentrations (x_c), while the coupled system achieves stability in a coordinated manner in both aspects. In a coupled asymmetrical simulation (not shown), a unique x_c is observed for each repressor or set of repressors with identical parameters, however t_c remains coordinated.

Plotting the time-to-capture of multiple simulations (N=1000 uncoupled, coupled) shows a distribution characterized by an early peak followed by exponential tail. The coupled system shows a steeper decay, while the uncoupled system exhibits a small second peak following the largest distribution bin (this observation persisted on each of three repetitions of this simulation) (Figure 5).

The effect of the period of monitoring the repressor concentrations on the time-to-capture was examined on the coupled system. A shorter period of 6 s produced a tighter distribution and a shift towards faster t_c, and a longer period of 600 s produced a broader distribution and a shift towards slower t_c. The longer period also exposes the periodicity, with grouped distributions aligned to the period (Figure 6).

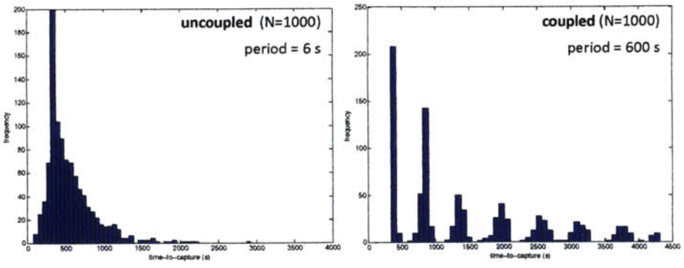

Figure 6 - *Distribution of time-to-capture in the coupled system when modulating the monitoring frequency of the protein concentrations from 60 s to 6 s (left) and 600 s (right). The longer period exposes the periodicity with grouped distributions.*

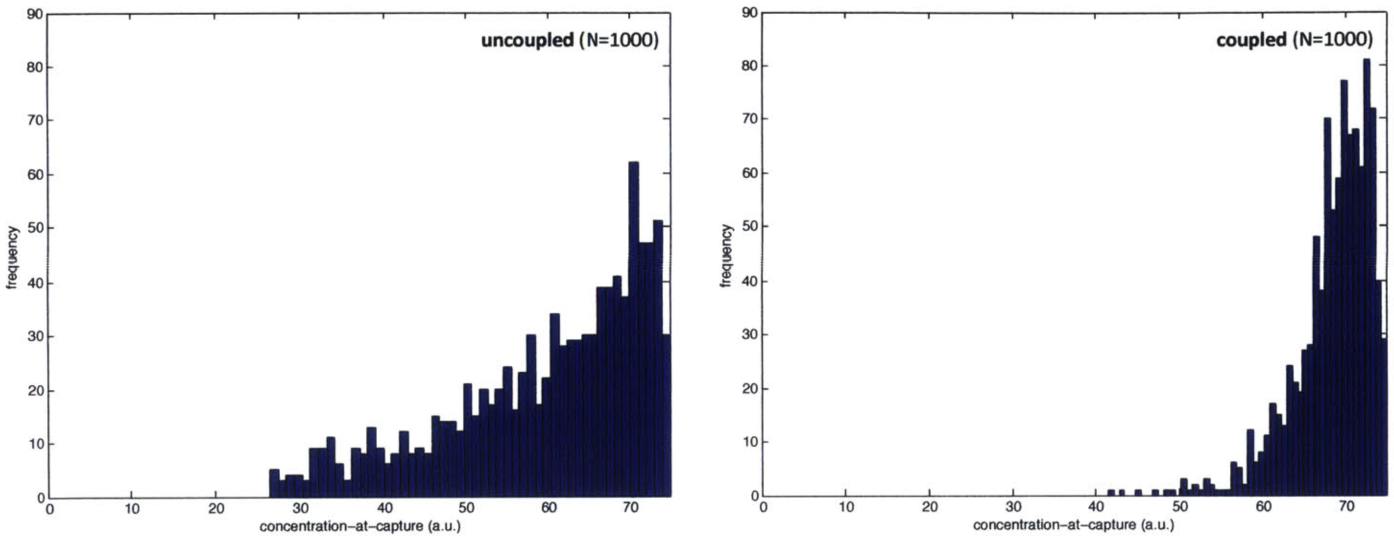

Figure 7 - *Distribution of concentration-at-capture for the coupled and uncoupled systems. Note that in addition to coordinating the capture of the four repressors, the coupled system also provides a narrower distribution of concentrations-at-capture.*

The distribution of concentration-at-capture (N=1000 uncoupled, coupled) for both systems is also exponential, however the coupled system shows a markedly narrower distribution as a result of the feedback network (Figure 7).

To compare the stability performance of the both systems, a simulation (N=100 uncoupled, coupled) progressively reduces the control range window size, from the initial baseline setting (defined in the analysis section above) to zero, and estimates time-to-capture, concentration-at-capture, and stability for each range. The mean time-to-capture values of both systems simulated for incrementally decreasing control ranges shows similar overall behavior of both systems, with faster performance of the uncoupled system. The mean concentration-at-capture comparison of both systems shows similar overall performance for both systems, with higher mean concentration-at-capture and a more focused distribution for the coupled system. The stability comparison of the uncoupled and coupled systems shows similar behavior with better stability performance (defined above as the fraction of trajectories that get captured in a "reasonable" time) for the uncoupled system (Figure 8).

Discussion

The additional negative feedback seems to constrain the range of available states to the system, making it slightly harder to force the repressors into a pre-defined range using inducer randomization, but making the actual distribution of capture concentrations within the control range more precise (narrower). An additional feature of the coupled architecture is that, while capture is slightly less probable than in the

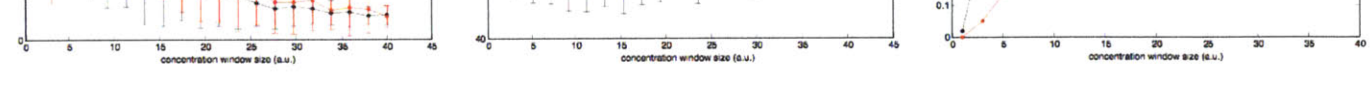

Figure 8 - ***To compare the stability performance of the both systems, a simulation (N=100 uncoupled, coupled) progressively reduces the control range window size, from the initial baseline setting (defined in the analysis section above) to zero (therefore any output value is out*** *the range, and inducer concentration is always randomized and no steady state is possible), and estimates time-to-capture, concentration-at-capture, and stability for each range. The mean time-to-capture comparison of both systems (left) shows faster performance of the uncoupled system. The mean concentration-at-capture comparison of both systems (center) shows higher mean concentration-at-capture and a more focused distribution for the coupled system. The bars in the left and center figures give the standard deviation of the respective distributions (not the error of the mean values). The stability comparison of the uncoupled and coupled systems (right) shows similar behavior with better stability for the uncoupled system.*

uncoupled case, the four repressors will be captured simultaneously. These can be useful behaviors towards the idea of adaptation or self-optimization, though I believe these potential behaviors were limited by the circuit design investigated by this proposal. Specifically, inducer randomization events triggered by an offending repressor affected all the functional units simultaneously.

The uncoupled system shows better stability performance, likely as the trajectories are independent and one trajectory running away doesn't influence the probability to capture of the others. Less interference from the neighbors seems to improve the probability of capture.

The digital monitoring of the system provides for a unique feature exposing periodicity, which was outside the scope of this proposal but could be be further characterized as a feature of the cyber-biological system.

Considerations for v2

In the homeostat, the state variables (needles) are physically confined to the control range interval. The machine reacts to the needle pressing against one of the ends until all the needles stabilize away from the ends. Repressor concentrations, on the other hand, are not confined to the control range, and if they drift too far away, they can't be brought back by inducer randomization.

In the system architecture as described, each functional unit was proposed to contain each one of the four promotors driving one of the repressors in order to pass the output of each unit to the others. However this effectively linked the state of all the units, and when a repressor strayed outside the specified control range, the corresponding inducer randomization event which followed affected all four units. This circuit design muddles the idea of four functional units with independent internal dynamics, taking as input the output signal of each of the others.

This should be further explored in circuit designs where the which functional units that "listen" and to each others' output and "share" their own state, but "respond" by only changing their own dynamic. The behavior observed in this investigation may be significantly different with the design in figure 9 (as opposed to the schematic in Figure 2), where the inducer randomization would only directly affect the local dynamics of one of the functional units, which should then change only it's own output signal, and communicate that as an input to the other units. A biological circuit would need to be proposed where in each unit the state variable being monitored is decoupled from the randomized variable affecting the dynamics and the output passed to the other units.

This architecture would more closely represent Ashby's homeostat, and could provide insight to the potential advantages of designing biological circuits with architectures that provide unique features of state adaptability.

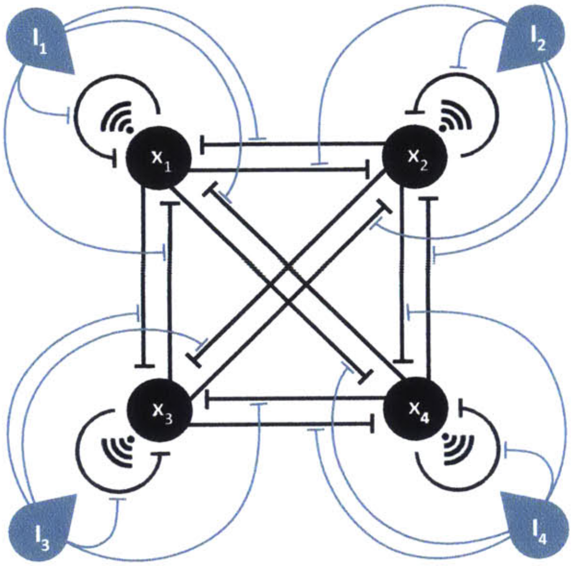

Figure 9 - *A schematic proposed for the next version of the Represseostat, where induction operates on the inputs to the functional unit, and a randomization event should not directly affect all functional units simultaneously.*

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