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Tuning Communication for functioning: sounding in/the - Naples 2020

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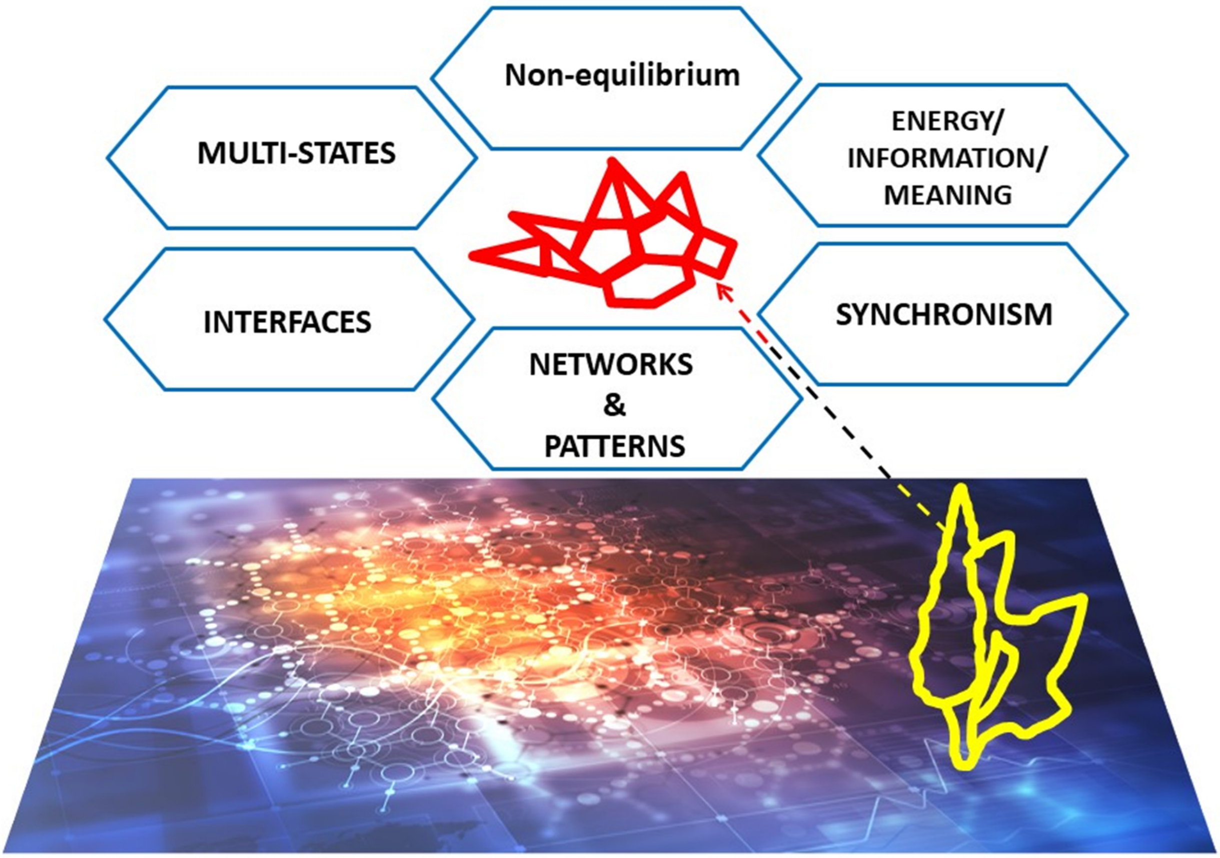

Schematic view of the concept of Stem Materials: understanding the functioning of living organisms and their interaction within the ecosystem (here represented in yellow as the marine gastropod mollusk named "pelican's foot") is approached through the integration of different aspects (in hexagons) which allows to identify building blocks (in red) to be assembled for a new generation of intelligent materials.

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Tuning communication for functioning: sounding in/the fluids

Preface

The concept of Stem Materials

In nature, living organisms consist of a limited number of primary components and chemical bonds organized in complex systems capable to adapt to diversified environmental conditions. Materials are very rarely adaptable, and often require a large number of components to achieve high performances in specific functions. In this comparison between organisms and materials, the approach to their respective life-cycles are also largely different, the former renewing in a continuous interaction with the environment, the latter mainly preserving from alterations. Indeed, materials able to perform different functions and to respond to external inputs will become increasingly important. They will play a fundamental role in the additive production to the extent that these are designed and structured to perform specific operations and self-adapt to varying external conditions, without any additional device. This generation of materials can substitute robots in some applications, i.e. when communication and electronics are considered vulnerable aspects. Materials able to perform as sensors and actuators, accordingly to external environmental conditions for fulfilling different requirements, are still a challenge. These intelligent materials should be flexible in any context and condition, and possibly consist of primitive units, containing the minimal and sufficient number of components to perform a basic function, whose combinations can respond to specific requests of multi-functionality and adaptability. This is the concept of STEM (Sustainable Transformative Engeneered Multi-functional) Materials (http://www.foresight.cnr.it/working-groups/wg-materials).

The challenges

Many scientist already met to identify what scientific challenges are needed to tackle to implement the concept of Stem Materials (see cover figure and also https://bmcmaterials.biomedcentral.com/articles/10.1186/s42833-019-0004-4).

In this context, one of the main scientific challenges to understand the operational functioning of complex systems, such as biological systems, is the role/meaning of the information, its transport and interaction between the different agents. Despite the large amount of data which can be accumulated on the transfer of matter and energy, the rules and processes which structure and organize the system in real networks that dynamically modify their topology in relation to external inputs, are still a matter of research in different disciplines. Whether you want to call it "semantics" or a **functional analysis of the dynamics of topology** (in space and time), the need is to understand how the transport of information can result into an action and in structure/organization. Understanding how a signal is generated, propagates towards the 'target' and turns into a response has innumerable cross-cutting implications: from social communication, to robotics, to the synthesis of functional materials or medicines. This understanding is therefore embedded in many different concepts, from information in life sciences to network topology and ecosystem functionality. "*Languages of nature*" are still far to be framed in a mathematical formulation capable to infer a universal grammar including biotic and abiotic roles. Some theories are still at the level of hypothesis and far to be demonstrated: "*Proving Darwin*" is probably the key challenge for introducing a bridge between information and meaning towards understanding the fitness of different components in a complex system. What we are learning is that if we want to build STEM

(Sustainable Transformative Engeneered Multi-functional) materials, we need to focus on the language of STEM (Space Time Energy Matter).

The context and the aim of these proceedings

One of the most unexplored aspects in the functioning of complex systems, are the understanding of how the information transfers within them, with the external environment, and realizes in a "meaning". This lack of investigation contrasts with the large amount of data usually accumulated to describe the transfer of matter and energy within the systems. Matter and energy distributions, however, constitute the information that structure the real networks that dynamically adapt to the overall dynamics of the ecosystem. Whether we want to call it "semantics" or functional analysis of the dynamics of the topology of the networks, the need is to understand how the transport of information can result in an action.

At sea, sound is the most effective means of transmitting signals, in comparison to chemical and light ones, over medium-long distances. In fact, both chemicals and light lose direction or vanish beyond a very short spatial scale. Understanding how a signal is generated, propagates towards the 'target' and transforms itself into a response has innumerable transversal implications: from social communication, to robotics, to the synthesis of functional materials or medicines.
In this context, a workshop was organized to identify clues from different research, mainly focused on what can be learned from recent investigations on communication in fluids (underwater environment, brain, bacteria etc.).

The workshop promoted short articles, which aim to stimulate reflections towards in a multi and inter-disciplinary cognitive path that can identify the understanding of the foundations of the functionality of signals complex systems. These proceedings report these short articles, which have been asked to briefly focus on scientific results or questions and provide references for entering into the details. The programme and the authors of the articles are included in this book too.

Further workshops will be organized to address other issues which can complete the mosaic of the aspects to understand the role of the information and energy flows to induce action/functioning.

Pier Francesco Moretti

From the Workshop on Stem Materials organized by the CNR S&T Foresight Project in Rome, December 2018: "We are nowhere" or "We are now here"? A space in writing, a time lag in speaking, a code and a context can change the meaning and the action. (Courtesy of Vasileios Basios).

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Sounding the sound in the ocean:
back-casting foresight to identify observing strategies to understand the ecosystem

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Keywords: Underwater noise, signal analysis, observation systems, complexity

Abstract

Scientific studies of the effects of anthropogenic impacts on the environment support the interventions aiming to match the conservation of the ecosystem with the socio-economic sustainability of human activities. In particular, marine challenges probably show one of the most complex systems in terms of environment, industrial sectors, regulation, governance, geo-political scenario. An ecosystem integrated approach, which should include societal, economic and political variables, is needed to identify appropriate interventions, which in turn require a knowledge-based scientific support to policy decisions.

In this article, we conduct a back-casting foresight exercise to identify the scientific strategies that need to be implemented in order to successfully tackle the problem of acoustic pollution in the marine environment. We choose a back-casting approaches because of its suitability to address complex problems and find solutions on a long-term basis. We identified the targeted scenario as the one with a model where data can be effectively used to provide knowledge of the status of the marine ecosystem.

We focused, therefore, on how knowledge borrowed from other scientific disciplines could help us build observation strategies that could allow us to understand the functionality of the signal coupled with the status of the ecosystem which is conceptually represented as a network of agents. We briefly review many examples on how data are collected and analyzed in order to provide relevant clues to understand the interconnection of signals. This brainstorming exercise can lead us to a reflection on the proper observing strategies that need to be implemented in the marine environment in order to understand communication in an ecosystem and its functioning. The appropriate observation strategy consists in the installation of fixed observing stations at different locations and heights (i.e. moorings) and proceed with a cross-spectral analysis between different variables, hopefully to identify indicators which can reduce the dimension of the phase space.

Context

Gathering data from observations is a fundamental step within the scientific method to acquire relevant information in any branch of natural science. This may give clues to understand the behavior of a system. In addressing the impacts of acoustic pollution in the marine ecosystem, a diversity of techniques such as animal tagging, and acoustic network technologies, lend themselves as the optimal solution to investigate issues such as the impact on marine life (Wright et al., 2007). The marine ecosystem is a result of a huge variety of different components which are interconnected and constitute the environmental contribution for a socio-economic value. Due to the long permanence underwater, to the high cost of performing research at sea, to the extent of the oceanic habitat, and to the unreachable nature of some areas, some animal species are poorly known, and many are data deficient (https://www.iucnredlist.org/search?redListCategory=dd). Research regarding these topics is a complicated task which could greatly benefit from a multi- and inter-disciplinary approach. It has been demonstrated that underwater anthropogenic noise exerts an impact on marine ecosystems: the problem the scientific community is facing, however, is to quantify and understand the signals in their role for the functioning and for the status of the ecosystem as a whole. Experiments are usually carried out on samples from few species, using hydrophones and other sensors or multipurpose instrument attached to the animal (TAG) and different methodologies such as spectrograms, correlations, waveform analysis are used for the analysis of the signal. Nowadays we are gathering a lot of data, however, in this particular field we are missing a comprehensive model to frame the interaction between different signals from living organisms and anthropogenic sources. Waiting for such a model, the observations should be at least designed in a way that could provide data useful to understand the functionality and sustainability of the marine environment.

How should we measure information? There is no unique way: what is more suitable will depend on what we are studying. Information can be broadly divided into elementary – such as technical ones – and advanced, which are the ones we extract with a process. The missing information on the state of a system is related to the elementary information called entropy which can be maximized in a system if suitable constrains are satisfied (Beck, 2009). To understand the meaning of the spatio-temporal energy dynamics of living organisms we need to identify fundamental aspects of communication and the role of noise.

Here we propose a back-casting foresight exercise: we fix a future desirable scenario and we try to identify scientific based methods applicable in order to solve it. It refers to the ability to predict what will be needed in the future in order to address a particular issue. This "foresight" decision-making process promotes trans-disciplinary research and engages stakeholders creating key networks (Popper, 2008; www.foresight.cnr.it).

Here we address underwater noise as a polluting factor in seas and oceans, and we focus on understanding its impact of marine fauna and how to move in the direction of the design of a fully-fledged model and not to observe just what we can. In order to move in the right direction, we need to change the approach to observation strategies and analysis of the signals and shed light on the role of synchronism in communication and functioning in the marine ecosystem.

State of the art

The term "Noise" comes from the Latin nausea and it is usually used for unwanted signals, while sound includes all acoustic waves. For the purpose of this paper we will use the term noise when referring to a negative perception.

Sound in seawater is pervasive and far ranging, therefore, activities generating it underwater can create synergies and cumulative effects, thus affecting their health and their role in the whole ecosystem. Other stressors such as habitat degradation, climate change, and chemical pollution might worsen the situation, however, we have not understood how to deal with yet, also because it is difficult to evaluate the impact on the whole ecosystem (Weilgart, 2007; Slabbekoorn et al. 2010).

Anthropogenic sources –that can be broadly divided into continuous, i.e. shipping noise, and impulsive, e.g. seismic sources - become very pervasive for marine animals who use sound in every aspect of their lives. Noise alters animal behavior and affects their fitness in many ways since marine animals use sound in almost every aspect of their lives such as foraging, communication, breeding. If we imagine to move away from a source, a simple model of nested zones can be considered (Richardson et al., 2013). A part from injuries, the zones of physiological effects include the Temporary Threshold Shift (TTS)- a temporary elevation of the hearing threshold- and the Permanent Threshold Shift (PTS), a permanent elevation of the hearing threshold at certain frequencies (Southall et al., 2019). Main impacts, especially at the population level, are not only physical, but also behavioural and can include masking of communications. Masking happens when the animal perceives only an acoustic disturbance and not other biologically important sounds – like the ones produced by predators looking for preys. Sometimes this is not only caused by anthropogenic noise, even biotic noise produced by one species can hamper the acoustic signals of another species (Römer, 2013). This phenomenon is difficult to understand and still much needs to be studied, in fact, a variety of factors must be accounted for, such as a combination of sender, environment, and receiver characteristics (Erbe et al., 2016). Masking can result in lower masking thresholds for signal detection mainly through co-modulation masking release and spatial unmasking (Cunningham and Mountain, 2014). The first can help to improve the detection of background signals allowing the listener to receive more information (Moore, 1990) while "spatial unmasking" happens when sounds appear coming segregated from the source and are perceived with different arrival times (Bee, 2007). In this area, if animals modify their behavior or their vocalisations, they can start cascade effects, creating an impact on the food web and on the ecosystem. More research is needed to assess the risk of masking created by various anthropogenic activities, the possible and anti-masking strategies developed by organisms, and effects at the population level. Another impact of noise, not always easily detectable is stress, a state which can seriously affect animal health, even when mating and nursing (Erbe, 2012; Wright et al., 2009).

All organisms experience cognition - the detection of the environmental conditions - and communication which results in the interchange of information carrying a functional message, leading to languages and semantics. Noise can impact both. Communication is important in cultural social learning, especially of marine mammal species (Rendell and Whitehead, 2003). Knowledge can be created through interactions among individuals, therefore bigger groups have an advantage, this is known as the 'pool-of-competence' effect and has a great impact on future decisions made by the group (Biro et al. 2016; Webster et al. 2017). Animals usually use modifications of amplitude, duration, pitch and timing to vary vocalisations: the level of interference between noise and the signal needs to be considered and it depends on the degree to which the frequencies of noise and the signal overlap (Hulse, 2002). Synchronized displays may sometimes play a role in emphasizing acoustic details or draw attention to the sound produced by the source, depending on the ability to focus the auditory streams. (Spence et al., 2000). This is important for auditory scene analysis and details evaluation. The fundamental problem in this field regards the ability of the receiver to identify the message generated by the source, decrypting the characteristics of the signal and understand the meaning of the signal the animal has chosen among a set if other signals coming its way. Signals contain information. Signal description, however, has not been successful because we don't study all the signal but we introduce means**.** For its reception both pressure and particle displacement –which carries information on direction of energy flow - need to be considered, especially in close proximity to a source. The propagation of a signal is limited by the decrease in signal-to-noise-ratio, a problem that could be solved by evolutionary changes in signal features that can lead to long-term adaptations (Brumm, 2005; Klump, 1996).

The European Commission through the Marine Strategy Framework Directive aims to achieve the Good Environmental Status through 11 descriptors, the last of which states:" Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment", (EC Decision 2010/477/EU, Directive 2008/56/EC). Indicators 11.1. and 11.2 have been identified respectively for distribution in time and place of loud, low and mid frequency impulsive sounds and for continuous low frequency sound. This increasing political attention is also connected with the economic side of this topic such as the costs of possible impacts on underwater noise and the damage on the society. However, it is still very difficult to quantify the ecological cost of underwater noise for the society, especially because we still need to create models to evaluate risks at the population level and on the ecosystem. The principal issue that needs to be taken into consideration is the cascading effect on the marine ecosystem and the damage produced on the ecosystem services (Merchant, 2019). Moreover, also the enforcing of mitigation measures has a cost and should also be computed together with a comprehensive model that could calculate cumulative and aggregate impacts (Söderqvist, 2014). Mitigation measures that should be implemented, in order to reduce the impact on marine life, could be expensive and have direct and indirect costs. These measures can be implemented in various ways such as abating the source, restrictions in time and space, using personnel as marine mammal observers, using passive acoustic monitoring. Therefore, this necessary process might be expensive (Merchant, 2019; Söderqvist, 2014). Furthermore, interventions need to be considered also in terms of efficiency, since other aspects of the problem can arise and collateral issues are introduced. In case of uncertainty, the precautionary principle could be applied. However, this depends on the culture (it is applied for European Countries (COM, 2000) and objectives identified at political level.

Reflections

We adopted a back-casting foresight approach to reflect on the meaning of noise, the way it is monitored and analyzed, and possible scientific paths to understand the impacts on the marine environment. We face a complex system, and we have to gather data and frame in a model where signals, noise and soundscapes are analysed all together. This will be of paramount importance to address interventions for conservation and management. This multidisciplinary problem will involve different expertises and will need advanced materials, tools and technologies. Nowadays, underwater acoustic studies are mainly performed in the frequency domain. However, this provides a limited point of view and loss of casualty because it is not taking into consideration the "semantics" of the signals, a crucial aspect to be investigated (Balázsi et al., 2011; Parbery-Clark et al., 2011). We should also take into consideration phase and coherency between different components of the network in the system (Strogatz, 2000; Watts and Strogatz, 1998). In fact, the synchronization between different signals is important for inducing a state of equilibrium of the system, not of the single components. Despite the efforts in investigating the cumulative effects of inputs on the marine environment (Gisiner and Simmons, 2011), the understanding of the tuning of the signals for the functioning of the ecosystem itself is lacking. In this regard, the role of the communication, meant as exchange of energy and information between the different components of the system (including organisms), has to be taken into account. What said suggests that data have to be acquired to allow an analysis capable to investigate this aspects and extract useful clues to evaluate the status of the system.

Despite the difficulty in comprehending the interconnections between the single components of the marine system and its global state, the economic interests in forecasting tipping points for the system and irreversible paths solutions are high. In this context, the scientific community has not yet provided robust models to understand the interaction of the underwater acoustic field with the ecosystem which can support policy decision which are fulfilling the diversity of involved pressures. On the other hand, the industrial sector is struggling with the acceptance of the precautionary principles. More detailed studies, thresholds and specifications are needed in legislation and insurance contracts in order to satisfy stakeholders and adopt effective interventions that can be also sustainable.

A change of point of view in tackling underwater noise issues is needed in order to identify the specific issues and address conservation globally. The approach needed based on observing strategies uses cross-spectral analysis a method used especially for temporal and spatial waveforms or discrete data. Its basic purpose is to decompose functions into their spectral components which are a convenient descriptive way. These techniques are being used in astrophysics to study the sun, for example in new research study about internal gravity waves (Calchetti et al., 2020, submitted for publication; Gardner, 1988).

For our purposes, we believe moorings should be used instead of Lagrangian observational methods.

Marine animals, especially mammals, are highly mobile, elusive and elicit the public opinion's concern. In order to avoid visual surveys which are costly and sometimes not very efficient, acoustics methods should be preferred since mammals are very vocal. Nowadays, passive acoustic monitoring (PAM) are the suitable method for the study of free ranging animals also because this technology allows data collection during nights in locations that are poorly accessible and while the animals are submerged. Researchers are using PAM to research the effect of noise, behaviour, abundance, and for much more (Zimmer, 2011). Moored hydrophones can be deployed for long periods and can record continuously, producing vast amounts of data (Erbe and King, 2008) which can be used to provide a more fruitful analysis. These systems are also used because they do not interfere with the animals' behaviour. Moreover, PAM systems implement mitigation measures of anthropogenic activities especially on board of seismic surveys (but also offshore exploration, military and civilian sonar, etc.). Costs and technological aspects have to be considered to design the proper system parameters and positioning of the instrumentation (such as animal behaviour and environmental characteristics, Zimmer, 2011).

Observing systems based on an Eulerian approach have enabled an unprecedented investigation of the acoustic field of the solar surface through a multi-layer and multi-variable data acquisition coupled with cross-spectral analysis (Lindsey and Braun 2017, Moretti et al. 2003 and 2007). This remote observing strategy has allowed to "sound the Sun" and extract information of the inner parts, despite the impossibility to install instrumentation on the field (Kosovichev, 1999).

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Order, Chaos, Rhythms and Patterns

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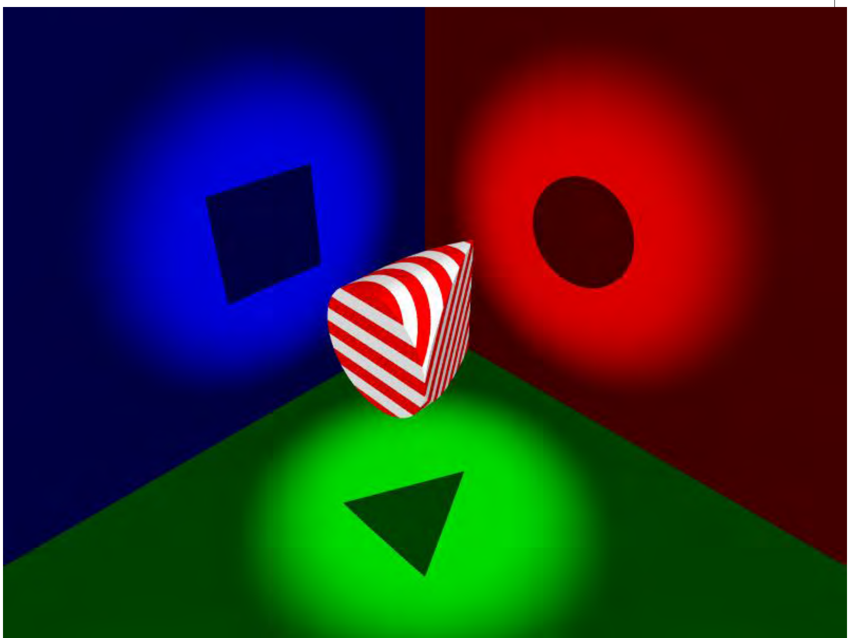
Keywords: complexity, non-linear dynamics, self-organization, information processing

Abstract

Complex and intertwined oscillations and vibrations are the underlying mechanisms of complex systems. The importance of the paradigm shift that emerges from the science of complexity and its importance for frontier, future and current, related research fields is sketched in this work. Some aspects of the historical account are presented. The role of out-of equilibrium systems, chaos, self-organization, pattern formation and emergent properties of collective oscillations is discussed. The role of multiple time and space scales, especially in biological information processing, is highlighted and its significance in future developments is addressed. As complex systems science offers a unique perspective in revisiting the concepts of equilibrium, dynamics and information processing this prospects are also briefly discussed.



"Nonlinear science introduces a new way of thinking […] through the process of continual switching between different views of the same reality these methods are cross-fertilized and blended into a unique combination that […] helps to identify the appropriate level of description in which unification and universality can be expected."

Grégoire Nicolis

"Introduction to Nonlinear Science", Cambridge Univ. Press, 1995

Context

'This is the century of complexity' more than a famous quote this statement becomes a reality as we traverse this century. Complex System Sciences, or the Science of Complexity, which has been heralded, among others, by Ilya Prigogine and Gregoire Nicolis has been characterized as the third scientific revolution of the 20th century after relativity and quantum mechanics. This revolution was not announced in the beginning of the previous century as the other two, though. Yet, it did take place around that time. A peculiarity of the scientific revolution of Complexity Science also lies in the fact that it took roots in a inter-disciplinary and cross-disciplinary fashion. It affected the foundations of our thinking in a horizontal way spanning almost every discipline by now.

Just having a look at complex system's organizational map (Fig,. 1) one can see the depth and breath of this new paradigm which has spread roots in contemporary science [i]. Every major development the over the last decades has confronted the demand of solving a non-linear, complex, issue in its respective field.

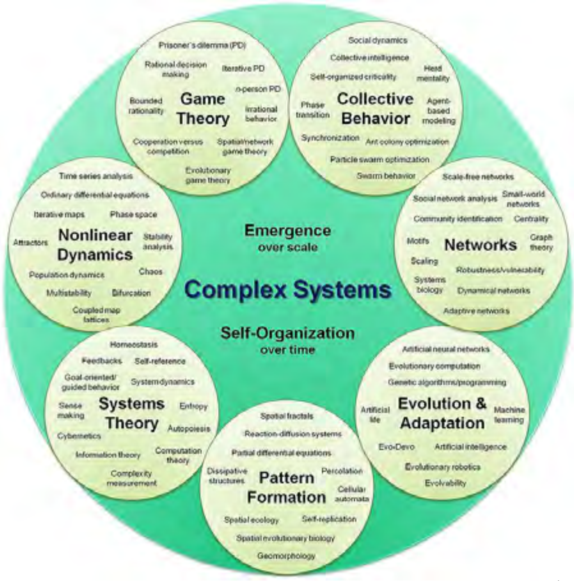


Fig. 1: The organization of Complex Systems according to scientific disciplines

For example, the idea of symmetry breaking, a generic nonlinear mechanism, coming from nonequilibrium statistical mechanics has been extensively used from the fundamental particles arena as the base of new particle discovery, up to their pinnacle the famous Higg's Boson via the *Brout–Englert–Higgs mechanism,* to wider area of pattern formation and morphogenesis.

Emergent properties, over spacial scales, and self-organization, over time-scales, are ubiquitously present from microscopic physics, e.g. nano-scale material synthesis, to photonics, e.g. laser arrays. Equally so they are present from biophysics and biology to social biology and even social sciences and recently in 'data-science'. The ideas, concepts, tools and vocabulary of nonlinear complex systems is indeed, by now, ever-present at the frontiers of knowledge.

But, what are the prominent concepts that characterize the scientific revolution of Complexity? What makes this scientific paradigm-shift distinct form the other scientific revolutions?
If one examines closer a comprehensive answer to the above questions is that Complexity changed our ideas about the whole and its parts, and also made us reconsider the long-held idea that determinism implies predictability. The paradigm of Complexity rests on the fundamental role of the non-linearity of relations and their spanning multiple scales.

The Whole is More and Different that its Parts
By definition a Complex system is a system, which is comprised of many nonlinearly interacting constituent parts. Complexity Science teaches is us that there are properties of the system that cannot be reduced to or attributed to solely in the properties of its parts. (Fig. 2 credits in [ii]).

Figure 2: A paradigm-shift for the whole and its parts: Emergent patterns, Self-organization, Superorganisms. Is this D'Alembert's dream or every naive reductionist's nightmare? (credits in the references)

Up to the advent of complexity the whole universe, the whole cosmos was though to be a well-tuned clockwork mechanism set in motion. In physics the emphasis was put in the parts, mainly harmonic oscillators coexisting and weakly interacting, mostly linearly. The same world-view and presumptions invaded biology and science at large.

The Second Law of Thermodynamics with its inevitable 'heat death', absolute equilibrium forever, was thought of as the overarching absolute law for the evolution of the universe. A Malthusian picture of history and a naive, blind, Darwinian evolution for biology necessarily followed this track of thought.

One of the first blow to this mental construction came from a set of extraordinary experiments for their time on oscillating chemical reactions. These chemical oscillators although reported from the times of G.T. Fechner and W. Ostwald in the 18^{th} century were severely criticised and totally ignored up to the middle of the 19^{th} century.

A chemical oscillator is a complex chemical reaction where the products feedback to the reactants and periodic changes set in. They are the prime example of non-equilibrium thermodynamics and they were thought of as a scandal and impossible. Impossible yet true this example heralded the new era of complexity.

"Eppur Si Muove": The importance of being Non-linear.

Boris Belousov, back in the 50's Soviet Russia, was searching to find a non-organic analogue to the Krebs cycle, and he discovered the first and most famous instance of a chemical oscillator. Yet, he could not get any credit for this discovery till the late 1960's. The detailed study of a young and bold graduate student, Anatol Zhabotinsky, resulted to the first major publication of what is now the celebrated Belousov-Zhabotinsky reaction (BZ reaction).

One of the many essential and fascinating aspects of the BZ reaction is that in a spatio-temporal setting and under the influence of small fluctuations –either on its enclosing boundary or at its intrinsic parameters– it develops patterns. Again this came as a surprise to the old school. The Second Law dictates a homogeneous outcome as the only equilibrium. Yet, the BZ reaction never ceases to move, patterns emerge and cease. It has also been observed that BZ reaction with special catalysts can be excited into self-organising activity through the influence of light. Moreover, its oscillations can even keep the time as a chemical clock. In modern terms the system exhibits excitability and adaptability via self-organization.

And here came Ilya Prigogine and his students Grégoire Nicolis and René Levefer. They developed the theoretical mathematical framework that explains these scandalous phenomena. They based their mathematical modelling on Prigogine's previous work on the thermodynamics of open systems, on 'Catastrophe Theory', and on Alan Turing's idea on morphogenesis. Their model was called 'the Brusselator' and although it met fierce resistance before being accepted it eventually made Prigogine win the Nobel price in 1977. By now, this too, is a cornerstone of our understanding of complex systems and self-organizaton.

Figure 3: The famous BZ reaction oscillatory chemical patterns. In this case, rotating spirals propagating across a Petri dish (credits in the references).

What comes out is the concept and theory of dissipative structures, i.e. structures that exchange energy, materials, and information with their environment in such a way that they maintain a new kind of order. An order out of chaos and far from equilibrium. We may call this new discovered kind of order dynamic, ever moving, equilibrium. This is the equilibrium of the living rather than the equilibrium the dead.

As the title of one of the papers that came out of the so-called Brussels School of Thermodynamics, puts it "The Brusselator: It does oscillate all the same".

The state of the art

When a dynamical system oscillates it goes through the same or almost the same set of states in a cyclic fashion. The simplest oscillation, of the pendulum or harmonic oscillator, is due to the balancing act of a positive and negative feedback. Oscillators are ubiquitous in nature, everywhere around us, in all scales and frequencies, we are surrounded by vibrations and rhythms. Each system has its own characteristic set oscillatory/vibrating patterns we call these eigen-frequencies or eigen-modes. Simple mechanisms have simple eigen-modes, complicated have complicated ones, and complex systems have complex modes of oscillations.

It is important that we emphasize here the distinction between the complicated and the complex. A complicated system can be reduced to and analysed by its parts, in its entirety. A complex system cannot be reduced to or described by its parts; any such endeavour can only be partial and approximative.
The reason behind this distinction is the interrelationship between the parts and the interrelationship between parts and the whole system. In complicated systems these interrelationships can be put on a linear superposition, i.e. any set of them is the same as their sum. In a complex system such a linear superposition fails due to its intrinsic non-linear relationships. The states of a complex system are coupled rather that merely summed. The informed reader will recognize, of course, that this is reminiscent of the situation in a quantum system, it is indeed so, but this discussion is far from the scope of this exposition.
In other words, the eigen-frequencies and eigen-modes of vibrations/oscillations in a complex system are non-linearly coupled where in a complicated system they are just linearly coupled or uncoupled. This fact is expressed also in their frequency-spectrum where in complex system it is continuous and in complicated systems discrete.

So, in a complex system different mutually influenced, coupled, mechanisms actively coexist during different phases of their oscillations. The oscillations of a complex system are, … well, complex. They can shift continuously between slow and fast frequencies, large and small amplitudes, and the phases among them can change from synchronized motion to entirely chaotic or even coexisting chaotic and synchronized ones. Most importantly all these oscillations coexist a very large interval of time and space scales and many regimes of motion are present. Technically speaking we say they span many scales in space, amplitude and time, typically across some orders of magnitude.

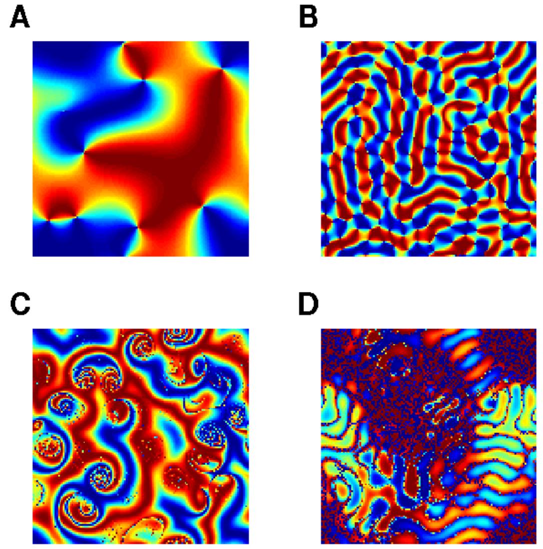


Figure 4: Four distinct synchronization patterns in a 128x128 array of Kuramoto-like oscillators with differing phase interaction functions and spatial coupling topologies. (A) Pinwheels, (B) Waves, (C) Chimeras, (D) Chimeras and waves. (credits: the Kuramoto Model entry in Wikipedia.org).

In the contemporary vocabulary these complex oscillations/vibrations including are called mixed mode oscillations (MMOs) and they might include chaotic ones. Recently co-existing, at the same system, chaotic and synchronized oscillatory patterns have been observed. These states are called 'Chimera States', typically but not necessarily, appearing in arrays of coupled complex oscillators (Fig. 4). Chimera States have been observed experimentally in a variety of complex systems in physics, chemistry, engineering and biology. They have been are detected and studied in laser arrays, mechanical systems, waves, bacterial or animal populations' collective motion, and in biologically complex organs –or regions of them– notably the brain. The mathematical understanding of 'chimera states' and the role that information flow among pathways of coupled sub-groups within these arrays of oscillators plays is a very active filed of research the two last decades.

As mentioned above, oscillators and patterns in complex systems depend crucially on the overall system parameters its topology of connections and its boundary or closure of the system as a whole. These global or 'order' parameters, coupling constants and topological constraints determine the overall behaviour of the system in an interplay with the local, individual parameters of each one of the constituents of the system. One of the great challenges that we are facing at this point is the mathematical modelling and understanding how exactly local interactions translate to global behaviour. This challenge it is similar to the flocking of birds where simple local rules give rise to a very complicated global behaviour. The development of novel mathematical tools and techniques to address the problem preoccupies several scientific communities and cross-fertilises a significant number of interdisciplinary research programs at the frontiers of our present knowledge.

Another topic of great importance and focal point of contemporary research is the control and prediction of dynamical regimes. To this end the traditional Catastrophe Theory has been revisited and its aspect of bifurcation analysis and dynamic stability augmented with non-linear time-series methods and signal-processing spectral theory. New phenomena along with the classical theory of resonance and resonant modes have been discovered in these scenaria of bifurcations, cascades of multiple rhythms driving to chaos, stochastic resonance, chimera-states new types of 'catastrophes' and transitions from one dynamic regime to the other.

In addition, recent research on oscillations and the genesis of rhythm or 'rhythmogenesis' have shed ample light in the processes and mechanisms of biological information processing. It has been established that a biological information processor in contradistinction with a computer has to be chaotic. The presence of a chaotic and mixed-mode oscillations regime actually does not impede but on the contrary makes a biological

processor more reliable, more adaptive and more functional in a changing environment. The dynamics of cognitive action require a slower super-cycle comprising from two dynamical regimes that contain faster sets of smaller cycles. A chaotic regime which enables exploration and observation, expanding via bifurcations to generate new chaotic attractors and a stable regime which serves as categorization and memory utilizing sustained stable oscillations.
The subject area of 'rhythmogenesis' has taken these insights to a fully developed research program where the detailed mechanisms are studied in small group of neurons the so-called central pattern generators (CPG) that produce and coordinate rhythmic activity; be it in muscular tasks (e.g. pacemakers, gait) or cognitive tasks (e.g. decision-making, judgement). Frontier research is also being contacted at the macromolecular and cell levels in particular on the role of the electromechanical vibrations of microtubules, always in relation to rhytmogenesis.

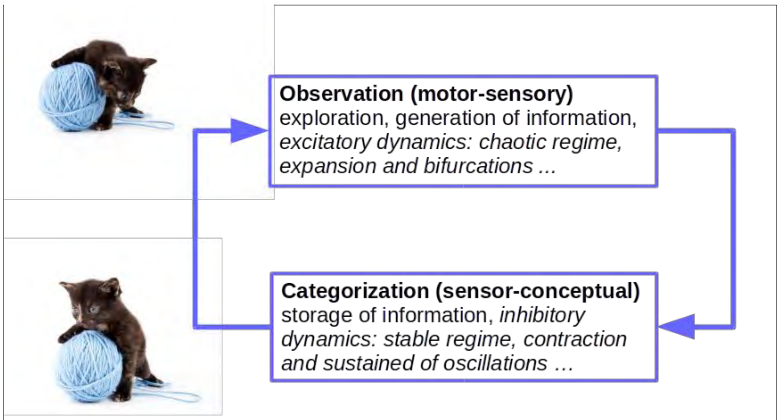


Fig. 5: The dynamics of biological information processing require a super-cycle containing oscillations both in a chaotic regime and in a stable one.

So, it is natural that new adapted Fourier-type techniques and theories had also to be invented. For example, the well established by now and proven of great utility recent advancements in Wavelet-analysis, Entropic measures and Symbolic Dynamics. Once again the concepts of entropy and information are rapidly becoming the crux of the matter in our understanding of the complex (coherent or incoherent, synchronized or not, chaotic or mixed-mode, global or local) coexisting variety of vibratory and/or oscillatory modes. In real-life applications these new concepts and mathematical tools developed for complex systems contribute the most in understanding which vibrations are due to random noise of due to deterministic fluctuations.

And this is not just a question of improving the 'signal to noise ratio' cannot only be merely improved, this discrimination between what is noise and what is meaningful information will prove a research field of paramount importance in the very near future. It might even trigger the long awaited 'semantic revolution' in our current paradigm of what information really is after all. Such a development will obviously have repercussions that will effect not only complex system research as such but also our currently established world-view at large.

Reflections

One cannot avoid bringing in mind that the very meaning of complexity. The word 'complex' derives from the ancient root word (in Latin and Greek) -plex/plek/pli which means weave together, the patterns of a fabric or interlacing, embracing. 'Com-plex' as a concept then points to an interwoven, embracing whole comprising of interlaced mode of vibrations resulting in surprising patterns and dynamical behaviours.

The study of the detailed non-linear feedback circuits that are part of the mechanisms that produce these interlaced oscillations that weave patterns in multiple scales of time and space are one of the most promising future trends in complex systems.
Moreover, in our data-obsessed era, the information carrying and processing capacity of a complex system is of paramount importance. Many insights of biological information processing are waiting or are readily being applied to certain cut-edge information processing, such as artificial neural networks, Bayesian networks and all of their variants.
Another important area of application would be the field of materials science that usually goes with the term "intelligent materials". How intelligent a material can be? Is this another passe-partout phrase like "artificial intelligence"? Or it really does hold some promise when describing materials with self-organization, excitability, memory, adaptability and real-information processing powers that make them amenable to

control and coordination? Their intrinsic spectrum of vibrations and the information flow inside and around them –within other coexisting components and with their environment– determines their meaning and function and in the end their actual utility and future possibilities of production.

These questions cannot be addressed in any traditional conceptual framework. They call for expanded and empowered conceptual schemes inspired by the advances of new paradigm of complexity science and must be guided by the study of, not only their constituent parts, but also by the study and the understanding of their interconnections, their interrelations among themselves, their boundaries and their interfaces between the whole system, or systems, where they are necessarily embedded.

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Understanding underwater acoustic communication: more than frequency

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Abstract

The intrinsic diversity between different types of underwater sound, namely natural sound, animal vocalizations and man-made noise, is heuristically evident. However, in order to reliably assess the prominence, or impact, of one type of sound into another, what is needed is a metric for evaluating objectively such differences in terms of their suitability to communicate some useful information: that is, to their functionality. Since their inception one century ago, studies in underwater sound have traditionally proceeded through analysis methods based on the frequency domain. However, the evaluation of information contents in underwater communication, that is of its functionality, may be effectively conducted by means of techniques, originated from and widely used for speech analysis, based on the combined evaluation of a set of quantities named prosodic parameters. These parameters refer to specific physical quantities and to their corresponding perceptive parameters, for which determining and preserving time modulations is essential. Such techniques may expand the conventional time-based or frequency-based approach into an unexplored multidisciplinary context blending various branches of acoustics, biology, and cognitive sciences together.

Introduction

Theoretical principles framing acoustic communication are easy to understand, but sensing and analyzing the signal can make the difference when the aim is the interpretation of the role of communication in the ecosystems. If we restrict our scope to underwater acoustics, at present we are still facing a number of unresolved questions [1,2,3]. Is ocean noise increasing globally? How man-made noise in the sea impacts on health and behaviour of marine animals, and on the marine ecosystem in general? What are the most effective and most sustainable ways to reduce such impact, and down to which threshold levels? Reasons for this lack of knowledge partly arise from technology lags: for instance, measurements of underwater sound pressure are affected by considerable errors (accuracy of better than ±10 % is seldom reached, while up to ±20 % is more commonly encountered). Moreover, most sensors are still based on the piezoelectric effect which is virtually only able to capture the time component of an acoustic field (acoustic pressure) and not its spatial components (acoustic particle velocity). This may not be always adequate, since tests done in the laboratory proved that fish are more sensitive to particle velocity than to pressure [4] while extensive measurement at sea is still lacking due to practical limitations of the presently available particle velocity sensors. Other facts limit the capacity in responding to the above questions: for instance, methods for acoustic signal processing are largely based on the frequency domain, since technologies for acoustic communication historically originated from narrowband systems (sonar system processing mimicked that of radar, concurrently developed). However, analyses based on frequency, despite being computationally very efficient, hinder the ability to detect features in acoustic communication which pertain to signal causality. In a real marine environment, frequency-domain characteristics and time-domain characteristics of sound are equally important and an overall impact on marine life can only be derived from a balanced combination of these two categories. Only recently, steps have been taken in fundamental acoustic analysis to account for this mutual time-frequency interplay, by officially introducing along with the traditional time-averaged descriptors (e.g. SPL, sound pressure level) other descriptors (e.g. SEL, sound exposure level) based on the equivalent of an acoustic 'dose' [5]. However, these first attempts are still insufficient to keep track of the complexity emerging from a typical underwater acoustic scenario, where natural sound (both continuous and intermittent) interplays with animal vocalizations (featuring more or less complex time patterns) and with man-made noise (which may possess any of the preceding features). With this in mind, it is clear that a new paradigm for underwater acoustic analysis, capable of accounting for all relevant features of communication, would be very welcome.

Context

For species living in air, including humans, sound (i.e. hearing) is just one of the possible communication path together with other sensory means: sight, smell, touch. Peformances in one or more of these senses has resulted in a specific evolutionary fitness. Consequently, information exchange between individuals of a given species, or within the environment at large, more heavily relies upon those privileged means. In addition, selectivity of both the transmitting part and the receiving part are mutually enhanced to increase efficiency: as an example, human vocal tract resonances are matched to the bandwidth of human ear’s maximum sensitivity. On the other hand, it is well known that species living in the sea environment can only rely on sound to communicate, other means being precluded for all but the shortest ranges due to the extremely high absorption of sound waves by seawater [6]. Those species have then specialized to convey into the acoustic path all the information they need, or desire, to pass on to other individuals. This generated the well-known complexity of voiced communication among marine mammals, which share many physiological aspects with humans, and is also coherent with more recent findings on sensitivity to sound of other species including fish, which may passively get information about their surrounding environment although not being able to acoustically interact with it [4].

Frequency analysis is the primary method for sound signal processing. As this method is applied to the marine environment, one key feature immediately emerges: the frequency distribution of quantities which have influence on, or are influenced by sound propagation, tend to be shaped by an inverse 1/f law in the entire bandwidth spanned by typical acoustic phenomena such as natural noise and underwater communication by marine animals. Figure 1 shows three types of either direct or inverse frequency dependence: of sound absorption by seawater, of the universal curves for ambient noise in the sea (Wenz’s curves) and of auditory sensitivities of a number of marine animals.

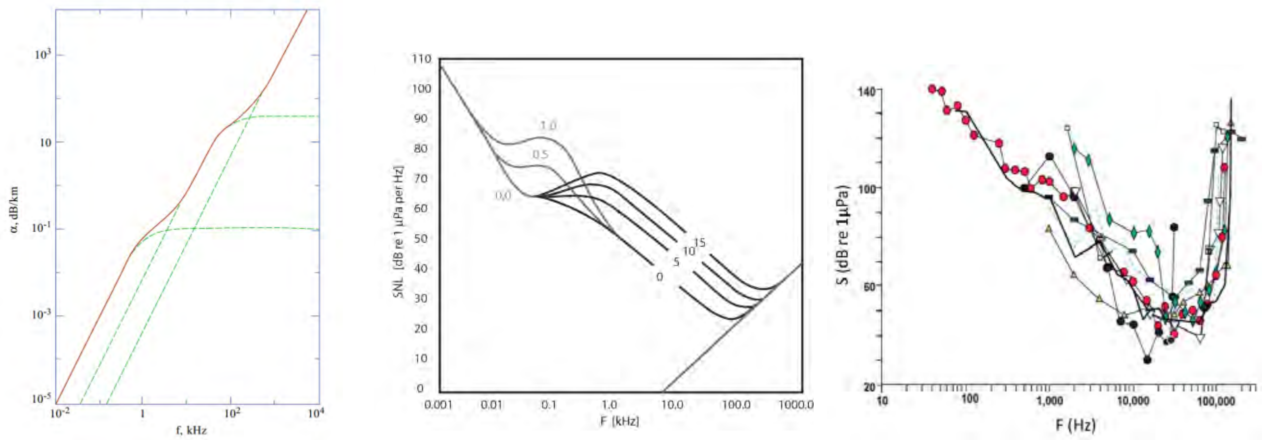

Figure 1. Frequency plots of sound absorption by seawater, showing contributions by $MgSO_4$ and boric acid (left); of underwater noise in terms of shipping noise (from absent=0 to intense=1) and of free surface agitation caused by wind in m/s (center); and of auditory sensitivities of some marine animals (right). All are shaped by either a direct or inverse frequency dependence within the underwater communication bandwidth typically used by living species including man.

Despite being a simple and powerful practical method, frequency analysis is not free of drawbacks when applied to acoustic communication. As opposed to natural sound produced by natural phenomena which are essentially random in time and memoryless, acoustic communication is intended to carry information, which must obey some even primitive form of causality in order to be understood properly. In other terms, the time arrow must be preserved in acoustic communication, possibly at all time scales within both speakers’ and receivers’ capabilities. However, frequency analyses usually do not account for such time arrow, at least within each time segment on which the frequency spectrum is evaluated. As an example, a given time series and its time-reversed version share the same frequency spectra. Reducing segment duration is not an optimal solution in the general case, unless each piece of information contained in the signal can be precisely assigned to a given periodicity. Moreover, frequency analyses of long time signals are usually averaged over multiple time segments, or at most treated using low-order statistical descriptors, to reduce data complexity: in such a way, cause-vs-effect features in the overall signal may be lost.

Acoustic communication and Prosody

Sound in nature may be either spontaneous or intentional. Spontaneous sound includes natural sound, which is a by-product of physical phenomena accompanied by relative motion of matter, either living or inanimate. Intentional sound includes sound generated by living organisms with a specific aim at communicating some information from a transmitter (speaker) to one or more receivers (listener), or in a more general sense to produce some effect on the receivers. A more general classification for intentional sound, which can be applied to living species in a broader sense, is ‘functional sound’. In functional sound, the acoustic wave acts as a carrier and the information is carried in one or more of its physical characteristics (amplitude, frequency, and so on). Since acoustic communication is a relatively slow process developing in time domain, to take advantage of effects arising from its slow propagation speed the information is carried more efficiently using time modulations of its characteristics, instead of using its steady state values. One may compare this with the fact that, as opposed to sound, the extremely faster light waves are normally functional even if their characteristics do not vary with time. As an example, sunlight on a clear daytime is neither useless nor harmful to any living organism on earth, while a fixed-pitch sound lasting for an indefinite time within one day is certainly useless and possibly harmful for any evolved animal and may be supposed to be so for the majority of living species. The information contents, or the functionality of sound produced and listened by living species is then closely matched to time modulations. Such modulations are more complex for organisms with higher ability either as a speaker or as a listener to exploit them: the complexity is highest for humans, and gradually decreases down the evolutionary tree.

The above considerations may be applied, as a special case, to how humans, and possibly other highly evolved species, ‘judge’ sound in nature. Considering natural sound – often referred to as background noise – some sounds may be judged either too repetitive (e.g. noise by rainfall or insects) or too chaotic (e.g. ocean surf or forest winds). The frequency (spectral) contents in this type of sounds is either concentrated (monochromatic) or widely spread (random or ‘white’ noise), and the resulting contained information is low. On the opposite, we may consider the most specialized types of sound produced and perceived by humans: speech and music. For these types of sound, evidences show that fluctuations in at least two of the major components, pitch (frequency) and loudness (intensity) tend to follow the above mentioned 1/f law (associated with ‘pink’ noise), regardless of different cultures and historical periods [7]. This fact suggests that, along with the evolution of life on the planet, there has always been a natural tendency to increase efficiency in acoustic communication by time-modulating its characteristics in the most suitable way.

Beyond this, ‘pink’ noise has been found in many other systems including human response to stimuli, natural images and mental states in psychology. It must be recalled that this behaviour is typical of systems in equilibrium [8].

Speech is the most straightforward context to introduce Prosody, which pertains to time modulations in acoustic communication as described above. Voiced communication among humans slowly evolved from primitive forms, imitating bird singing, to the present astonishing complexity of speech and singing in all its worldwide declinations [9]. A common feature is that physiological properties of the vocal apparatus (vocal tract, lungs), even very subtle ones such as the motion and stiffness of internal parts (e.g. the larinx) are exploited in more or less conscious ways to produce concurrent modulations in time of a set of parameters which collectively produce perceivable effects. Such variables are referred to as prosodic parameters and are recognized to be relevant to identify the information contents – that is, the meaning – of voiced communication [10]. A fundamental set of prosodic parameters is given in Table 1: for each parameter, evaluation is done by measuring either an auditory quantity, related to subjective judgement by a listener, or an acoustic quantity, related to the corresponding physical property of sound.

Auditory (perceptual)	**Acoustic (physical)**
Pitch	Fundamental frequency (Hz)
Length	Time duration (s)
Loudness	Intensity ($W \cdot m^{-2}$) Intensity level (dB re I_{ref})
Timbre	Spectral density ($Pa^2\,Hz^{-1/2}$) Spectral density level (dB re $Pa^2 \cdot Hz^{-1/2}$)

Table 1. Examples of prosodic parameters expressed as auditory (perceptual) quantities and their corresponding acoustic (physical) quantities. For the latter, units are given in parentheses where I_{ref} represents the reference intensity (1 $pW \cdot m^{-2}$ in air).

Prosodic parameters are said to be ‘suprasegmental’, that is they refer to a higher level than the single unit (‘segment’) carrying semantic meaning in the communication stream. In speech, segments are vowels and consonants, and suprasegmental analysis is done starting at syllable level up to words and phrases. Prosodic features may also be divided into personal features (e.g. one individual within one species may intrinsically emit higher pitched sound) and intentional features (e.g. parameter modulation due to a change in emotional state): only the latter carry some meaning, while the former need to be excluded in any semantic analysis.

In addition to the fundamental parameters listed in Table 1, it is possible to introduce more complex parameters which combine some of them: e.g. intonation mostly refers to pitch (frequency), but is also affected by changes in both loudness (intensity) and length (duration). As longer and longer portions of the communication stream are analyzed, from syllables to words and phrases, more features emerge such as stress (at a word level) or rhythm (at a phrase level). All these parameters carry some information which are increasingly intentional by the speaker and therefore are expected to be understood by a listener.

One key feature of prosody is that it precedes linguistic comprehension in the interpretation of vocal utterance by another individual [11]. Information may be transferred even beyond semantic meaning by prosodic modulation: in other terms, knowledge of the semantic contents may be superfluous to determine, if not the precise information, at least the attitude of the transmitting individual. This recognition is done with a relatively high confidence by the receiving individual [12].

The above considerations suggest that the analysis of prosodic parameters might be a powerful tool to extract information about the ‘speaker’ from utterances by any living species, for which the semantic meaning is generally unknown or may be only partly derived for specific pieces of communication which may be related with suitable stimuli. Therefore, an effective analysis of sound produced by living species in general should proceed by means of tools and techniques which fully exploit a suitable number of prosodic parameters, in order to come closer to the intended information contents, made up of both communication meaning and speaker attitude. Regarding knowledge of the latter, a more complete prosodic analysis certainly yields much better confidence than only analyzing few basic parameters such as frequency and duration, as it is customarily done for classification of sound produced by marine fauna.

When it comes to the assessment of underwater noise and to its impact on the marine ecosystem, it is of paramount importance to keep track of a great diversity of sources, each carrying different amounts of functional (i.e. intentional) information. Such sources may range from the natural ones possessing specific characteristics (typically, either ‘white’ noise or monochromatic components) which usually do not carry functional information, to animal vocalizations which are inherently functional and possess more or less developed prosodic features [13]. These sources are pre-existent to the exploitation of sea by man, while new man-made sources came progressively into play in the present industrialized era, whose prosodic features are often closer to natural sources. Indeed, since man-made noise is only a by-product of human activity at sea, it is not meant to be functional (i.e. to carry specific information) so it tends to possess generic features such as being monochromatic (also known as ‘tonal’ noise, which is typical of machinery noise by ships), random (as in the case of cavitaion noise by ships) or highly repetitive (as in the case of high-intensity pulsed sound of sources used in seismic prospecting, or in pile driving). The need for a faithful assessment of the impact of man-made noise on the marine environment would then benefit from prosodic analysis, which is a powerful tool for extracting features of the acoustic communication possessing most of the information contents, that is the highest functionality. New methods for noise impact reduction at sea based on this approach would be expected to concentrate on preserving functionality as a whole, by exploiting a balanced modulation of relevant prosodic parameters in an optimal and sustainable way.

Conclusions

A number of open questions may be derived from the above discussion, with an aim at increasing confidence, reliability and robustness in the assessment of the impact of underwater sound on the marine ecosystem:

- Is there a unique 'safe' sound level in the ecosystems in terms of behavioral impact on life?
- Is loudness reduction alone optimal in reducing behavioral impact?
- Are other prosodic parameters (e.g. pauses, pattern repetitions) relevant for the health of the ecosystems? Do they interplay with each other?
- Is there room to compensate loudness reduction with other parameters' variation, at a lesser cost?
- To what extent living organisms may react to sound signal prosody as we do? At which time scales? Are we able to predict such ability from biological features (e.g. size, age) or other features (e.g. population density, stress factors)?
- Are we able to acoustically 'shape' anthropogenic noise sources by varying their prosodic features to reduce pressure or impact towards animals?
- Is our aim achieving environmental silence *per se* or preserving/restoring a more natural acoustic environment?

Searching for answers to the above questions would help in reaching both a better understanding of communication and a better environmental status. An appropriate observing strategy is needed to infer a general theoretical formulation for the meaning of communication for functioning of the ecosystems.

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The sounds in the marine and freshwater ecosystems: the most effective mean to receive and transmit information

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Abstract

The sound is the most effective means of communication in the marine and freshwater ecosystems. Here, we provide a brief introduction about the importance of sounds in these ecosystems and summarize some case studies as examples of the potentialities of acoustics used both by animals in their natural life and humans to explore the ecosystems. The first case study shows use of passive acoustic monitoring to investigate the spatio-temporal distribution and the population composition of a north Atlantic fish species, the haddock, in the Arctic fjord of Kongsfjorden (where the climate changes are rapidly affecting the ecosystem). In the second case study, the temporal variations of the soundscape, included the human impact component, in a typical Mediterranean shallow water ecosystem is showed. The last study aims to shed light on the role of social communication in the group dynamics of the striped dolphins and to evaluate the hypothesis that the vocal patterns can function as a cue to acoustically distinguish social activity. Sounds in the sea carry an huge amount of crucial information for many animals species, while researchers, listening and applying increasingly innovative techniques, have only recently begun to understand these acoustic "languages" part of the ecosystems.

Introduction

If we close the eyes and listen to the sounds surrounding us in a terrestrial environment, we discover, almost surprisingly, to be able to capture the acoustic aspects of the reality that surround us: for example, we will perceive different sounds if we are in an urban or rural context, if in a closed environment (in a school, in the house or in the supermarket) or outdoors (near the sea or in the mountains). But not only this: through the sense of hearing, we can also obtain information about "when" we are, that is the temporal dimension that we are living in that specific moment. The set of sounds that surround us allows to guess whether it is day or night, whether it is morning or afternoon, or eventually in what season we are. This is because we link the experience of the past and our "anthropological machine" to the live sensing.

Thanks to the intrinsic physical properties of water, in the marine environment the sound speed is four times higher than in the air and sound travels for long distances. Acoustics is therefore one of the most exploited channels by marine animals, not only to communicate at the intraspecific level, but also to collect information from the surrounding world (Tyack 1998).

It is widely demonstrated that not only marine mammals (such as dolphins, whales, and seals), but also a large number of fishes and crustacean species, actively use sounds during certain vital activities (Ladich 2014; Popper et al. 2010). Studies carried out in the natural environment and in the laboratory have identified and described signals emitted by several species in relation to different behavioural context. The meaning of such sounds is in some cases still debated, but scientific studies have allowed to formulate some hypotheses that vary depending on the species and the context studied: dolphins for example, produce two main categories of sounds, tonal whistles and impulsive clicks, used respectively during socialising and feeding contexts (Herzing 2014). Among invertebrates, lobsters produce pulsed sounds called "rasps" that are thought to have the function as contact calls or to warn the conspecifics of the presence of predators (Buscaino et al. 2011). Among fish, in the Scienidae family drum signals are emitted by males to communicate to females their availability for mating or to acoustically delimit their territory sending a signal to rival conspecifics (Ramcharitar et al. 2006).

The sounds emitted by different marine species are characterized by frequencies ranges within or outside the sound bands audible to the human ear. In some cases, the signals can extend over wide frequency ranges, from hundreds Hz to hundreds of kHz. The frequency characteristics of the sounds emitted can vary accordingly to the different communication ways and the spatial distribution of the different species. For example, some whales performing long migrations between the hemispheres, maintain contact between the individuals and look for the potential partner using signals at very low frequencies (of the order of few Hz), which can propagate even for several kilometres (Širović et al. 2007).

Not all the biological underwater sounds are emitted with a specific communicative function. In some cases, acoustic signals are involuntarily produced during certain activities, such as feeding, competitive clashes or swimming. On these occasions, the occurrence of such sounds may be a cue of the level of activity of the species that produces them. This includes the sounds produced by the so-called snapping shrimps, a term used to indicate the set of crustaceans belonging to the Alpheidae family. The snapping shrimps are characterized by a big claw, whose opening and quick closing generates impulsive signals in the frequencies between 2 and 40 kHz (Versluis et al. 2000). Such signals are produced during feeding activities and are generated at such energy that is thought to stun the prey. In temperate seas, the number of pulses recorded is such as to dominate the acoustic environment, reaching a rate of 2000 pulses per minute and following seasonal and circadian trends indicating the level of crustaceans activity (Lillis and Mooney 2018, Buscaino et al 2016).

Recent acoustic researches are shading light to more and more fascinating phenomena. It has been revealed that acoustic signals can be generated not only by animals, but also by some plant species. For example, a relationship has been found between the process of seaweeds photosynthesis and some impulsive sounds (Freeman et al. 2018).

The sounds produced by marine organisms can therefore be the expression of the life present in the seas and they constitute what is called an acoustic community (Farina and James 2016). This concept, translated from ecology and projected into a purely acoustic context, reflects all those ecological phenomena that occur under the sea surface and varying in time and space.

However, sounds in the marine environment come not just from biological origin. Some geophysical phenomena, such as rain, wind and earthquakes, or ice movements and their melting, determine sounds that become peculiar of some environments and change in relation to the different periods of the year (Wenz 1962). These sounds can be perceived by the organisms inhabiting the sea, that obtain key information for their survival.

Also humans carry out activities that produce acoustic signals, which interfere with natural sounds. Not only boats using, but also industrial activities, linked to energy production or the development of new infrastructures, generate noise affecting marine species (Hawkins and Popper, 2017).

The magnitude of these effects may depend on several factors, such as the intensity of the noise or the duration of exposure to the sound stimulus. One of the most obvious effects is the reduction of the perception space of species, through a phenomenon that is called masking. This can makes both communication between specimens and the perception of environmental sounds less effective (Clark et al. 2009). The noise can also interfere at the physiological level, causing both an increase in stress levels, and, in extreme cases, physical damage that can lead to the death of organisms (Kunc et al. 2016).

Many studies are now showing the negative effects of underwater noise on species. It should be noted that the reaction (physical or behavioural) of organisms to the acoustic impact, not only can be direct and immediate, but can also have effects on subsequent generations, interfering on the survival of populations (Blom et al. 2019). New environmental protection strategies have recently defined anthropogenic noise as a form of pollution, that needs to be monitored to avoid the exceedance of acoustic thresholds with the risk of irreversible impact on different marine species (EU Marine Strategy Framework Directive)

The sounds described so far can be categorized as of biological (biophones), geophysical (geophones) and anthropogenic (anthropophones) origin and they characterize and determine what, in recent years, has been identified as acoustic landscape or soundscape. Defining the soundscape concept, even in a marine environment, underlies that the different sound sources cannot be considered only as components independent of each other, but as part of a system in which the elements are all interconnected and related, reflecting the life of the ecosystem they characterize. The different sources coexist, influence each other and they are both produced and perceived at the same time by the species.

In complex ecosystems, such as forests in the terrestrial environment or Posidonia meadows in the marine environment, where the number and intensity of biological sounds is very high, strategies of partitioning of acoustic activity have been observed to reduce sounds overlap and allow effective transmission of information (Ceraulo et al. 2018, Buscaino et al 2016). In such ecosystem, the acoustic space is divided in terms of time windows and/or frequency bands. For example, when the songs of different species occupy the same frequencies, they are emitting shifting temporally to avoid overlapping, starting only at the end of those of the previous species; or again when several species share the same temporal windows, the signals are emitted on frequency bands that do not overlap. Such time and frequency subdivisions define the so called “acoustic niches” (Krause 1993).

The set of sounds occurring in a certain underwater environment determines its “acoustic signature”, that makes identifiable peculiar acoustic habitats (Farina and James 2016). Recent researches have highlighted as several fish and invertebrate species use such signature for orienting in the space and for heading towards the environments functional to their vital development (Slabbekoorn and Bouton). Studies on crustacean species are showing, for example, how the choice of suitable environments for recruitment and growth is conveyed by the sound of conspecifics, may be inhibited by the occurrence of predators sounds and disoriented by boat noise.

The field of study that deals with these concepts is the soundscape ecology, a discipline that is developing in recent periods and is opening our eyes (and ears) to a greater understanding of phenomena that are difficult to grasp as a whole. This has been possible thanks to the development of new technologies for building up autonomous acoustic recording systems adapted to the aquatic medium, which, released in the bottom of the sea, can collect acoustic information, even outside the range of human ear hearing.

The identification of the signals and their attribution to the different species lead to obtaining relevant information on the ecosystem ecological status. For example, it is possible to detect the presence of certain species, their spatial and temporal distribution and, in some cases, the reproductive or courting periods, critical for their survival.

One of the greatest strengths of the acoustic method is the ability to gather information without any direct influence on the environment, revealing phenomena on a large spatial and temporal scale. Thanks to autonomous long-term recorders, humans can become a silent listener, able to grasp events in the marine environment, which would not be perceptible with traditional methodologies.

Case Study 1: Passive acoustics to study the spatio-temporal distribution and the population composition of haddock (Melanogrammus aeglefinus) in the arctic fjord Kongsfjorden (Svalbard) (modified from Buscaino et al. 2020)

The haddock (*Melanogrammus aeglefinus*) is a Gadidae fish and is a significant species for the fishery industry. It lives at depths between 80 and 200 m and at temperatures between 2 and 10 °C (Olsen et al. 2010) in the Atlantic and the Barents Sea. Analogous to other gadoid species (Hawkins & Picciulin 2019), the haddock produces sounds containing of a series of pulses (knocks) composed of two short pulses at low frequencies (Hawkins & Amorim 2000). Studies made in tanks discovered that the sounds are usually produced by males during patrolling displays, which are both a territorial and a spawning behaviour in haddock (Casaretto et al. 2015). Also, female and juveniles produce sounds. Casaretto et al. (2016) demonstrated that the structure of the double pulses varies according to sex and fish maturity (Fig. 1). Differences in haddock sounds are caused by sexual dimorphism in the generating mechanism, with drumming muscle size varying according to sex, age, maturity and season (Templeman & Hodder 1958, Casaretto et al. 2016).

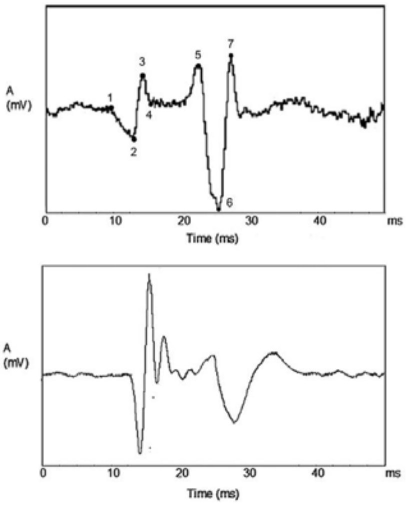

Figure 1. Oscillograms of female (above) and male (below) haddock sound units. Credit: Casaretto et al. 2016.

In the Svalbard Archipelago (Norway), the haddock represents the third most important fish species captured by the fishing industry. Recently, an increase in haddock captures was recorded. This can probably be partially attributed to the climate change with a northward shift of some species (Berge et al. 2015, Misund et al. 2016). Although the haddock areal comprises the Svalbard Archipelago, Kongsfjorden (79° N) is the northernmost limit of its distribution (Pethon & Nyström 2005, Dalpadado et al. 2009). In this fjord, the inmost colder Arctic water masses (glacier site) coming from glacial mix with warmer ouside Atlantic waters (open water site); therefore, an environmental Arctic-Atlantic gradient is generated from the inmost part of the fjord to its mouth (Svendenson et al. 2002).

In this study, using autonomous recorders sited in three sites (glacier, middle and open water sites, see the Appendix for more details) of the Artic Kongsfjorden, we (I) characterize haddock sounds (II) show the spatio-temporal distribution of the haddock by using its calls, (III) discover the correlations between haddock sounds and external physical factors such as tide and solar elevation and (IV) use acoustic data for identify fish age and sex.

In total, we analised 7751 hours of recordings covering a period of one year (from April 2014 to March 2015). We did not record any haddock sounds near the glacier site, whereas were more abundant in the open water site than in the middle fjord (see Fig. 2).

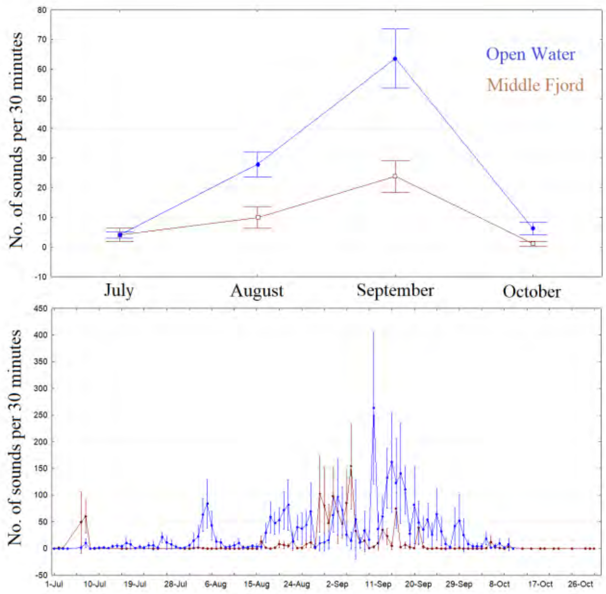

Figure 2. Upper: Mean (±standard error) number of haddock sounds in 30 minutes for each month of acoustic activities. Lower: Mean (±standard error) number of haddock sounds in 30 minutes on each day of sampling. Credit: Buscano et al. (2020).

The haddock sound abundance showed strong temporal pattern with a correlation with the cycles of neap tide (15 days) in August, with a clear diel cycle (24 hours) in September and October (Figure 3). This result suggests that in this extreme environment with 24 hours of light during summer, haddock adjust their acoustic activity according to the main available oscillating external physical driver, such as tide during the polar summer, while when the alternation of light/dark starts, they shift the periodicity of their calls to a diel cycle.

Calls were recorded outside the spawning period (from July to October), and their characteristics indicated non-reproductive communicative contexts. By using a detailed sound analysis based on previous laboratory studies for the first time, we suggest that the monitored population contains mainly juveniles (44% compared to 41% females and only approximately 15% mature males), showing the predominance of females in the middle fjord and juveniles at the open-water site (Figure 4).

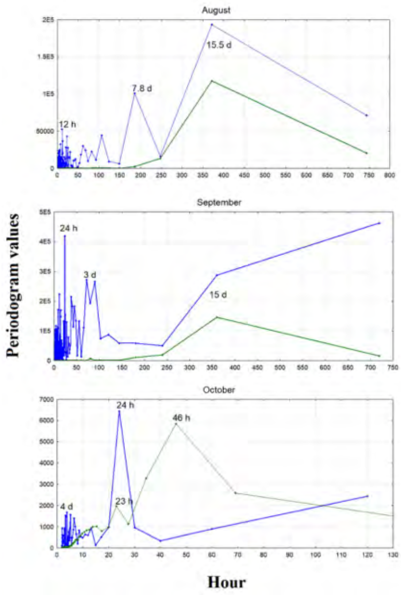

Figure 3. Periodograms, as showing the results of spectrum analysis, for haddock sound count (blue line) and DE-Tide (green line), considering August, September and October of open water site. Modified from Buscaino et al. (2020).

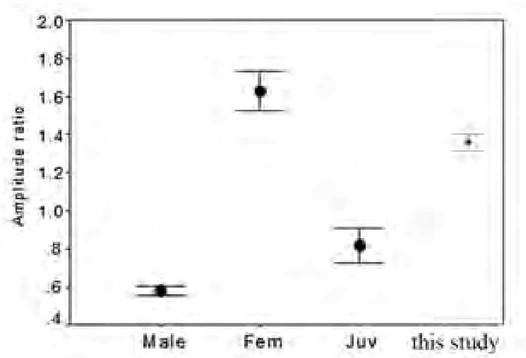

Figure 4. Amplitude ratio of haddock sounds recorded in an aquarium (Casaretto et al 2016) and in this study. This study: Mean (error bar: 0.95 confidence interval) values obtained for the open-site (light blue) and middle-fjord site (brown). Modified from Buscaino et al. (2020).

Case Study 2: the temporal variation of soundscape and human impact (modified from Buscaino et al 2016)

This study explores the soundscape of a typical Mediterranean shallow water ecosystem sited in the Capo Grecale Marine Protected Area (MPA) of Lampedusa (Sicilian Channel).

The main aims of this study were a) to investigate the seasonal and circadian patterns of octave band sound pressure levels (BSPLs), b) discover the main biological, physical and human sound sources c) test the automatic acoustic complexity index (ACI) (Pieretti et al. 2011) to describe the biotic contribution to the soundscape, and d) quantify the percentage of time in which fish choruses are masked by vessel passage noise.

Figure 5 shows the mean power spectrum of the whole data set, one-year (black line), or the winter (grey line), or the summer (blue lines). For all frequency bands, significant differences between summer and winter were found (Mann-Whytney U test, p<0.05). This seasonal variability was mainly attributable to the sea state for the lower frequencies up to 1 kHz (goodness of fit of the linear regression r > 0.49 and p-value<0.05) and to the activity of snapping shrimp for the higher frequencies (goodness of fit of the linear regression r > 0.49 and p-value<0.001).

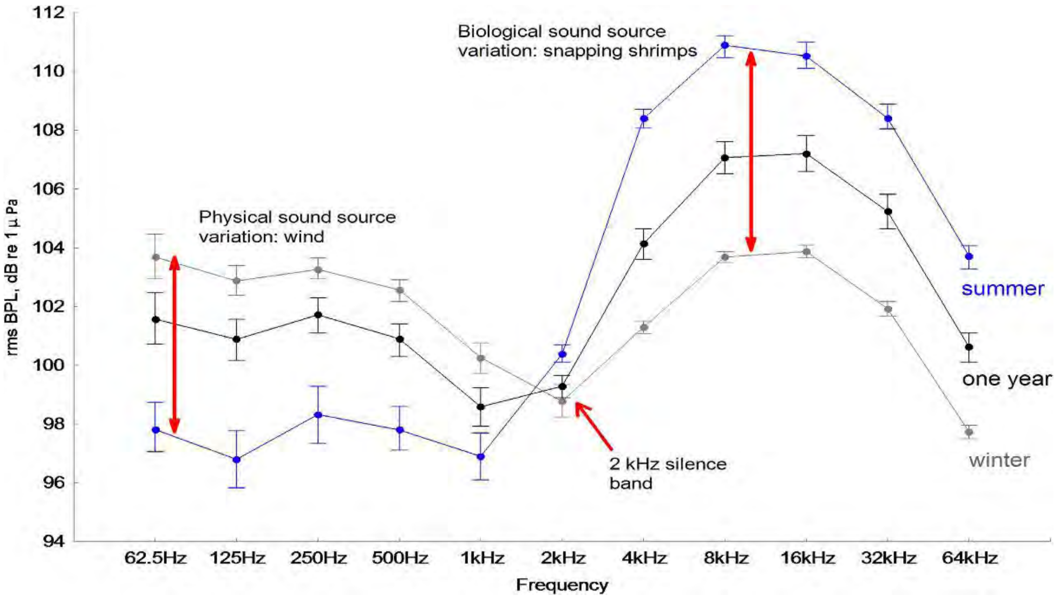

Figure 5. Seasonal trends of rms BPLs for all data (one year), summer data (July, August and September) and winter data (December, January and February) (Median; Whisker: 45th–55th percentile). The differences between the summer and winter for each BPL are significant (Mann-Whitney U test, p < 0.001).

In Figure 6 (upper panel) we show the fish vocalizations count (the grey area), the 1 kHz BSPL (black line) and the ACI (blue line). The fish counting peaks at sunset are in line with the ACI peaks (blue line) and with the correlations in the octave bands occupied by fish sounds. In the lower panel of Fig. 6 the number of pulses produced by snapping shrimp (grey area), the 4 kHz BSPL and the ACI are shown. Here, a marked circadian pattern, with peaks during sunset and sunrise are well visible. The number of snapping shrimps pulses and the circadian pattern decreased during the winter. Snapping shrimp sounds (Fig. 6) were well-correlated with both the BPL and ACI values in the corresponding octave bands.

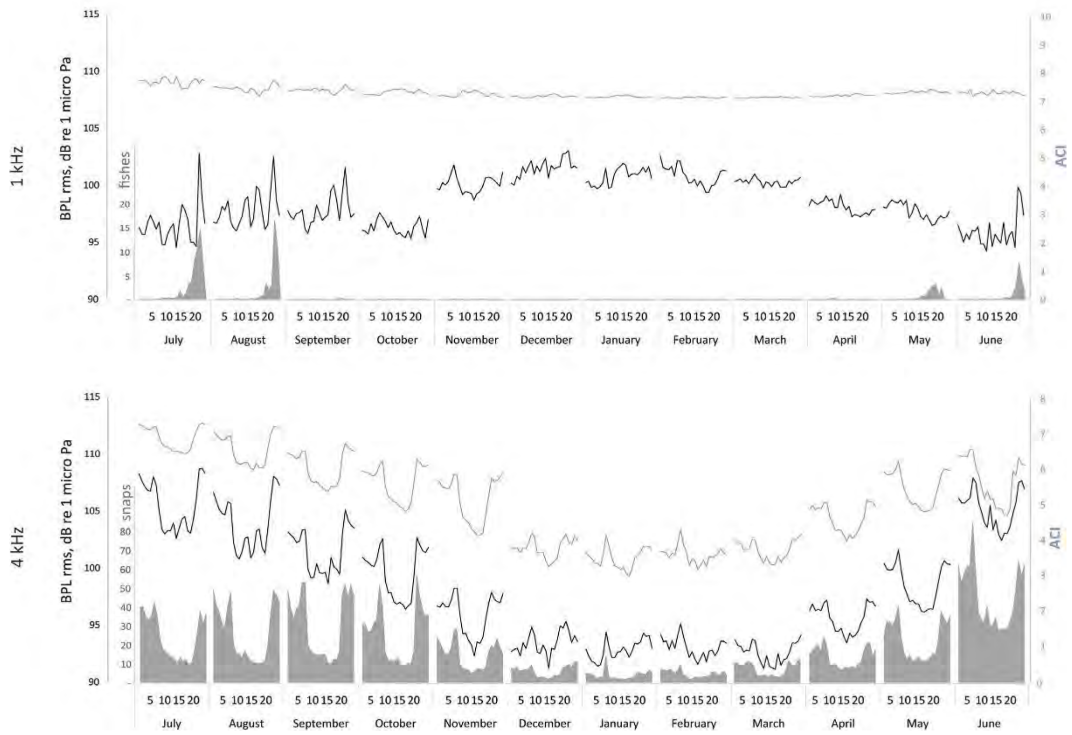

Figure 6. Upper panel: BPL-1 kHz (black line), ACI (blue line) and fish vocalization counts per minute (grey area). Below panel: BPL-4 kHz (black line), ACI (blue line), and snap counts per minute from snapping shrimp (grey area). X-axis: hour of the day for each month. Credit: Buscaino et al 2016.

The main source of anthropogenic noise are the vessels with a mean of 13 passages per hour. During the fish choruses' season (mainly summer at sunset), 46% of the files included vessel passages with a potential masking effect. In Figure 7 the spectrogram of two continues recording is shown. Here, we have an overview of the frequency band partition for the different components of the soundscape. Snapping shrimp (S), the grey clouds up to 2.5 kHz, showed increased activity during sunset and sunrise. Fish choruses (F), below 1.5 kHz, are represented by the smallest grey clouds during sunset. Vessel passages (V), which very often masked all the frequency bands (see the vertical black lines), represented the strongest sound in the soundscape (blackest signals).

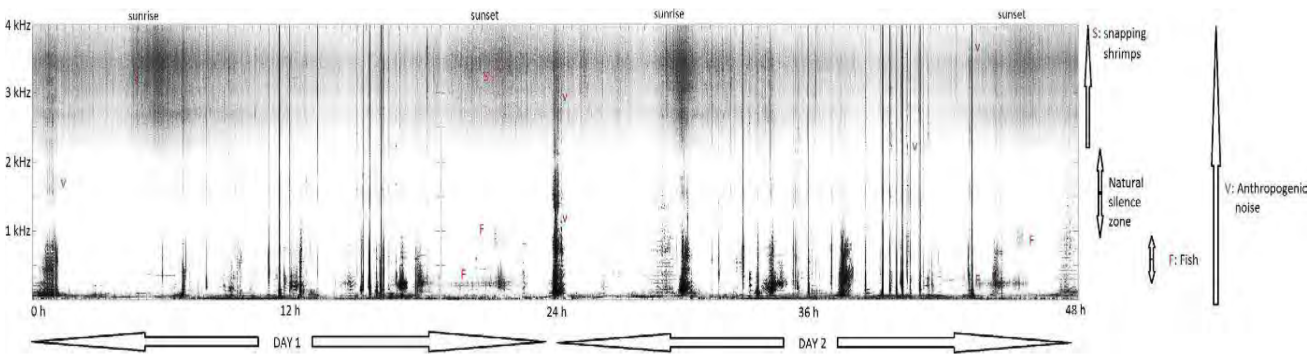

Figure 7. Spectrogram of two-day continuous recording (11 and 12 July 2014) showing: the snapping shrimp sound (S), fish choruses (F) and anthropogenic noise caused by the passage of vessels (V). The spectrogram

was obtained using the XBAT software (Cornell University. USA). x-axis: time; y-axis: frequency 0–4 kHz; SPL intensity is shown in greyscale. Credit: Buscaino et al 2016.

Case study 3: Striped dolphins' vocal complexity: the pivotal role during social context (modified from Papale et al. 2020)

Gregarious species require complex patterns of communication for maintaining coordinated behaviors, group cohesion and an articulated social structure (Brudzynski, 2014). Dolphin species can represent a useful model for studying social communication, since they exhibit extensive size range in social group and aggregations, in which individuals maintain dynamic relations and affiliations (Gazda et al 2015). Vocalizations are a pervasive feature of odontocete social life and are used to interact with conspecifics. Increased vocal complexity has been observed in terms of calling rate (dos Santos et al., 2005), whistle modulation (May-Collado et al., 2007) and presence and number of complex signaling (Nemiroff 2009; Wellard et al., 2020). These latter were defined as the combination of more than one signal or component, often stereotypically repeated and overlapped (Hebets and Papaj, 2005), that have been suggested to provide directionality and identity cues, crucial during social interactions in large groups (Filatova et al., 2013; Papale et al., 2015; Kaplan et al., 2018). Even if *Stenella coeruleoalba* is a worldwide species that lives in large social groups, there is a distinct paucity of studies about its vocal complexity. For the species, the study of communication during different social activities can reveal dynamic changes in signal structure across contexts (Wilkins et al. 2015, Papale et al 2017). To that regard, this study aims to shed light on the role of social communication in the group dynamics and evaluate the hypothesis that the vocal patterns can function as a cue to acoustically distinguish social activity.

Acoustic recordings of *S. coeruleoalba* were collected during 32 encounters: 5 during resting, 13 during socializing, 10 during travelling, and 4 during feeding. In Table 2, the presence and number of vocalizations were summarized. Complex signaling seems to be more present and flexibly used than previously thought since they were never reported for the species. They were made up of a combination of whistle with burst pulsed, whistle with click train, or two tonal fundamental frequencies and prevalently occurred during socializing activity (Table 1, Figure 8).

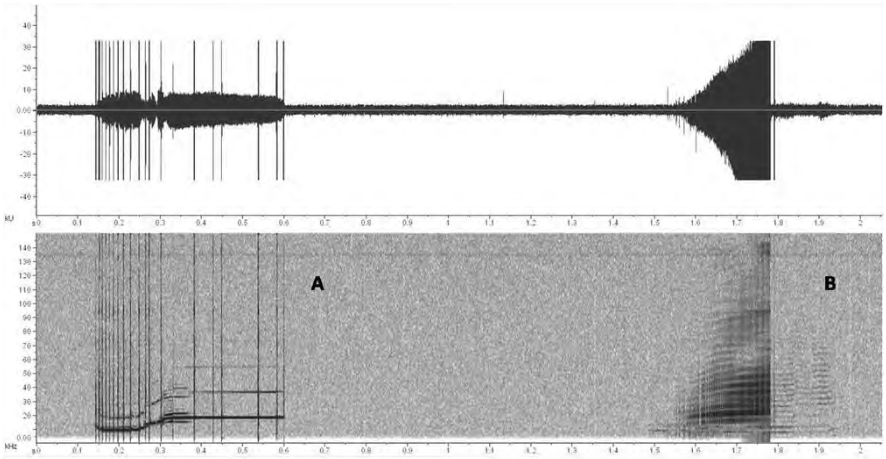

Figure 8. Waveform and spectrogram of complex calls: A. Biphonic signal combination of whistle with click train, B. Biphonic signal combination of whistle with burst pulsed (from Papale et al 2020)

The calling rates of clicks did not show variations during the different activity classes (estimate among -0.37 and 0.31, t-value among -0.62 and 0.58, p>0.5), while the one of whistles and burst pulses were significantly higher during socializing encounters (Kruskall Wallis test p<0.001, p= 0.03 respectively) (Figure 9). The observed differences were in the range of what reported for *S. frontalis*, *T. truncatus* and *Delphinus delphis*: a higher number is usually recorded during high-intensity social activity (Jacobs et al., 1993; Jones and Sayigh, 2002; Cook et al., 2004; dos Santos et al., 2005; Gridley et al., 2016).

	# encounters	# clicks	mean clicks rate (sd)	#whistles	Mean whistles rate (sd)	# bursts	mean bursts rate (sd)	# biphonic calls	mean biphonic calls (sd)
Travelling	10	30637	8.96 (6.41)	1728	0.35 (0.28)	341	0.18 (0.38)	258	0.10 (0.15)
Feeding	4	12358	5.76 (8.54)	486	0.21(0.35)	180	0.07 (0.13)	21	0.01 (0.01)
Resting	5	5690	5.27 (6.35)	262	0.17 (0.10)	48	0.04 (0.05)	22	0.01 (0.01)
Socializing	13	102293	10.48 (6.63)	7445	0.99 (0.61)	1857	0.34 (0.33)	819	0.16 (0.12)

Table 1 Number of encounters and vocalization (mean (Sd) calling rate (calls/[minutes*group size]), and number of calls) per activity class.

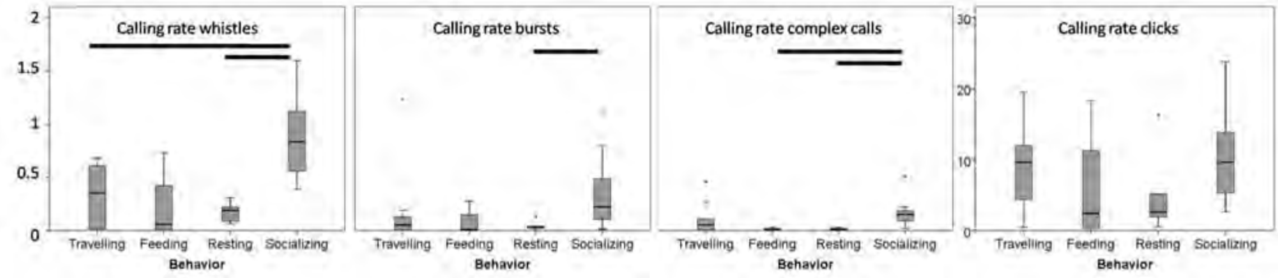

Figure 9 Box plots of the calling rate of the different vocalizations in relation to the activity classes (from Papale et al 2020).

422 whistles were selected for analyzing acoustic parameters. Whistle modulation represented by the number of inflection points, the number of steps and the number of minima and maxima, supported the discrimination of the activity classes. During socializing, whistles were characterized by a more complex modulation compared to signals emitted during feeding (Tamhane test: number of minima and of maxima $p < 0.001$) and travelling contexts (Tamhane test: number of maxima $p = 0.006$) (Figure 5). The activity classes significantly affected the number of maxima (LM: Conditional model Estimate = 0.285, $p < 0.001$), while duration and the number of minima were influenced by the combined effect of both activity and group size (LM: Conditional model Estimate = -0.153, $p = 0.008$; Conditional model Estimate = -0.448, $p = 0.002$). As already observed for *S. frontalis* and *T. truncatus* (López and Shirai, 2009; Azevedo et al., 2010; Papale et al., 2017), also the striped dolphins emitted whistles more modulated when engaged in social activity and when the number of individuals increased. The increase in whistle modulation during social events, as the presence of stereotypically repeated biphonic signals, suggests that animals use more complex calls to communicate signaler's position, identity or group belonging. Even if a relation among the group size and the activity is not clear, the number of individuals influenced the signal modulation. Large groups engaged in other activity classes than socializing, imply a high level of interaction among the individuals. Therefore, it is likely that social interaction functional to different behaviors, could be the driving force of the acoustic complexity more than the pure socializing. Results of the study suggest that complex vocal patterns, in the form of high calling rate, signal modulation and biphonic signaling, can mediate social interactions in *S. coeruleoalba*. The outcomes from this study state how vocal complexity carries out a pivotal role during social context and support the possibility to detect and remotely investigate potentially sensitive periods and areas for social interactions.

Discussion

The sea is not a silent world and every ecosystem has its own soundscape signature changing with time, for example following the day-night cycle or alternation of seasons, and along the space (vertically, horizontally). Sounds can be generated by animals (biological sounds), natural events (geophonies), and humans (anthropogenic noise). The sum of these sounds composes the soundscape. In some ecosystems, the dominant physical and biological components of the soundscape are known but, for most of them, we are far from an accurate understanding of underwater sound "code". Collecting more data and improving the automatic analysis, also applying data analysis techniques already used in fields other than bioacoustics, will improve this understanding. Passive Acoustic Monitoring (PAM) is a valid technique for monitoring and explore all the ecosystems, especially those that are extreme (for example the deep sea, the high latitudes environments) where others methods cannot be achieved for logistical difficulties, requiring very high cost/time effort. Moreover, studying the recordings, often it is not possible to attribute the origin of a peculiar sound. For this reason, the development of new techniques and data analysis, preferably coupling sea and tank experiments, is needed to identify sounds emitters, generating mechanisms, meanings of the signals and the coupled functions.

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Appendix

Materials and Methods Case 1

Kongsfjorden is a glacial fjord of the Svalbard Archipelago (79°N and 12°E; Fig. 10). In this fjord, the inmost colder Arctic water masses coming from glacial mix with warmer ouside Atlantic waters; therefore, an environmental Arctic-Atlantic gradient is generated from the inmost part of the fjord to its mouth (Svendenson et al. 2002). Although the haddock areal comprises the Svalbard Archipelago, Kongsfjorden is the northernmost limit of its distribution (Pethon & Nyström 2005, Dalpadado et al. 2009).

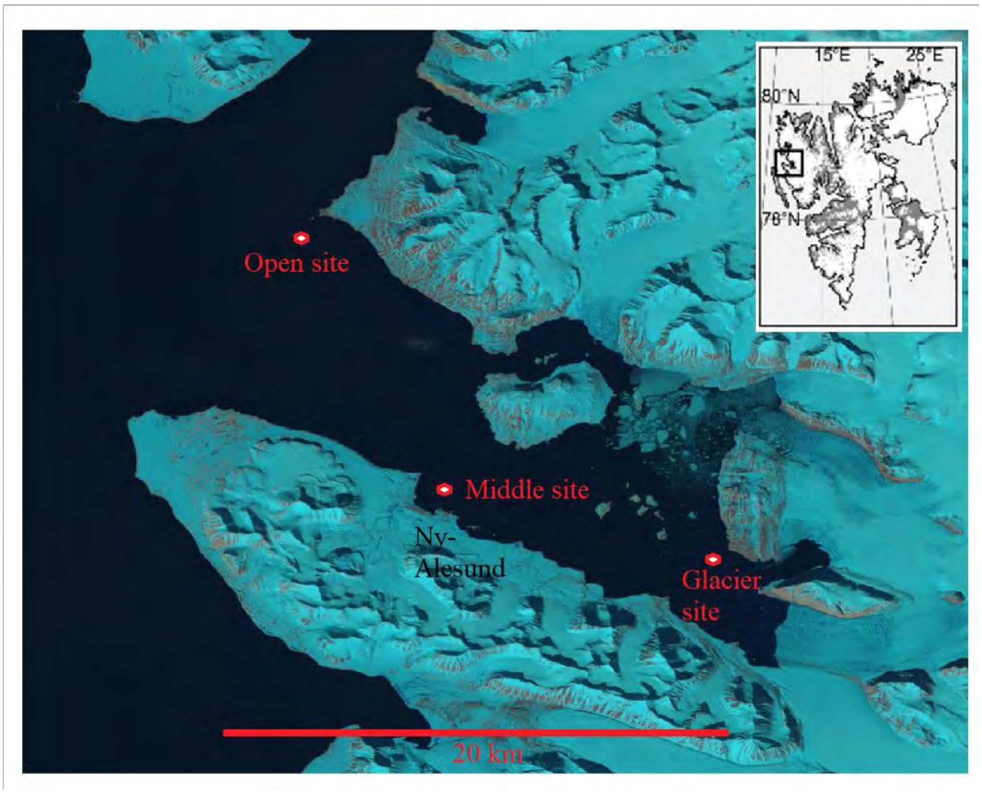

Figure 10. Study area: in the insert, the Svalbard Islands are represented, while in the principal image, Kongsfjorden, Western Svalbard, with the position of the recorder sites (red dots). (Credit: U.S. Geological Survey, Department of the Interior/USGS).

Data were collected from April 2014 to March 2015 by using three autonomous passive acoustic recorders deployed at three different sites (Fig. 10): the glacier site, was located in the inner part of Kongsfjorden at 4 km from the Kronebreen glacier front; the middle-fjord site was positioned in the middle part of Kongsfjorden; and the open-water site was positioned at the mouth of the fjord. We set the sampling frequency at 48 kHz with a resolution of 16 bits.

Data were examined using two Matlab codes with the aim of detecting and characterizing the haddock sounds. Following the studies of Casaretto et al.(Casaretto et al. 2015, 2016), we measured these parameters (see Figure 11): (1) pulse duration: total duration of the double pulse measured as the temporal difference between points t7 and t1; (2) the frequency of the first pulse of a knock, calculated as $f_1 = 0.5(t3 - t2)^{-1}$; (3) the frequency of the second pulse of a knock, calculated as $f_2 = 0.5(t7 - t6)^{-1}$; (4) difference in amplitude between the first and second pulses, calculated as (a7 −a6) (a3 −a2)$^{-1}$; and (5) the pulse interval, i.e., the interval between the start of the first pulse and the start of the second, calculated as t6 −t2.

Variations in pulse amplitude are indicative of the maturity and sex of the fish. Accordingly to Casaretto et al. (2016), pulses with an amplitude ratio lower than 0.8 were assumed to be produced by haddock males, sounds with an amplitude ratio ranging between 0.8 and 1.1 were assumed to be produced by juveniles, and all the sounds with an amplitude ratio higher than 1.2 were assumed to be produced by haddock females.

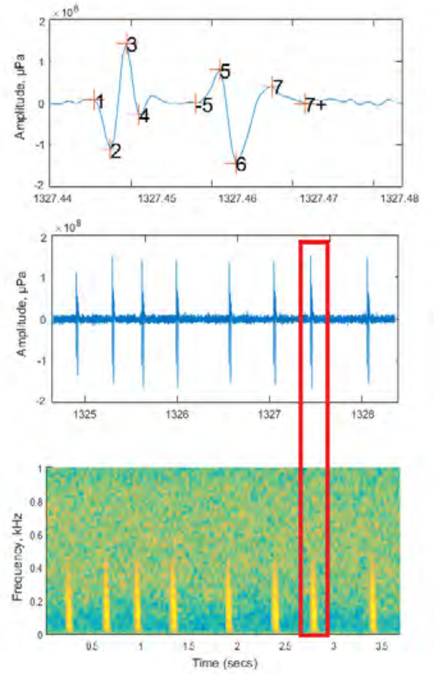

Figure 11. Upper: waveform of a double pulses sound, the knock, from a train. The numbers indicate the points at which time (t) and amplitude (a) measurements were taken. Middle: Waveform of the knock train (red rectangle is the selected sound). Lower: Spectrogram of the knock train (FFT length: 128, overlap: 100, sampling frequency 2000 Hz). This visualization was used by an operator to check the accuracy of the detection and the localization of the points from 1 to 7+ in the waveform. Credit: Buscano et al. (2020).

We extracted the online tide tables for Kongsfjorden (https://www.kartverket.no/), then to obtain the down envelope tide (DE-Tide) we used the MATLAB function Envelope (designed by L. Wang). DE-Tide is the oscillation of tide amplitude (in this case, the lower amplitude, or neap tide). Neap tide occurs every 15 days, when the sun and the moon form a right angle.

We also calculated the solar elevation angles with respect to the horizon line. Positive and negative values of the solar elevation angles indicate the presence and absence of solar radiation, respectively, while the absolute value is a proxy of the distance from the horizon.

To explore temporal patterns in the abundance of haddock sounds, we applied single-series spectrum analysis.

Materials and Methods Case 2

The Sicilian Channel (see Fig. 12) connects the Eastern and Western Mediterranean basins and is considered an area of high biogeographical (Bianchi 2007) and hydrodynamic importance (Béranger et al. 2004). Lampedusa island is an area where coexists flora and fauna of the warmer eastern basin and the west, which is influenced by cold Atlantic currents. In the Capo Grecale MPA human activities have been prohibited (no-entry zone) since 2002. This MPS covers a sea surface area of approximately 0.81 km^2 (see Fig. 12). The bottom in this area consists of a mix of sand, rocks, and Mediterranean seagrass (Posidonia oceanica).

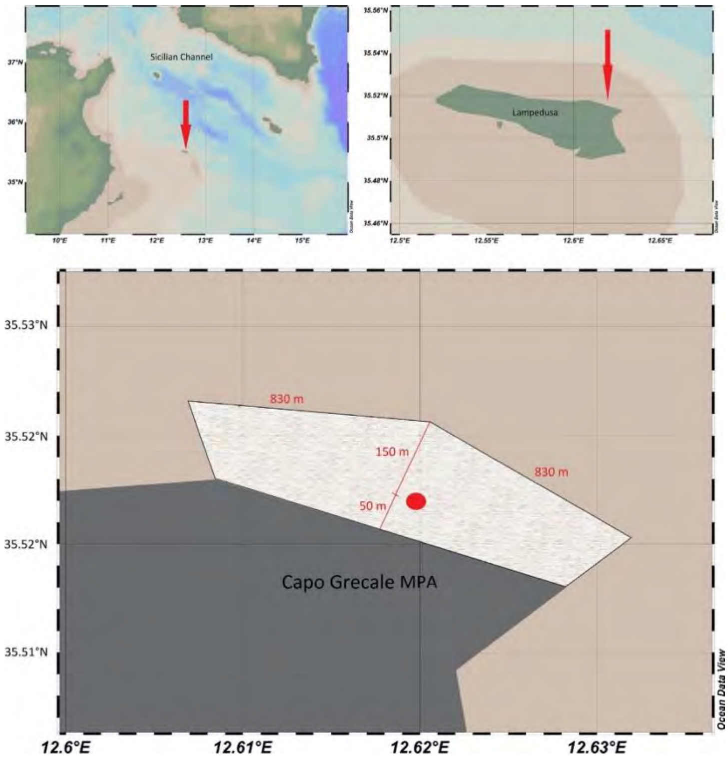

Figure 12. Top: The study area: Topleft-Lampedusa Island in the Central Mediterranean Sea; Topright-Lampedusa Marine Protected Area (red arrow). Below: Capo Grecale MPA delimited by yellow buoys with the spacing shown. The recorder position (red point) was near the midpoint of the MPA. (Map source: Schlitzer, R., Ocean Data View, odv.awi.de, 2015). Credit: Buscaino et al. 2016.

Data were collected for one year (July 1, 2013, to June 30, 2014) using an autonomous recorder (SM2, Wildlife Acoustics, US, high frequency option). The recorder was placed at the halfway point of the longer side (approximately 1600 m) of the Capo Grecale MPA (see Fig. 12), 50 m from a cliff and 150 m from the borders of the protected area (Fig. 1; 35°31.27′N, 12°37.67′E). We set the sampling frequency to 192 kHz (resolution 16 bits). We sampled all day, setting a duty cycle of 2 minutes of recording (wav files) and 28 minutes of no recording.

For each 2-minute file, we calculated the average octave band sound pressure level (BPL, dB re 1 μPa, rms) beginning at the 62.5 Hz central frequency. We used a non-linear frequency band partition to obtain higher resolution at low frequencies, which showed greater variability than the high frequencies. Moreover, 1/3 octave band analyses were performed within the framework of Descriptor 11 of the European Marine

Strategy Framework. The Mann-Whitney U test (Statistica v.8 software package, USA) was used to assess significant differences between daytime and night-time during different seasons at different BPLs and between winter and summer.

To better characterize the biophonic component of the soundscape, we processed the dataset using the acoustic complexity index (ACI) (Pieretti & Farina 2013). In this study, ACI was tested as a metric for detecting the possible presence of biological sounds, with the aim of isolating the biophony from the anthropophonic and geophonic components of the soundscape. To define the correlation of the main soundscape components on the different BPLs and ACI, linear correlation analysis was performed using the Statistica v.8 software package (USA).

The files were analysed by an operator and/or by dedicated automated analyses (*ad hoc* developed Matlab codes) to identify the main biological (snapping shrimps and fish pulses), physical (wind and wave noise) and anthropogenic (vessel passages) sound sources and to count each acoustic event.

Materials and Methods Case 3

Acoustic recordings and behavioral data were collected on board of a catamaran research vessel during standardized surveys in 2016, 2017 and 2019. The focal group was defined as aggregation of dolphins engaged in the same activity within 100 m from the boat. Since the group size estimation ranged from 10 to 80 individuals, three classes were considered as: group 1 = $x < 20$ individuals, group 2 = $20 \leq x < 35$, group 3 = >35.

The sampling of activity state of the focal group was carried out by instantaneous scanning and assigning the predominant group activity state. This step was repeated every 3 minutes for at least 5 times (for a total session of 15 minutes). The prevalent behavioral category during the entire session was considered as the activity state of the focal group during the encounter (Table 1).

Activity class	**Observed behaviours**
Feeding	Dolphin involved in chases or captures of prey items close to the surface, showing erratic movements at the surface, multidirectional diving and rapid circle swimming.
Resting	Dolphins observed in a tight group (<1 body length between individuals) stay close to the surface, emerging at regular intervals and swimming very slowly.
Socializing	Physical interactions ranging from chasing to body contact, such as rubbing and touching or copulation among dolphins. Aerial behaviours such as breaching frequently observed.
Travelling	Dolphins persistently swimming in the same direction at sustained speed and making noticeable headway.

Table 1. Activity class defined by observed behaviors (Papale et al 2020).

Acoustic data were collected simultaneously to behavioral data using a mobile recording system made up of a pre-amplified omnidirectional hydrophone (ColmarGP0190) with a sensitivity of -175±5 dB re 1V/µPa among 5 and 170 kHz. Also, an autonomous recorder RASP12 (Nauta^{tm}) was deployed during summer 2017 from the boat by using the same data collection protocol. It was made up of a pre-amplified omnidirectional hydrophone (Sensor Technology SQ26-05), with a sensitivity of -168.8±5 dB re 1V/µPa among 100 Hz and 50kHz.

Data analysis

For the single clicks and the train of clicks identification and count, the Pulse Train Analysis in Avisoft SAS-Lab Pro (Avisoft Bioacoustics, Germany) was applied. In order to exclude possible false positive errors due to noise presence or clicks emitted in burst pulses, an expert operator visually corrected data for all the recordings. Whistles and burst pulses with a good signal-to-noise ratio were detected and counted through visual inspection by two expert operators in iZotopeRX3. Overlapped multiple frequency-modulated and or pulsed components were detected and classified as complex calls. The call was considered only if it was clearly independent by other signals or if it was stereotypically repeated, since signals can randomly overlap because of simultaneous emissions by more individuals. The overlap of the components could not necessarily start and end together but they were never recorded separately.

The calling rates of signals were calculated for each sighting as the (number of signals/minutes*group size).
In order to measure the parameters and describe signal complexity, whistle not overlapping with others and completely recognizable in their time-frequency contour were selected. Whistles with similar contours were measured only once. Ten parameters (time duration (in seconds), beginning frequency, end frequency, maximum and minimum frequency of the contour (in Hz), frequency range (as the difference among maximum and minimum frequency), number of inflection points (defined as the point where a slope change occurred), number of steps (a discontinuity in frequency), number of maxima and minima in the contour (local maxima and minima within the contour)) were measured by visual inspection of the spectrogram.
Clicks calling rate under different behavioral contexts was examined by using Generalized Linear Mixed Models with a Penalized-Quasi Likelihood with *glmmPQL* package in R (R core Team) because of the different features of the two hydrophones. A Stepwise Cross-Validated Discrimination Function Analysis was performed to investigate the possibility of distinguishing behavioral activities classes through the parameters of whistles. Finally, to evaluate the single and combined effect of the activity classes and of the group size on the features of whistles Linear Models were used.

Functioning of brain waves: unexpected excitable behaviors in astrocytes

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Abstract

Consolidated evidences have contributed to shift the classical neurocentric paradigm of brain understanding towards a more holistic vision, that newly introduces astrocytes as a functional component of the neuronal network. Here it is presented an overview of the results obtained by multidisciplinary and international collaborative efforts, aiming at exploring and understanding the molecular mechanisms underlying astrocytes signaling and dynamic "wave". The innovative approaches pursued aim at exploiting the potential of advanced materials interfaces and devices, to allow selective and diverse modality to trigger astrocytes functionality by electrical, photonic, mechanical and topographical stimuli and to achieve unprecedented recording of astrocytes bioelectrical oscillations. In this presentation, we trace these findings obtained by using different strategies. Our results might provide insight into the astrocytes biophysical mechanisms and dynamics that have clinical relevance in several cerebral disorders, such as epilepsy, brain tumours, Alzheimer and Parkinson diseases. Uncovering dynamics in astrocytes might also allow novel understanding in brain communication processes and over a longer term represents a blueprint of wave-like processes occurring in others "non classically excitable" cells of our body.

Keywords: astrocytes, glial interfaces, ion channels, calcium waves

1. Introduction

The study of bioelectricity called electrophysiology is based on the evidence that our body mass is mainly composed by ions and water, which are unequally distributed in the intracellular and extracellular compartment. Ions flows through the plasma-membrane by means of transmembrane proteins forming ion channels, following their concentration gradient. Ions dynamics and electrochemical equilibrium determine the cell membrane potential. Accordingly, the cell membrane potential has a negative value that is peculiar of different cell types. The variation of action potential occurs differently also depending on the cell type and function. On the basis of their electrical properties' cells are classified as excitable or non-excitable. **Excitable cells** are cells that can elicit and propagate a specific form of bioelectric wave called action potential. The latter is an all or nothing event that occurs only in those cells equipped with high density of ion channels, which are gated by variation in the plasma-membrane voltage. The amplitude of the action potential cannot be tuned, but the response to specific stimuli can alter the frequency of the bioelectric pulse.

Small amplitude (few millivolts) variations and oscillations in voltage membrane potential occur **in every living cell, even if they are uncapable of generating action potential**. Cell life cycle and processes such as differentiation, proliferation and death are accompanied by changes and oscillations in plasma membrane potential[1a]. Also, external stimuli, such as bioactive molecules, changes ions and water concentrations, mechanical stress, osmotic stress or temperature rise can induce alteration in the voltage membrane of cells. These variations, called graduated potentials, are directly correlated with the intensity of the stimuli. In light of the recent discoveries regarding non-excitable cells in the brain, variation in membrane potential of non-neuronal glial cells might represent a new path for understanding mechanistic beyond brain communication processes and function such as complex learning and memory.

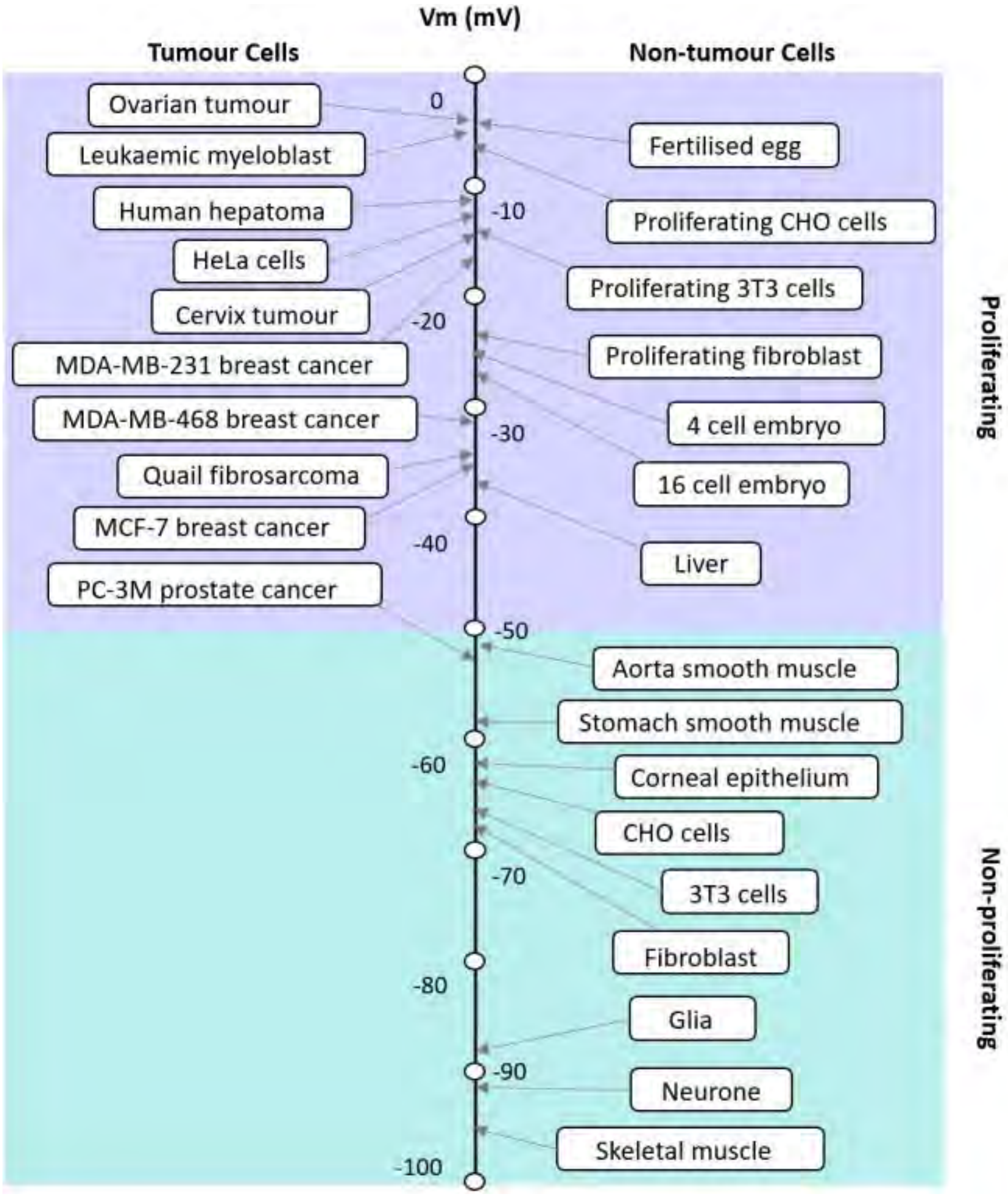

Figure 1: membrane potential values in different types of cells (from [1a], permission requested)

Indeed, within the last four decades the traditional understanding of brain functionality and organization has been dramatically revolutionized. The neuronal doctrine, which classically places neurons at the centre of Nervous System as the elementary components of transmission and processing of brain information, has now been widely overcome by a new concept in neurophysiology and neuroengineering. Currently available neural interfaces aiming at the study, diagnosis and therapy of the Central Nervous System (CNS) functions and dysfunctions target mainly neuronal action, but emerging literature is directed to the study and development of technological tools specifically devoted to the mechanistic investigation of non-neuronal cells, called glia. [1,2]

In the vertebrate Nervous System, glial cells are more numerous than neurons. Although glial cells have long been considered as **electrically silent "non-excitable" cells**, consolidated evidence highlighted their crucial functions in brain physiology and pathophysiology. [3-5] Far from being merely passive cells providing structural and trophic support to neurons, glial cells are active and dynamic elements of the neuronal circuitry. Among glial cells, astrocytes establish tight contacts with blood vessels at the glio-vascular interface and envelop synapses forming the glia-neuron interface. *In vitro* and *in vivo* studies support the relevance of astrocytes in the modulation of synaptic transmission and control of brain homeostasis. Astrocytes commonly respond to many brain injury and disease by becoming reactive, triggering a complex cascade of inflammatory events called astrogliosis. These evidences have contributed to shift the classical neurocentric paradigm of brain understanding towards a more holistic vision, that newly introduces glia as a functional component of the neuronal network.

In this proceeding it is highlighted that astrocyte proliferation, structural changes and ion channel activity are crucial functional targets for understanding and explaining the physiological and biophysical mechanisms beyond the *functioning of brain waves*. In this respect, the role of potassium and chloride channels, water transport, calcium signaling, and structural protein expression in astrocytes physiology is reported. It is then proposed that ion channels and water channels as molecular candidates to be considered for probing, sensing and modulation tools of the nervous system and to understand deeper the sense of brain waves. Uncovering the role of ions and water "wave" and possibly their coordinated and collective dynamics in astrocytes might serve as a blueprint for communication processes occurring in all "non classically excitable" cells of our body.

1. 1.1 Physiology of astrocytes

Astrocytes are star-like shaped cells in the Nervous System (Figure 2). The complexity and heterogeneity of cortical astrocytes is the main distinguishing feature of the adult human brain compared to rodent one. [6] Astrocytes play crucial roles in a large variety of physiological and pathological processes in the brain. They are responsible for the maintenance of ionic and chemical homeostasis of the cerebral environment, through the numerous ion channels, aquaporins and transporters present in their processes. (Figure 2). [3,8,9,10,11] Astrocytes also contribute to the formation and integrity of the blood brain barrier, a structural barrier that protects the brain from any microorganism or toxins circulating in the blood. Due to their strategic position, astroglia act as a bridge that mediated the cerebral bidirectional flow of ions, water and metabolites at the glio-endothelial interface (Figure 3). [1,3]

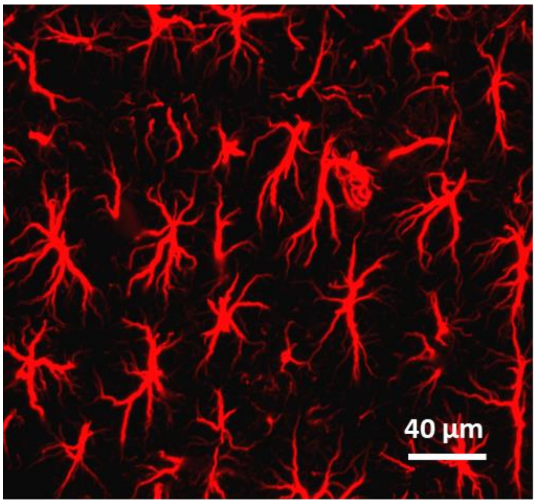

Figure 2 Immunofluorescence image of astrocytes in the Nervous System. Reproduced with permission [7] under the terms of the Creative Commons Attribution License, Copyright 2012 Butenko et al., Public Library of Science.

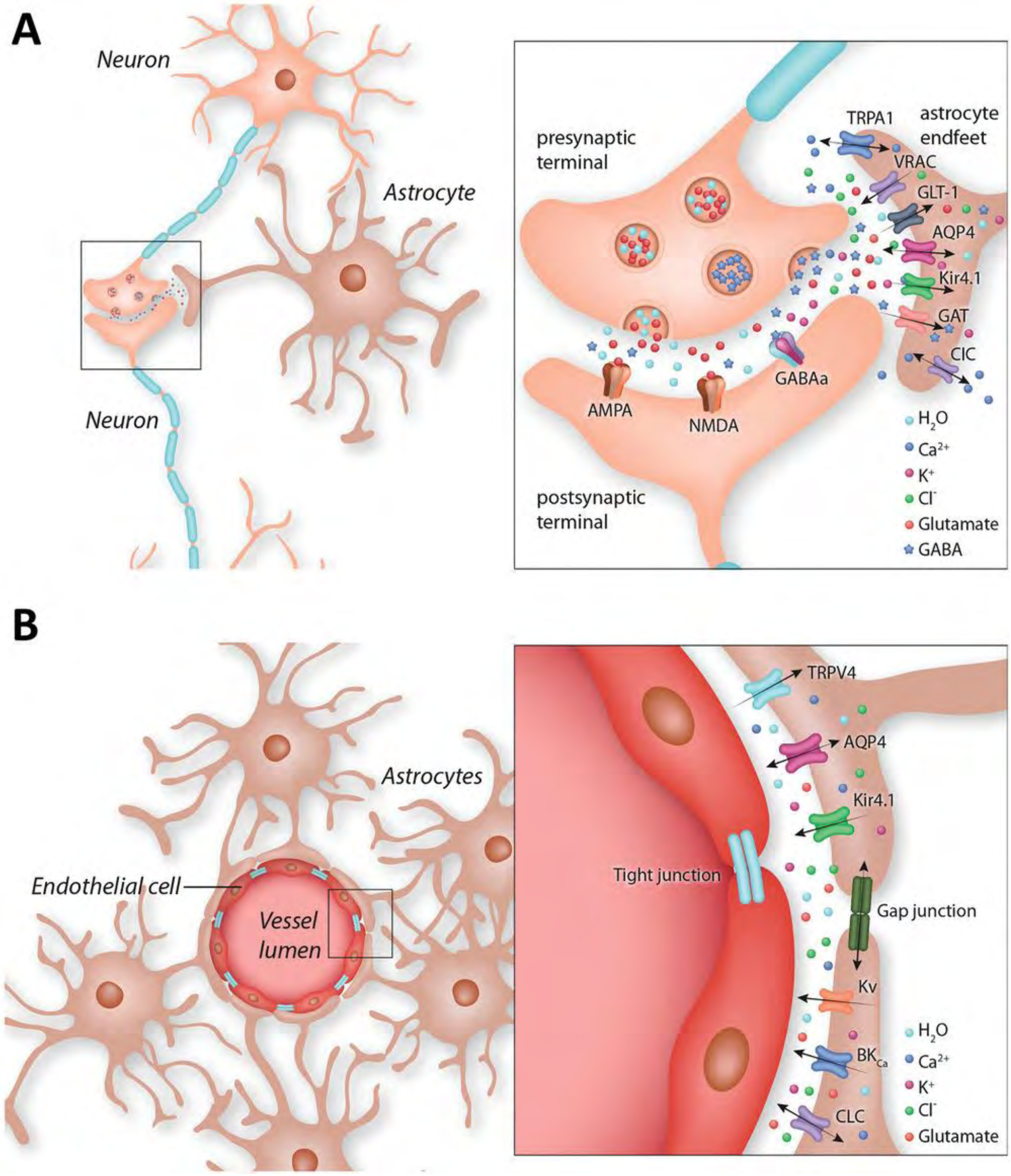

Figure 3 Scheme representing the expression of membrane proteins in astrocytes at distinct microdomains. A) At the perisynaptic astrocytic domain, B) At the gliovascular astrocytic domain. Reproduced with permission. [1] Copyright 2020, Wiley-VCH GmbH.

Astrocytes maintain potassium (K^+) homeostasis through the uptake and spatial distribution of K^+ ions. This mechanism, named "K^+ siphoning and buffering'' [8] aims at removing excess K^+ from areas of neuronal activity and dissipating it on distal sites into the vicinity of blood vessels. The mechanism is allowed by changes in membrane potential of astrocytes. Notably, alteration in this astrocytic process are the molecular basis of many brain electrical disorders or spreading of epileptic seizure. Among K^+ channels expressed by astrocyte, delayed rectifier K^+ current, Voltage gated K^+ channels and inwardly rectifying channels are mainly responsible for K^+ homeostasis. Besides K^+ channels, astrocytes express calcium (Ca^{2+}), sodium (Na^+), chloride (Cl^-), and water channels, by which they sense neuronal activity and modulate the biochemical composition of extracellular environment. [3] Astrocytes participate to Cl^- homeostasis by activating anionic channels. Additionally, astrocytes face the cytosolic fluctuations in the Cl^- concentration occurring upon anisotonic conditions, by the opening of volume-regulated anion permeable channels allowing the efflux of Cl^-. [9]

Astrocytes express channels for the water transport, called aquaporins (AQPs). Among the members of the AQPs family, AQP4 channel is the predominant channel expressed in astrocytes processes. AQP4 is involved in the regulation of the concentration and volume of the extracellular environment and in K^+ spatial buffering, to facilitate the osmotic movement of water across the plasma membrane. [9,10] The synergic cooperation between AQP4 and Transient Receptor Potential (TRP) calcium channels is essential to promote volume regulation mechanism through osmosensing, water transport, Ca^{2+} signaling and osmolyte efflux in cortical astrocytes.[11]

Ca^{2+} signaling is central for the regulation of homeostatic functions, as well as for the transmission and processing of information in the CNS. Perisynaptic astrocytes respond to neuronal activity with variations in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and release of so called gliotransmitters. The ability of astrocytes to show increases in $[Ca^{2+}]_i$ in response to several chemical-physical extracellular stimuli (such as mechanical, electrical or light stimuli, osmotic gradient, neurotransmitters) might represent an alternative (non-classical) path for cell functioning. A modality for cell excitability that could be cell distributed in specific microdomains as well as having a functional importance in astrocyte-astrocyte collective behavior as well as in astrocyte-neuron intercellular communication.*[3,12-16]*

Spatial and temporal diversity of astrocytes $[Ca^{2+}]_i$ elevation dynamics reflects distinct molecular mechanisms of Ca^{2+} signal generation. The Ca^{2+} signals originating in the astroglial processes, develop differently from those arising in the soma, showing different kinetic profiles. [16-18]

$[Ca^{2+}]_i$ elevations in astrocytes are derived from distinct compartments, including intracellular stores and the extracellular space. $[Ca^{2+}]_i$ signals can occur as rapid oscillations of typically 30 seconds duration, resulting from inositol 1,4,5- trisphosphate (IP3)-mediated Ca^{2+} release from cytoplasmic organelles or as slow and long-lasting transient on the scale of hundreds of seconds induced by Ca^{2+} entry from extracellular space. [3, 17,18] In response to neuronal activity, Ca^{2+} signaling can be limited to a single cell (at so called microdomains) or propagated as “waves” through astroglial syncytium by gap junctions, allowing the modulation of high cognitive functions such as memory and learning.

In particular, extracellular Ca^{2+} can mainly enter into the cell through members of the TRP family. **TRP are plasma-membrane sensors** able to sense and transduce different chemo-physical extracellular stimuli into intracellular Ca^{2+} variations. Among these, the transient receptor potential vanilloid 4 (TRPV4) and ankyrin 1 (TRPA1) are the main channels expressed in astrocytes plasma membrane mediates mechano-, osmo-, temperature and volume sensitivity in astrocytes and regulates the release and uptake of neurotransmitters. [11,19]

The pathophysiological importance of these signals in astrocytes derives from their ability to respond to all forms of CNS insults through inflammatory reaction called astrogliosis. Reactive astrogliosis is a common hallmark of different neurological disorders, such as epilepsy, cerebral tumors, Alzheimer and Parkinson diseases, as well as the typical *in vivo* reaction of astrocytes to the insertion of implant device or electrodes.[4,5,20-22]

Given the importance of astrocytes dynamic waves and structural components, it is evident that their probing and sensing in a spatiotemporally precise manner is extremely relevant for understanding cognitive function or to determine mechanism beyond brain dysfunction. Interestingly, TRPs channels and AQPs are unequally distributed in brain astrocytes, being mainly localized in nano- or micro-domains. The interplay between AQPs and TRPV4 mutually regulates their expression in the membrane and the Ca^{2+} and water permeability of the cells.[11]

2. Probing functioning of astrocytes

It is highlighted that chemical, electrical and nanomechanical stimuli and interaction between AQPs and TRPVs can be targeted to alter channels function in real time or their expression over longer terms. However, most of the technological tools used to probe and sense astrocytes are derived from those developed to study neurons.[23] Thus, studying the signal dynamics of astrocytes is difficult because they do not generate action potentials, the size of distal thin nanoscale processes in contact with synapses is below the resolution of traditional optical microscopy and fluorescent dyes-based methods lack cellular specificity.[24-26] In light of these considerations, our aim is to explore and understand ***the molecular mechanisms underlying astrocytes signaling and dynamic "wave" response to several types of stimulation, such as electrical, light, mechanical and topography stimuli, in attempt to gain insight into the role of astroglial cells in brain function and dysfunction.*** The vision beyond our work is that advanced nanomaterials interfaces and devices can provide precise (nanoscale), fast (ms, sec), control of ions and water dynamics and wave, and possibly collective behavior occurring in astrocytes, thereby allowing selective manipulation of TRPV4, TRPA1 and AQP4 channels expression and function.

In this work, we overview our steps to achieve this goal by the use of advanced biomaterial interfaces, electronic, photonic and optoelectronic strategies as tools for selective stimulation and recording of astrocytes functionality in the healthy and pathological brain.

2.1 Probing and driving potassium dynamics

At first, we demonstrated that expression and dynamics mediated by K^+ channels in astrocytes can be modulated and driven by interaction with biomaterials interface. [27,28,29] It was showed that the release of guanosine, a mole from silk fibroin biomaterial induces the increase of inward current in astrocytes as well as particular the up-regulation of Kir 4.1. On the contrary, analysis of K^+ channel conductance reveals that growth of astrocytes on bare silk film does not alter the resting K^+ current in astrocytes.[27] The interface of astrocytes with nanostructure films of hydrotalcite nanoparticles (HTlc) films with primary rat neocortical astrocytes nanostructured surface drive astrocytes differentiation that was paralleled by molecular and functional up-regulation of K^+ channel called Kir4.1 (Figure 4). The effect was accompanied by induction of F-actin fibre alignment and vinculin polarization. [29] A similar effect was observed by using a forest of gold coated silicon nanowire (AuSi/Nws) as glial interface. Kir4.1 expression and function were increased in cells grown on AuSi/Nws.[30] Thus, K^+ channels molecular and biophysical features and protein levels in astrocytes depend on the surface, the topography and on the geometry of the interface/scaffold.

However, the correlation between K^+ channels expression and nano-structural clues of the surface is not trivial. Indeed, we found that topography and morphological features of the substrate drives astrocytes adhesion and elongation nanostructured electrospun nanofiber of Poly-capro-lactone. However, despite the morphological change primary astrocytes grown on electrospun fibres showed no alterations of electrophysiological properties.[31] On the other hand, as observed with disordered HTlc and AuSi/Nws films, we found that a dramatic actin-cytoskeletal rearrangement as well as focal adhesion point number and distribution occurs, when astrocytes are in contact with nanostructured interface. [30,31] Thus, it is plausible that the cytoskeletal rearrangement is react to the extracellular environment but independently by the expression and function of K^+ channels. At the same time***, it is possible that K+ channels expression and dynamics reflects the "state" or the "fate" of the cells***, in a balance between differentiated astrocytes expressing more Kir4.1 and migrating, polygonal, ameboid reactive astrocytes expressing more delayed rectifying K^+ (KDR) current. [27]

These results open the route to engineering novel nanostructured *interfaces capable* ***to drive K+ channels conductance expression and function****, which are essential in* astrocytic brain homeostasis. Nonetheless, given the importance of the dysfunction of astroglial K^+ channels in pathologies such as glioma and epilepsy, these biomaterials interface can be seen as a novel therapeutic approach to treat these devastating diseases.

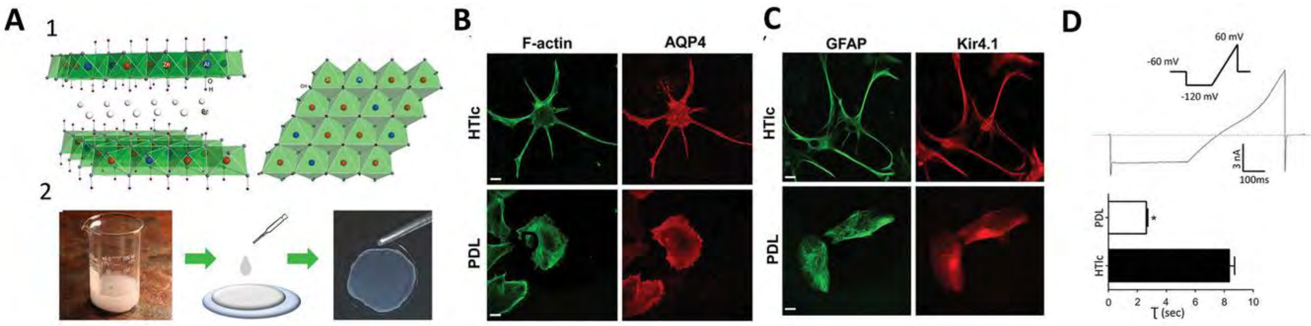

Figure 4 A) Schematic representation of structure 1) and preparation 2) of HTlc films. B, C) Confocal images of astrocytes grown on HTlc (upper panels) stained for AQP4 or Kir 4.1 (B, C, red), and Actin or GFAP (B, C, green). D) Functional properties of astrocytes grown on HTlc showing inward conductance (top) in response to ramp stimuli and increased the swelling rates (τ, bottom). Reproduced with permission [29] under the terms of the Creative Commons CC BY license. Copyright 2016, the Authors. Published by Nature Publishing Group.

2.2 Probing astrocytic water transport

Known the importance of AQP4 in water transport and in the brain water homeostasis as well as in the pathogenesis of astroglia-related acute and chronic diseases, we shed light on the mechanistic of the effect induced by nanostructured biomaterial interfaces on AQP4 expression and functions. We reveled that the molecular and functional expression of AQP4 protein significantly increase in astrocytes grown on HTlc nanoparticles films (Figure 4). [29] This unprecedent finding suggests that water transport processes can be successfully promoted by the nanotopographical and mechanical features of lamellar patterned HTLc, which represents an interesting substrate platform to grow astrocytes *in vitro* in a *in vivo* like condition. Interestingly, ***water transport and regulation volume decrease can be triggered by infrared (IR) stimulation pulses in astrocytes***, as an efficient tool that can activate water dynamics and regulatory volume response during brain homeostasis processes. In line with the evidence regarding the molecular and functional interplay between AQP4 and TRPV4, we showed that Ca^{2+} currents strongly depend on water transport and AQP4 expression is determinant for the dynamic and the amplitude of IR-elicited Ca^{2+} response. It is possible that exposure to IR stimulation can alter the mechanical properties and permeability of the astroglial plasma membrane or osmotic gradient, triggering water transport dynamics and swelling or shrinkage through AQP4 pathways. [32]

2.3 Eliciting Ca^{2+} signaling by photonic and electronic devices

With the discovery of the unexpected diversity of astrocyte $[Ca^{2+}]_i$ signaling, with different spatial locations and temporal dynamics, the mechanistic investigation of new molecular pathways by which astrocytes $[Ca^{2+}]_i$ can be generated to modulate brain function is rising. On this regard, we identified specific molecular players involved in Ca^{2+} response of astroglial cells upon the effect of IR stimulation. As consequence of AQP4 activation, we can activate a cascade of molecular events involving IP3, TRPVA1 and TRPV4 which contribute to shape the magnitude and dynamics of IR evoked $[Ca^{2+}]_i$ signals. Cell volume changes and/or mechanical membrane stress induced by IR stimulation might trigger the TRPA1 mediated entry of Ca^{2+}, which consequently promote the Ca^{2+} release via IP3. IP3 receptors can bind TRPV4 and sensitize it to initiate regulatory volume changes. Lastly, Ca^{2+} waves can spread to adjacent astrocytes by gap junction connections. [32] The possibility to control and modulate astrocyte biophysics at subcellular scale by IR light stimulation, can be useful to generate high spatial resolution and temporal precision tools supporting the study and triggering of astrocyte roles in edema, ischemia, glioma progression, stroke, and epilepsy.

According to these findings, we showed how we can elicit and monitor Ca^{2+} dynamics and channels in primary rat neocortical astrocytes, using an organic cell stimulating and sensing transistor (O-CST) device. The O-CST architecture, previously validated in neurons [23] can provide the real time stimulation and recording of bioelectrical signals coming from astrocytes cultured on the P13-based substrate (Figure 5). By performing patch-clamp recording and Ca^{2+} imaging experiments, we uncovered the contribution of TRP superfamily channels as critical transducers and mediators of $[Ca^{2+}]_i$ transients, which can be selectively activated by application of extracellular electrical stimulation. This our pioneering work has opened a novel scenario in the design and validation of organic bioelectronic devices for glial interfacing and measuring, by which we might selectively control glial molecular events and, thus, clinically intervene to restore the physiological pathways in damaged or compromised biological target systems.[33]

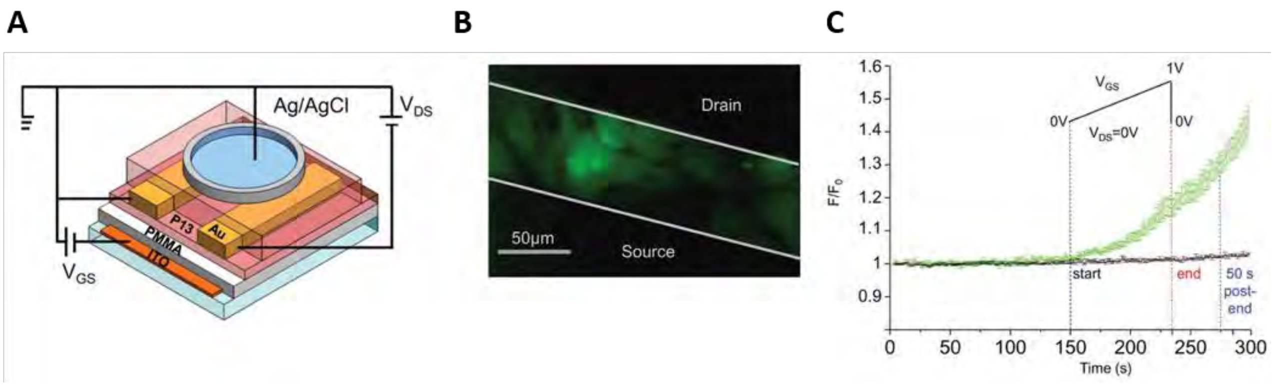

Figure 5 A) Scheme of Organic Cell Stimulating and sensing transistor based device B) enabling extracellular electrical stimulation and calcium imaging of fluo-4 loaded primary astrocytes. C) The electrical stimulation evokes calcium signaling in primary astrocytes. Reproduced with permission.[33] Copyright 2020, Wiley-VCH GmbH.

2.4 Probing chloride dynamics by optoelectronics

In order to explore the phototranduction mechanisms and biological pathways involved in astrocytes at the biomaterial interface, we have showed that photoexcitation of organic intrinsically light sensitive P3HT:PCBM blend can be exploited for manipulating astrocyte functional properties.[34] Compared with the cell growth on Poly-D-Lysine -coated control substrates, the organic polymeric interface can significantly improve the long-term survival of rat primary astrocytes and fully preserves their fundamental electrophysiological characteristics. Newly, the photostimulation of astrocytes by photovoltaic P3HT:PCBM interface is able to evoke a significant increase in whole-cell inward current, whose density is proportional to the illumination intensity (Figure 6). As supported by biophysical and pharmacological characterization, we observed that the photo-transduction mechanism is possibly mediated by the upregulation of an inwardly rectifying ClC-2 channel conductance. Among the members of ClC family, ClC-2 channel is involved in the regulation of cell volume because of its intrinsic volume sensitivity and, in particular, is thought to be important for ion homeostasis of the subretinal space. Thus, exploiting photostimulation approaches targeting astrocyte ion channels might have a clinical potential to uncover and control the role of non-excitable cells in physiological vision mechanisms as well as in pathological molecular alteration and reactive astrogliosis resulting in retinal degenerations. [34-36]

Figure 6 A) Scheme of P3HT:PCBM based device B) enabling photoexcitation with visible light and C) eliciting chloride ionic current in primary astrocytes. Reproduced with permission.[34] Copyright 2014, Wiley-VCH GmbH.

Besides single channels activity, global and possibly collective dynamics occurring in astrocytes are an elective target to understand the role of astrocytes in brain function and dysfunction. By using inorganic glial-silicon nanowire interface formed by a forest of randomly oriented gold coated-silicon nanowires we have shown non-invasive extracellular recording of the slow-frequency oscillations generated by differentiated astrocytes (Figure 7). [30] The physiological significance of these waves is still elusive, but the idea is that they might contribute to the regulation or the amplitude of brain rhythm and that can tune its excitability depending on/inducing/regulating the brain state.

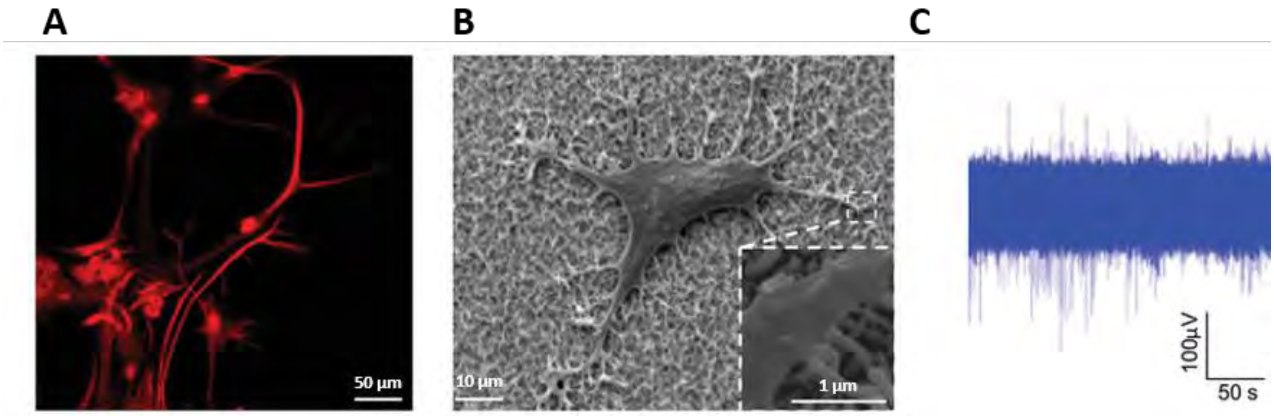

Figure 7 A) Confocal imaging of astrocytes stained for Kir4.1 on Au/SiNWs B) Scanning Electron Microscopy (SEM) micrographs of a differentiated astrocyte plated on Au/SiNWs. Inset: higher magnification images showing astrocytes process enveloping a nanowire. C) Extracellular recording obtained from astrocytes grown on Au/SiNWs MEA. Reproduced with permission.[30] Copyright 2020, Wiley-VCH GmbH.

3. Conclusion

In this presentation, we highlight the recent achievement in the understanding of biophysics mechanisms underpinning astrocytes activity, such as ion, water and structural dynamics, as functional targets of glial interfaces, glial engineering, and glial photonics.[1] We believe that multidisciplinary strategies and common effort toward understanding the significance of astrocytes waves is needed to enable significant advancements in the field of neuroscience and neurology and to generate driving knowledges also neural engineering and material science in the near future. Above the others, the development and validation of interfaces, devices and theory approaches to probe and sense astrocytes dynamics with high spatiotemporal resolution in organ on a chip and in humans, and novel modelling and machine learning approaches that predict or describe the mechanistic beyond their function, will open novel path for understanding the role of "non-excitable" cells in the nervous system and possibly serve as blue print knowledge on the communication processes also occurring in other apparatus of our body.

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Nanomotion with AFM-cantilevers: clues towards understanding life

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Abstract

The insurgence of newly arising, rapidly developing health threats, such as drug-resistant bacteria and cancers, is one of the most urgent public-health issues of modern times. This menace calls for the development of sensitive and reliable diagnostic tools that are capable of analyzing on a nanometer scale the responses of single cells in biological systems to chemical or pharmaceutical stimuli. Recently our team demonstrated that nanometric scale oscillations exerted by biological specimens reflect the status of the microorganism metabolic activity. Atomic force microscopes (AFM) or low-noise homemade dedicated devices can highlight these oscillations and follow their modifications upon exposure to different chemical or physical stimuli. The method consists of attaching the organism of interest onto a cantilever sensor and following its nano-scale motion as a function of time. We exploited this nanomotion sensor to monitor the activity of several types of bacteria, yeasts and mammalian cells. Here we will review this technique in details, presenting results obtained with many different microorganisms, with a view to highlighting the capabilities of this tool and the potential applications of nanomotion in fundamental research and medical microbiology.

Introduction

There is a strong correlation between movement and life. In every living system, the energy consumption is translated in large or small-scale motions or vibrations, which are a very specific means to understand the characteristics of the system and evaluating its status. In fact, higher organisms perform most of the vital functions through movement, to the point that the coordination of motor activities is often aimed at satisfying needs such as searching for food, reproduction, defence from predators and other important activities. The implementation of the movement is a very complex activity that requires the coordination and the transfer of information between several parts of the organism. These movements are often large enough to be monitored with simple sensing techniques but are also inherently complex, influenced by the combination of many endocrine and behavioural parameters, as well as by contextual or environmental variables and are, thus, difficult to evaluate in terms of the information they can provide on the status of the biological system.

An interesting scientific theme is the transposition of the paradigm of movement towards smaller organisms and approaching the nanoscale. For much smaller systems, such as molecules and macromolecules, the importance of molecule dynamics in the emergence and in the modulation of chemical-physical and biological properties is well known and many tools are available for their modelling and characterization. Indeed, molecular motions are studied from both an experimental point of view, employing spectroscopy techniques that range from IR to X-rays, and from a theoretical or modelling point of view, through the methods of the molecular dynamics.

On the other hand, knowledge at the micro and nanoscale behaviour of more complex biological systems is much more scarce. In this sense, current questions concern how it is possible to conceive the movement of biosystems on the cellular scale and whether it is possible to measure it. On one hand, pushing towards the investigation of small complex biological systems allows limiting the parameters affecting the system under investigation, even though the complexity of the overall system is often not reduced. On the other hand, though, small specimens produce smaller movements and vibrations whose origin and information content may be different from those extracted from larger systems and their characterization requires different analysis procedures and more sensitive investigation systems.

Other intriguing questions might regard the possibility to re-propose some typical aspects of macroscopic systems, such as the coordination of movement between different cellular entities, the transfer of information and the integration of a response at the supra-cellular level. Questions like these can find interesting deductive ideas thanks to the development of new technologies with inherently special sensitivity to phenomena on the nanoscale coupling nanoscale sensitivity to the continuous monitoring of the biological activity.

Nanomechanical sensors [1] are extremely versatile devices that hold the promise to provide this means of investigation. For instance, one of their most remarkable applications is as micro-balances to determine the weights of extremely small masses that are directly deposited upon their surfaces. Other uses of such microscale sensors involve the coating of one of their faces with a molecular layer, this causes a static bending that depends upon the abundance, the interactive properties and the nature of the molecules. In this way, these nanomechanical sensors can be used to produce extremely sensitive devices, artificial noses and these measurements can monitor with high sensitivity the binding of targeted molecules to biomolecular receptors[2-5].

In the framework of the investigation of the movements of biological systems at the nanoscale, a breakthrough was proposed by Dietler's group, which demonstrated that nanomechanical sensors, such as atomic force microscope (AFM) cantilevers could be implemented to characterize, on a nanometer scale, the fluctuations that are produced by the movements of living organisms.[6, 7] Indeed, even if the AFM was initially developed to produce high-resolution topographic images of surfaces in the order of 0.1 Å[8], the scope of its applications has recently broadened to exploit the sensitivity of the cantilever. This includes the monitoring of the nanomechanical properties of samples[9-15] and a localization of the effects of drugs on biological specimens[16]. Building on the versatility and sensitivity of the cantilevers, several groups have now proposed their use to produce real-time measurements of the nanoscale movements of any sample, from bacteria to entire cells, within air or liquid medium, with sensitivity in the Angstrom-to-micron range. This nanomotion technique can determine the viability of the organism: the nanoscale movements of the viable specimens will induce dynamic deflections of the sensor, which are collected in the form of a time-dependent chart of its vertical movements. If the specimens are exposed to a stimulus that induces their death, the sensor's oscillations are strongly reduced, providing a fast and reliable viability assay. Indeed the accumulated evidence indicates that the nanomotion fluctuations produced by a living system correlate with their metabolic activity. The principles of this method of detection are presented in Figure 1.

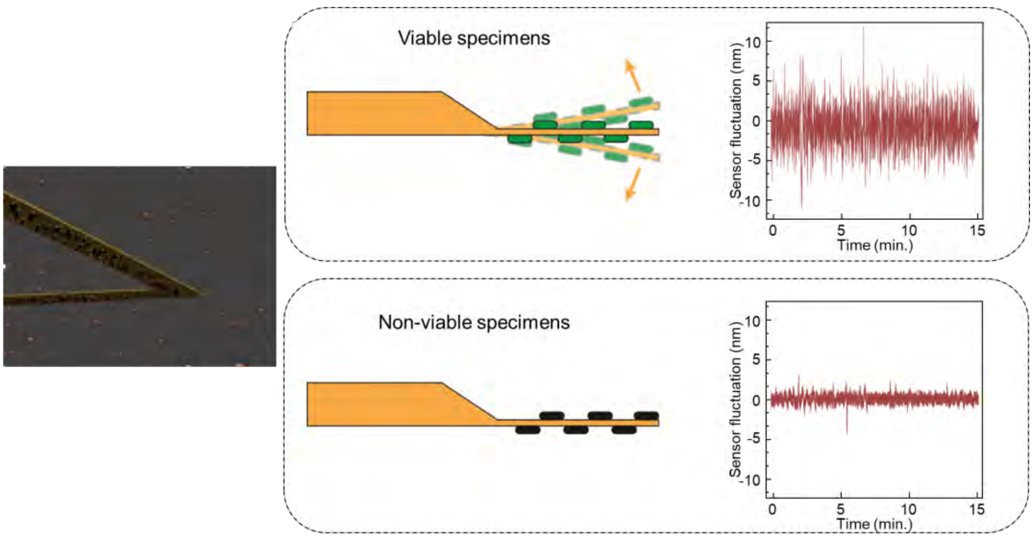

Figure 1. Simple schematics of the nanomotion sensor. When the sensor bears viable specimens, the cantilever oscillations are strong and measurable. When the specimens are rendered non-viable, the oscillations disappear.

Nowadays, the potential applications of this versatile technique are vast. The simplicity of its working principle lends itself to implementation in medical centers and research laboratories with applications both in the basic research and in the applied clinical fields.[17-23]

Here we describe AFM based nanomotion detection on different organisms, the possible mechanisms that produce the nanomotion and discuss the potential applications of this technique in studies involving biology, biomedicine and in the characterization of collective movements in cell populations.

AFM based nanomotion detection

Historically, the development on nanomotion on the submicron scale can be traced back to the pioneering work of Radmacher et al, who demonstrated in 1994 the use of AFM cantilevers to sense biological phenomena.[24] In this seminal work, the authors adsorbed lysozyme molecules onto a mica surface and approached an AFM tip above the proteins. Upon exposure of the lysozyme molecule to one of its substrates (e.g. oligoglycoside), the cantilever oscillated. It was speculated that the oscillations were caused by the lysozyme conformational changes. Several years later, a similar experiment was carried out by Alonso et al. by coating an AFM cantilever with topoisomerase II, a protein involved in mitotic chromosomes scaffold as well as DNA unfolding which changes conformation by oxidizing ATP. The oscillations of the cantilever first without ATP and then after addition of ATP evidenced a drastic increase which was associated to the conformational changes of Topoisomerase II induced by ATP.[25]

Nanoscale vibrations were also exploited to evaluate the viability of different microorganisms. The first report of this is by Pelling et al., 2004, who demonstrated how living yeast cells produce a measurable vibration.[26] In this study, an AFM tip was brought in close contact with the cell wall of *S. cerevisiae*, which induced nanoscale oscillations of the cantilever at relatively high frequency. Further characterizations showed that the recorded signal was not due to Brownian motion or noise but had a biological origin. Since then, several studies have explored the nanomotion of various microscopic organisms.

In 2013 Longo et al demonstrated that microorganisms attached onto an AFM cantilever can produce oscillations of the sensor that are directly correlated to the state of the organisms.[6] The time-dependent chart of the vertical movements of the sensor form a coloured noise signal, a nanomotion pattern, whose amplitude can provide a real-time determination of the metabolic status of the specimens as a function of different physico-chemical stimuli, and can therefore be used to distinguish almost instantaneously life–death transitions. Importantly the very same observations were reproduced by different groups on bacteria [27-30] and on other biological systems [25, 31-33], defining the robustness of the nanomotion sensor in the research laboratory.

The experimental procedure of a nanomotion analysis is quite simple; first, the organism of interest is attached onto an AFM cantilever, which was preliminarily functionalized using a molecule that promotes

adhesion such as fibronectin, poly-lysine APTES or glutaraldehyde. The choice of the compound depends on the organisms as well as the surface of the cantilever following optimized protocols[34].
Previous works; have demonstrated, by using FEM[6] and by studying conformational changes in quaternary protein structures[25], that the energy of two ATP molecules is sufficient to produce a 1 nm oscillation in a cantilever. Thus, as the complexity of the biological and biochemical activities is extremely high, the number of cells needed to produce detectable oscillations is very low and, as a matter of fact, it depends on their size [31] and not on their replication rate[19]. For instance, a single mammalian cell is enough to obtain exploitable signals whereas hundreds of bacteria are necessary for an equivalent signal to noise ratio (Figure 2).

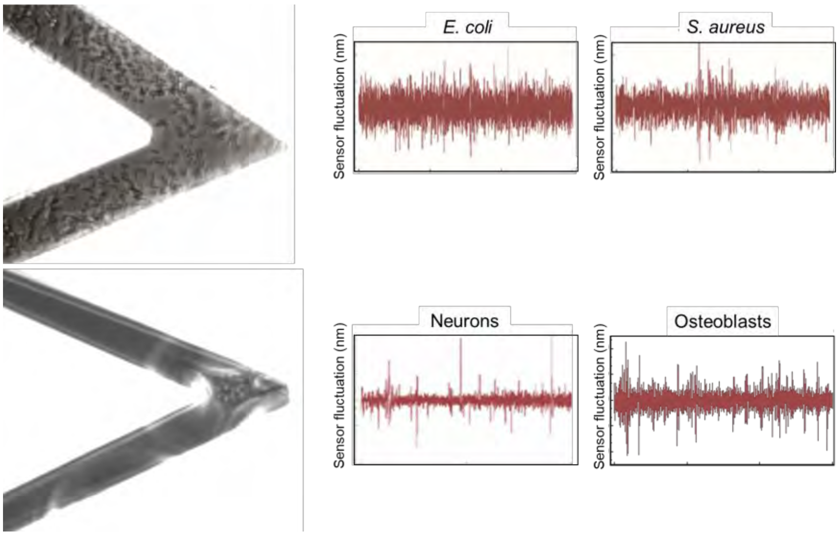

Figure 2. Application of nanomotion detection in the case of bacteria and cells. Left: Images of AFM cantilevers bearing E. coli (top) and a single osteoblast cell (bottom). Right: typical nanomotion signals when the nanomotion sensor is bearing viable E. coli, S. aureus, Neurons or Osteoblasts.

Cellular mechanisms inducing nanomotion

The origin of the nanomotion signal and its connection to the activity of the biological specimens under investigation are still not fully understood. The nanomotion signal is formed by contributions arising from many different activities occurring on the sensor's surface. The experiments involving the conformational changes in proteins indicate that the signal reflects the motion of sources that combine energy consumption with local movement or with molecule redistribution. A living biological system has an extremely complex activity and there are many diverse origins for such movements. For instance, such activities can include cytoskeleton reorganizations, focal adhesion movements, metabolically active organelles (such as mitochondria in animals and chloroplast in plants), vesicles production and trafficking, opening of ionic channels and can include the membrane interaction with the sensor.[25] All these concurrent activities contribute to the nanomechanical oscillator response, which appears as coloured noise, which must be studied to understand the depth of the information available and to extract more data on the specimens' status.

One of the most probable sources of nanoscale movement of biological systems comes from the larger motile components of the specimens. Some prokaryotes and eukaryotes possess flagella, pili or cilia, which are motile organelles that allow cells to move. Their movement could transfer a momentum to the cantilever either directly or via liquid turbulences, producing specific pattern in the nanomotion signal. For instance, Stupar and coworkers showed how the inhibition of the movement of flagella in *E. coli* could produce a reduction of the overall nanomotion signal [31].

Another possible origin of the cellular nanomotion involves the role of the plasma membrane. This is the most external cellular component of the specimens, consisting of a highly dynamic entity composed of a flexible lipid bilayer that supports glucolipids and proteins [35, 36]. The plasma membrane is the border between the specimen and the environment and can reflect, with its biochemical and mechanical properties, the status of the specimen. For instance, early stages of cytotoxicity cause a change in the viscosity of the cell membrane and morphology, both affecting their adhesion to the cantilever and the membrane's ability to transduce the innermost vibrations.[30] In a nanomotion experiment, Kasas and coworkers showed that, using actin-depolimerization drugs on mammalian cells, some components of the nanomotion signal disappear and the observable pattern changes. This data, on one hand suggests how the membrane movements can affect the overall measured oscillations signal and the importance of this gateway in the nanomotion experiments while on the other hand indicates that the overall nanomotion signal can be imagined as a superposition of specific, pathway-related, patterns.[31] Indeed, Long-Range Surface Plasmon Resonance (LRSPR) has been used to measure cell membrane vibrations, which appear to be in the range of nanometers.[37]

Many organisms, including bacteria, yeasts and plant cells, possess thick and rigid cell walls that are less motile than the cell membrane of mammalian cells. These walls contain several protein complexes, which are used by the cells to maintain a concentration gradient of specific ions inside the cytoplasm. Opening and closing of such ionic channels requires conformational changes from the proteins themselves [38, 39], which can produce detectable oscillations of the nanomotion sensor. This suggests that ionic channels could be an important component of the overall signal arising from a nanomotion experiment involving cells or bacteria.

A further factor to take into consideration is the cellular organelle, which acts as source of energy for the entire cellular metabolism: the mitochondria. Stupar and coworkers isolated these intracellular organelles and studied the nanomotion arising from them [40]. Preliminary experiments demonstrated that active mitochondria also induce cantilever oscillations, which change upon exposure to different molecules (malate, pyruvate).

Overall, there are several possible district of a cell that can be responsible, at least in part, of the nanomotion oscillations, which are then transduced by the sensor. Considering that mammalian cells can range from 5 to 100 μm, and possess multiple organelles in their cytoplasm, this could sum up to many components of the resulting, complex, coloured, noise nanomotion signal.

Selected applications

Bacterial susceptibility test

The very first experiments that were performed using nanomotion sensors involved an assessment of the responses of bacteria to antibiotics. The topic is of the utmost importance as it is foreseeable that in the near future antibiotics will lose their capacity to destroy pathogenic bacteria, owing to the development of resistance - a natural process whereby bacteria evolve to respond to a drug's selective pressure. Since the development and testing of new antibiotics takes several years, there is an urgent need for sensitive diagnostic tools to evaluate novel anti-bacterial agents and to track rapidly the development of resistances. The nanomotion sensor has demonstrated to be capable of monitoring the bacterial response to antibiotics in few hours, compared to days or even weeks of conventional techniques. Remarkably, Stupar and coworkers demonstrated how the detection of nanomotion can rapidly and reliably determine the response of bacteria to antibiotics [41].

Figure 3, illustrates a typical cantilever that is coated with cells of *S. aureus*, which are either susceptible (left) or resistant (right) to cefoxitin, a bactericidal antibiotic.[6] The oscillations of the sensor were measured, first in a growth medium and then upon exposure to cefoxitin. In less than 1 hour, the addition of the antibiotic caused a significant reduction in the oscillations of the susceptible cells that can be quantified in terms of the variance of the cantilever vibrations over time. This observed reduction of the vibrations suggests that the antibiotic compromised the metabolic activity of the attached bacteria. On the other hand, within the same period, the metabolic activity of the resistant *S. aureus* rose, which was indicative of a strong metabolic response to the antibiotic pressure.

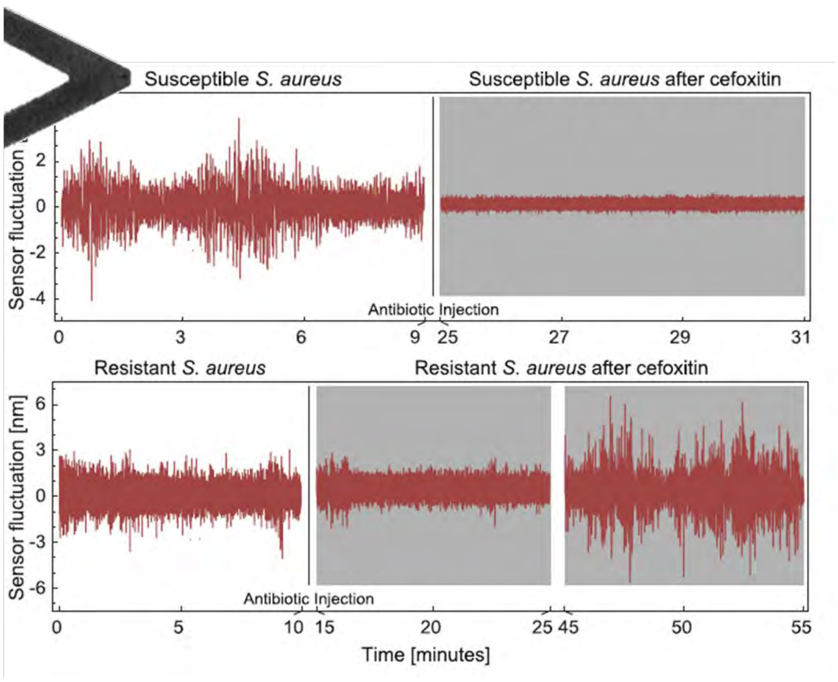

Figure 3. An example of the application of the nanomotion detection method. The response of the attached cells of S. aureus to cefoxitin (susceptible strain: top; resistant strain: bottom).

Evaluation of collective movements of bacteria

The ability of the nanomotion sensor to provide a high time-resolved overview of the movements of biological specimens allows evaluating not only the final effect that a particular stimulus has had on the biological specimens but also evidencing the steps that lead to this final outcome and the time-resolved response of the bacteria or cells. This has allowed showing some interesting patterns in the bacterial response to antibiotic pressure. For instance, as shown by Mustazzolu et al., some bacterial species, when exposed to an antibiotic insult (*Mycobacterium bovis* in isoniazid in Figure 4), can initiate a collective response, which involves a series of movement bursts followed by an overall movement reduction[19]. Such oscillatory pattern is indication of a collective response of the bacteria and could shed some light in the pathways that bacteria follow to respond to the bactericidal attack.

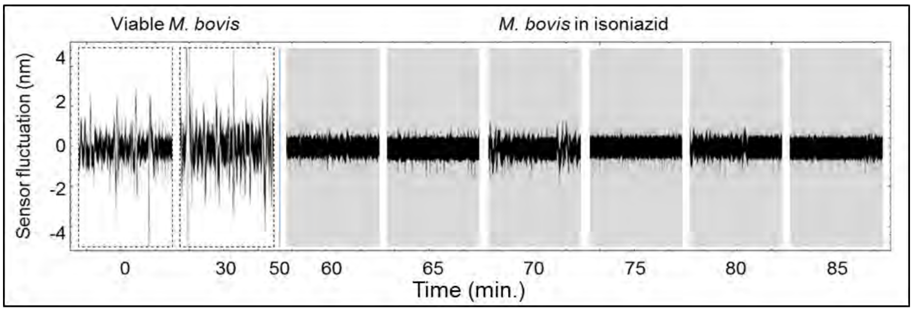

Figure 4. Exposure of *M. bovis* to isoniazid can produce an oscillating response pattern, which is indication of a collective strategy of the germs in the fight against the antibiotic.

Cancer cell susceptibility test

Following the experiments with bacteria, several researchers explored the magnitude of the movements that the sensor could detect, monitoring with sensitivity and high time-resolution, the metabolic activity of single cells or small clusters of cells**.** In particular, Kasas and coworkers and Wu and coworkers employed nanomotion sensors to monitor the responses of different cancer cells to anti-cancer drugs [23, 32]. To explain the relevance of this topic, we recall that the efficient treatment of cancers depends upon the diversity, the heterogeneity and the drug resistance of the targeted cells. The development of a tool that could be used to monitor the responses of specific populations of cells to specific drugs would be an important step towards susceptibility screening in individual cases i.e. personalized medicine.

Figure 5 depicts data appertaining to two lung carcinoma cell-lines, one of which (NCI-H460/R) is resistant to doxorubicin and the other (NCI-H460) susceptible to it. For these experiments, several cells were directly attached to the sensor's surface following a protocol similar to that used to perform single cell force spectroscopy experiments.[42, 43]

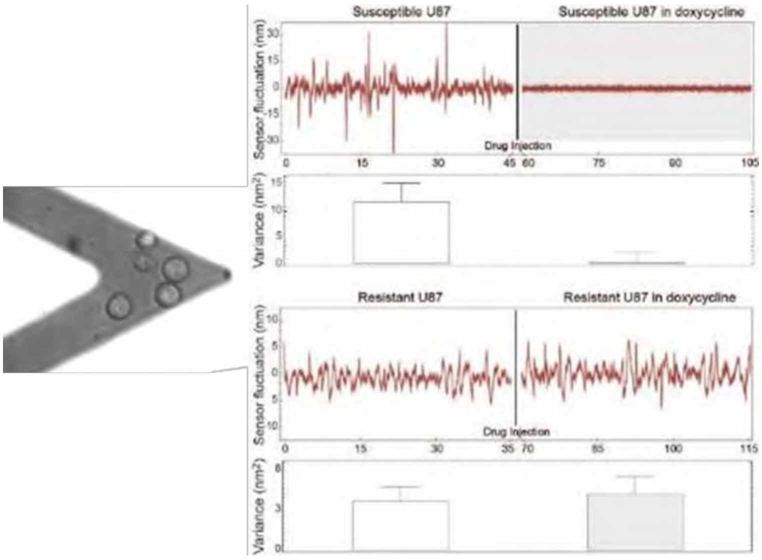

Figure 5. Application of the nanomotion sensor to cancer cells. Left panel: Images of an AFM cantilever bearing glioblastoma cells. Right panels: Response of U87 carcinoma cells to doxycycline, susceptible (top panel) and resistant strains (bottom panel). For susceptible cells, the variance decreased rapidly upon exposure to the drug, while the resistant cells were still viable after more than 2 hours.

When the cells had attached to the sensor, their oscillatory movements were tracked for several hours before and after their exposure to doxorubicin. Whilst the cells were bathed in a nourishing medium, they spread on the surface of the cantilever and evoked a strong nanomotion signal. After exposure to anticancer drugs, the susceptible cells died within approximately 90 minutes, whereas the resistant ones continued to thrive, producing measurable oscillations until the end of the monitoring period. Overall, as in the case of the bacteria, the analysis of the variance of the nanomotion signal permitted a rapid phenotypic indication of the resistance of the specimens to specific drugs.

Red blood cells

In subsequent experiments, Venturelli and coworkers analysed a biological system that is of great relevance in human physiology and health: Red Blood Cells (RBCs) or erythrocytes.[21] These cells are one of the simplest and the most frequently studied biological specimens. In their mature state, they assume a biconcave form with a diameter of about 8 microns and a height of typically 800 nanometres. Erythrocytes are in possession of a few peculiarities that render them extremely interesting for nanomotion experiments: the lack of a nucleus and of nucleic acids (and consequently of a synthetic apparatus), adapted metabolic pathways that utilize glucose instead of oxygen to synthetize ATP, and a unique architectural structure: the membrane skeleton.

The cells, prepared using a protocol that is described in detail elsewhere [44-46], were monitored for several hours, generating fluctuations constantly throughout the monitoring period (**Figure 6**). The optical images displayed no signs of morphological changes or of whole-cell motility. At the end of the monitoring period of several hours' duration, the osmolarity of the buffered physiological saline was drastically reduced by the addition of ultra-pure water, thereby rendering it hypotonic instead of isotonic. Within 10 minutes of the decrease in tonicity, the fluctuations were reduced and thereafter ceased altogether. This change in the nanomotion profile was coupled with a rounding of the cells. This morphological change in the erythrocytes reflected the physical influx of water, which raised the intracellular pressure and increased the mechanical stress of the membrane skeleton.

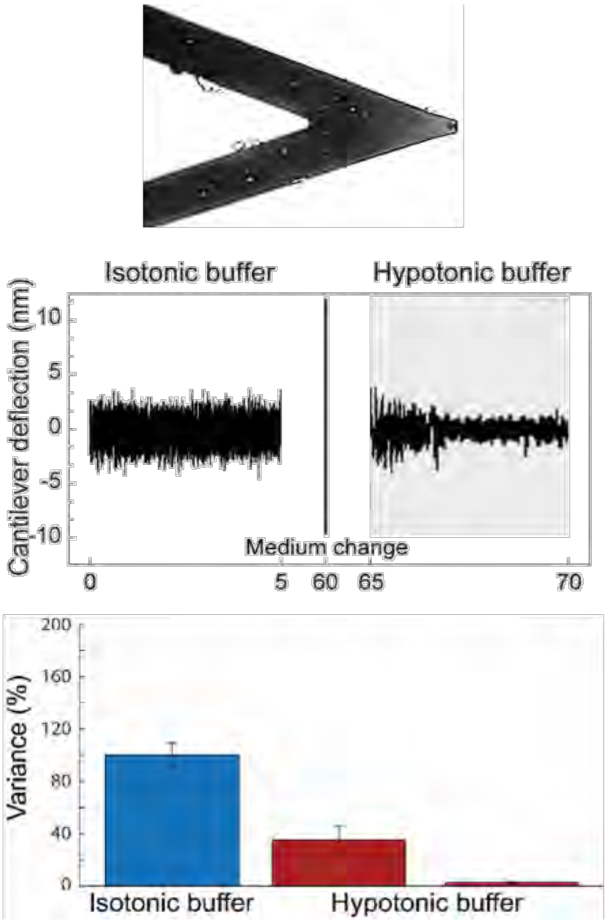

Figure 6. Nanomotion analysis of immobilized erythrocytes after their exposure first to an isotonic buffer (left) and then to a strongly hypotonic one (right). Within a few minutes of the switch to the hypotonic medium, the amplitude of the oscillations decreased; after 15 minutes, the movements of the sensor barely exceeded the level of background noise. The optical image depicts the morphological appearance of the red blood cells after their exposure to a hypotonic medium (rounded profile).

Discussion

The present work describes the methodology, and some applications, of a new investigation technique based on a non-morphological and non-conventional use of the atomic force microscope. As reported, this novel technique, called nanomotion sensor, allows translating the movement and vibrations at the nanoscale of simple (macromolecules) or complex (single cells or cell clusters) biosystems into measurable signals associated to the instantaneous deflection of the AFM microlever. The capability to measure directly the motion of nano-biosystems provides an innovative method that gives access to radically new information and data on the behavior of biological systems.

For example, in the present work we provided evidence of how, in an operatively simple way, the technique allows to associate the life condition of bacteria or other cellular systems to their ability to move. In this framework, the nanomotion sensor enables the investigation, in real time, of the life-death conditions of biosystems including cases of microbiological interest. Thanks to the great sensitivity of the technique and the possibility to detect the response in real time, this type of application has considerable advantages over conventional methods, which necessarily require cellular duplication.

Simple and typical examples of this class of applications can be located in the field of the development of new antibiotics as well as in the assessment of new antitumor drugs. In this sense, a nanomotion sensor-based approach was found to be also suitable to reveal specific information on the development of the death-pathway. For instance, it can also reveal how and, inprinciple, according to which biophysical/biochemical pattern, a bio-system dies. This kind of information can actually be precious in understanding the relationship between stimulus and response of the cell under extreme stress conditions.

Of course, the technique allows applications that go beyond the investigation of life/death cases. In fact, and probably even more intriguingly, the nanomotion sensor allows probing the response to sub-lethal environmental stresses in biological systems. In other words, it allows observing the time scale and the sensing and signaling mechanisms through which the cellular response is articulated and that permit the cell survival in harsh conditions.

However, to understand better the information that the nanomotion sensor can deliver, the signal should be interpreted using more complex mathematical tools. The nanomotion signal, in fact, results from the coupling of the cantilever with the mechanical impulses associated with the motility of the biosystems. In this sense, the resulting colored noise signal represents the synthesis of many molecular events, of elemental mechanical (adhesion, membrane channels), of structural (disassembling-reassembling of the cytoskeletal networks) and of biochemical nature associated, for instance, with the patterns of metabolic response (e.g. production of energy or of cellular resources). Indeed, a predominant role is certainly played by the metabolic activities associated with energy consumption. This last observation, in particular, is in accordance with the paradigm that the energy of a biosystem is also spent in kinetic terms, although obviously, in different proportions and ways depending on the specific state of the system under study. In this sense, understanding the mechanisms underlying the formation of the nanomotion signal and isolating specific cellular conditions could allow to analyze, in a completely new perspective, the relationships between an external stimulus and the cellular response

Projecting the potential of the nanomotion sensor technique in different contexts, some fundamental questions that animate the discussion on the functionality and behavior of biosystems can be answered. Some fundamental questions can be addressed in terms of dynamics of complex systems: for example, when a biosystem is stimulated and responds by activating or developing a specific metabolic pattern, can the measured nanomotion signal be considered as an emergent property of the system? If this were the case, we could hypothesize (or analyze) the existence of critical cellular conditions that determine qualitatively different responses. In this context, it would be of great importance to investigate and understand the role of environmental conditions and the cellular micro-environment.

In summary, the nanomotion sensor is a new, relatively inexpensive, technique that has the potential to shed some light with exceptional sensitivity and in real time, on the nanoscale response of biosystems in terms of their motility. This kind of information reflects the status of a biosystem and can be associated to different responses to an external threat: development of specific pathways, synthesis, metabolic adaptation and conformational transitions in isolated macromolecule.

Despite the potential to unveil entirely new views of the cell and of the interaction between cells in a cluster, there is still work to be done in order to isolate the single components of the measured signal and to correlate them with specific molecular events that trigger and regulate the cell response.

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Knowledge-based support to policy: beyond environmental complexity, foresight and governance to integrate ecosystem approaches.

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Abstract

Global challenges are a matter of fact.

Policy makers struggle with scouting and building effective solutions. The global challenges involve complex systems in terms of a diversity of interconnected aspects, such as culture, environment, legislation, industrial sectors, media, resources, finance, and stakeholders. Science is often asked to support decisions and provide solutions. An eco-system approach has been proposed as a crucial framework to be adopted in tackling these challenges, but this has been often limited to address solely the environmental aspects.

There is a lot of science which can provide clues to enable effective processes to find specific solutions. We briefly report results from different disciplines, focusing on the roles of governance and back-casting foresight. We call to promote a renewed meaning for the ecosystem approach when meant as an enabler for solutions to complex challenges. It has definitely to include the multitude of interconnected variables, focus on governance and be supported by network science.

Introduction

Global challenges are a matter of fact.

Climate, energy, health, demography, safety, security, pollution are just some of the many challenges humanity faces daily within a diversity of contexts. These linked global threats ask for effective and innovative solutions. In some cases, digitalization, circular economy, artificial intelligence, specialized skills have been identified as possible solutions, which in turn can introduce further global challenges.
Interconnectedness and indivisibility of different aspects are the dominant traits of most of these challenges. Most of the stakeholders invoke *complexity* as a justification for the difficulty to find solutions, while policy makers look for a drastic simplification of the systems and ask scientists to provide options for effective solutions.

But, why the global socio-economic-political system cannot escape from complexity?

The roles of space and time have drastically changed with globalization: events are quickly interconnected and impact everywhere, transforming the decision and intervention processes from sequential in time and consequential in space, to mostly immediate and ubiquitous. In addition, production and elaboration of data have introduced a deluge of information which can be accessible by the majority of stakeholders. Technologies have definitely accelerated this transformation, but while there is a huge attention to their development, the effort in the identification and adoption of appropriate processes to manage and tackle the challenges are very rare. Only marginal aspects are touched at institutional level, i.e some kind of coordination mechanisms, stakeholder participation, citizen engagement, data access (see as an example the European approach to implement the Sustainable Development Goals-SDGs, Niestroy et al. 2019). This has been also confirmed by the EU-JRC report on the impacts of Artificial Intelligence on public services (Misuraca and van Noordt 2020): even emerging technologies which are largely transforming many sectors have negligible impact on organizational reforms.
These mechanisms or procedures attempt to improve the process aimed at finding solutions to the problems. Rarely the process which is adopted is designed to enable solutions which are feasible and match the desired objectives. Any socio-eco-political-environmental system involves people (meant as skills and workforce), things, information and energy (meant as financial and natural resources). These variables are distributed in space, change in time, and are embedded in different legislative frameworks and cultures.
The challenge to identify and manage the interconnectivity between the multitude of aspects has consequently addressed the need first of multidisciplinarity, then of an inter-disciplinarity, cross-sectoral, multi-variable approach.

In summary, the recognition of the complexity of environmental systems has asked for the adoption of an "ecosystem approach" to account for all the components and interactions that drive the dynamics of the systems.

Nevertheless, solutions are still difficult to scout, to develop or to apply. Is the ecosystem approach ill-posed?

We propose to reflect on some explanatory cases: climate and marine pollution.
Both address complexity in terms of the environmental system. Physicists, chemists, biologists, engineers...they all provide contributions to identify and understand the fundamentals, and predict the dynamics of the ecosystem.
Again, the real ecosystem is not limited to the environmental one (O'Neil et al. 2014, Porter and Kramer 2011, Porter and Pfitzer 2016, Riahi et al. 2017), i.e., is not limited to what we define environment. Human activities, producing CO2 and pollutants, are taken into account by the models as physical and chemical fluxes at the boundary. However, the dynamics of these fluxes strongly depend on variables which are generally not included in the models themselves.
Human activities in fact are mostly influenced by political and economic drivers, which can bring "forces" to the evolution of the systems making it difficult to predict in the long-term. In this context, political options are assumed and posed as boundary conditions for the models. Identification of scenarios and forecasting have become a new market for scientists and policy makers, thanks to the fact that predictions for the future can not be contradicted in the present. Often fiction has been more successful in predicting the future than models (Bina et al. 2017). On the other hand, goals agreed at political global fora have rarely be achieved.
The globalization has in fact allowed the main drivers of the human society to behave as a fluid, while the management and governance of the political and organizational activities seem to be crystallized.
We propose to look for the knowledge-based support to policy adopting a scientific methodology. In fact, there is a lot of science in supporting policy, management and organizational processes. There is also a lot of science on complex systems. Rarely a equal-footed collaboration between different disciplines has been promoted to reflect on what are the essential aspects and the interventions which can be effective. Psychology, sociology, history, economy, and political sciences have often been neglected in many proposals for solutions. Many options, in turn, have been considered not politically correct and, therefore, not suitable to be communicated to the civil society.

We do believe that **a scientific methodology to address the overlooked variables of global challenges is mandatory**. This should not result in a proliferation of consultations or hierarchy between scientific disciplines. Diversification of interests, objectives and assumptions can in fact provide different options. Solutions are difficult, but any process has to be designed adequately to provide the product.

We believe that **governance and back-casting foresight** are crucial aspects to enable decision-makers to identify and implement actions.

In the following, we briefly report the scientific clues for the governance of complexity and reflections on how to expand the concept of the ecosystem approach.

The governance of complex system: a multi-dimension and multi-level network problem.

It is not the aim of this paper to write a treaty on complex systems. The word "complexity" is often used to refer to the emergence of unexpected collective properties, a priori not predicted from the analysis of known microscopic interactions. On general grounds, complex systems are characterized by: many heterogeneous interacting parts, multiple scales, transition laws, unpredicted emergence, path-dependent dynamics, networked hierarchical connectivity, interaction of autonomous agents, self organization, non-equilibrium dynamics etc. (San Miguel et al. 2012). There is not a general mathematical formulation for complex system to be applied or solved: that is, we are not dealing with a theory but with a concept.

Here we report few relevant aspects which are very useful to approach a system that is considered to be complex:

- it cannot be linearized, that is, its properties cannot be predicted from the sum of its parts;
- it is difficult to predict its evolution with high accuracy in the long-term;
- it can show abrupt changes.

Many efforts have been dedicated to modelling complex systems. The recent availability of huge amount of data has revived the inductivist approach, which relies on evidence (the past) by finding analogues and invoking big data as a fundamental instrument for much better forecasts. However, this point of view can be in practice useless: even if complete and precise information about the system is available, the required analog cannot be expected to be found in due time. In fact, the mean recurrence time is exponentially large in the system phase-space dimension so that a recurrence is never observed (Hosni and Vulpiani 2018): such an approach may be of little use.

So, the crucial aspect is how to identify essential variables, connect data and select parameters which can provide useful clues about the evolution of the system.

So, a complex system could be monitored and predicted, but requires intelligence.

Under some conditions chaos can also be controlled by means of small system perturbations. In this case, perturbations must be tiny, and properly synchronized. The result is that although the evolution of a complex system is difficult to be predicted for long time scales, the otherwise chaotic motion can be rendered more stable and predictable (Scholl and Schuster 2007).

The most famous methods of stabilization of chaos (i.e Pyragas and OGY methods) have been successfully applied (Boccaletti et al. 2000).

In this context, the reason why solutions to challenges involving complex systems are difficult to build becomes clearer. These challenges show interconnected aspects, and these aspects address different dimensions (finance, politics, environment, legislation, technology, media, natural resources, etc.). They can be represented via different levels which can represent their roles, dimension, spatial distribution and timescales. This multi-dimensional and multi-level space results in a representation of a network (see figure 1).

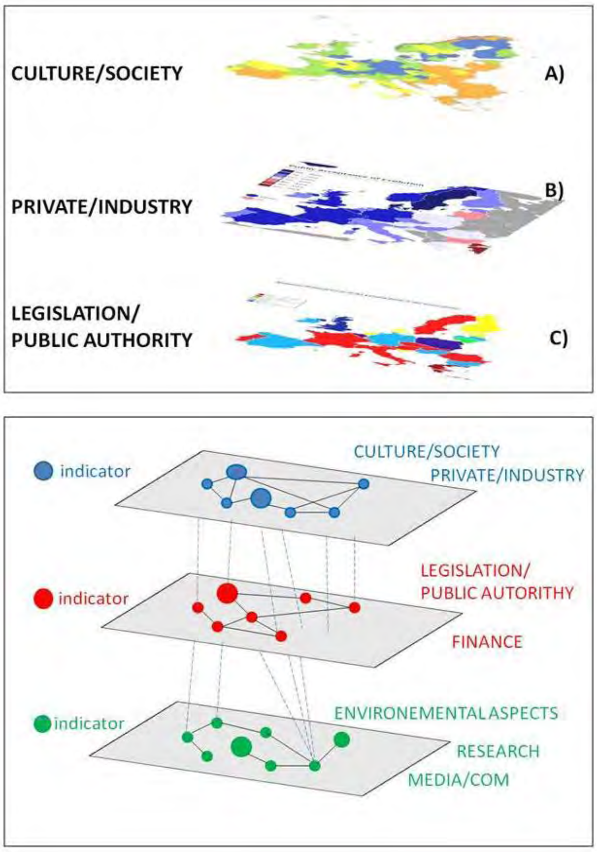

Figure 1: A graphical representation for a multi-level and multi-dimension system. The example focuses solely on the spatial dimension for an imaginary European context. Levels and dimensions can be selected accordingly to challenge to be tackled. For each dimension, an indicator that is considered relevant in the process is identified, and a map is produced. In this example, culture, industry and legislation have been identified. On top, from A) to C), three maps associated with the identified dimensions are shown. They refer to the selected indicators as follows: the leading political parties, innovation scoreboard, and cultural openness to innovation. On bottom, the relevance and links between different agents within different dimensions are represented as nodes of an interconnected network. Such an approach, which results in a network representation, facilitates the analysis of the dynamics of the system and the appropriateness of the governance and interventions.

The management of this network, when translated into a policy framework, is named governance.
Despite a deluge of academic studies on different interpretation of the word "governance" and modes of governance, a general and simple scientific-based support to how to design and manage is missing. Different

models for the governance of complex systems have been introduced by social sciences and hard sciences. The analogies from systems other than human society suggest to revise the mania of both control and forecast, as well as the identification of the spatial boundaries for local and global scales. Control, prediction, and identity of the territory where they refer to: these are the main foci of the governance. Governance is often meant or confused with power. This is driven by the perception of a hierarchical structure or a linear approach, while should indeed aim at guaranteeing the functioning of the system in a complex context.

For instance, amoebae do not rely on any leader cell (Kauffman et al. 1978): they assemble and dissemble without any programmed design. Birds do not rely in chiefs, but they organize in flocks to survive in migrations or against predators. Such self-organization have been successfully simulated by network science (Kauffman 2019). Crucial parameters are the number of connections and their level of channelling, which is the number of prevalent outputs respect to the possible ones. In many biological complex systems, few commands are functional to enable the community to self-organize (Couzin et al. 2005). Many other examples can be found, all framed within the concept that: more is different (Anderson 1972).

So we can ask why we, as humans, are reluctant to copy, or at least to learn, from systems that show some kind of complexity and have adopted strategies to maintain their functionality and identity. We are hominids, evolved in limited environments framed within cause-effect dynamics. Similar contexts facilitated structured societies, in primates and other mammals (Grueter et al. 2012).

Markets are a recent example of how globalization has asked humans to design other forms of governance for high dynamic systems. In analogy with the military approach, organizations of companies have followed some form of a hierarchical structure, mainly based on roles, discipline, and linearity/control of communications. Computer networks have then suggested a variety of topologies to be translated into organizational structures as a star, small world, random or scale-free networks (Atkinson and Moffat 2005, Albert and Barabasi 2002). In these cases, the unstoppable power of leaderless organizations is demonstrated (Brafman and Beckstrom 2006, Laloux 2015, De Toni et al. 2012). This being said, the form of governance has been positioned at a level higher than the product or the strategy (Hamel 2007, Ostrom 2010).

Reflections

Institutions are focusing on robustness and not on resilience.

Robustness is the capacity to resist external stresses, maintaining the internal structure. Resilience is the capacity to transform the organization/structure to adapt to external changes in order to maintain the identity of the system. In both cases, the main functions are preserved. Policy makers, when dealing with complex challenges, are more frequently invoking a resilience of the system: unfortunately, they often forget that resilience could also imply a restructuring of the governance. That is, "queens and kings" should be ready to give back their thrones. Governance is often confused with an organizational structure for humans, but this is not sufficient. When we in fact address a complex ecosystem, we have to take into consideration the multitude of interconnected variables which contribute to the dynamics of the system. These variables could include aspects from different sectors, difficult to be quantified and to be interoperate. Self-organization does not mean anarchy or lack of governance.

Some attempts for more integrated strategic approaches to design appropriate modes of governance have been promoted (Carayannis et al. 2012, Meuleman 2018). This proposals remain at the level of ambition and political guidance: operationally, they are difficult to implement: the system is difficult to control and to predict.

The future does depend on the present, but different scenarios can be realized depending on actions from different agents.

Foresight is a methodology for shaping the future (Popper 2008, Georghiou 2008). Back-casting foresight is a planning method that starts with defining a desirable future and then works backwards to identify policies, programs and action that will connect that specified future to the present (Brandes and Brooks 2005, Brandes and Kriwoken 2005). It does not identify multiple scenarios, but different paths/roadmaps to achieve the desirable future. One of these paths is the design of the appropriate governance which will be functional to the achievement of the goals. Back-casting foresight results in a systemic approach and it is based on a strategy to implement feasible and impacting interventions.

Any ecosystem approach should therefore address the multitude of interconnected variables, if it is meant as an enabler for solutions to anthropogenic complex challenges. The process should include a knowledge-based support to identify objectives and paths (through back-casting), and particular care in designing the management of the different agents, not only environmental aspects, which will enable the path to be implemented (through the appropriate governance structure).

Let us reflect in particular on the ecosystem approach traditionally suggested to tackle the challenges for seas and oceans. Human activities, such as fishing and transportation, impact on the marine environment, eventually decreasing availability of resources and increasing pollution. Regarding pollution, the contribution from land is the main source in many cases. A healthy marine environment, in turn, can provide valuable resources for human activities. Health and productivity are strictly interconnected. At the end, it is a matter of a sustainable balance between conservation and use of resources.

Sustainability can be evaluated at economic, environmental, social or political level. Priorities and capacities at territorial level can then show a diversity of path and goals. In practice, we address again a multi-dimensional and multi-level network, where local choices/actions can influence the emergence of global priorities and global trends can facilitate the evolution of the fragmented paths.

The ecosystem approach has been usually promoted when dealing with environmental challenges and often supported by models which include environmental variables solely (D’Alelio et al. 2019, Coles et al. 2017). Complex environmental system themselves can show abrupt changes and unexpected properties. In different contexts, this complexity can result in political asymmetric shocks or financial crisis. The economic interest in predictions of tipping points and irreversible paths is huge, since precautionary approaches are usually refused by the industrial sector, asking in turn for more detailed studies, thresholds and specifications to be included in legislation and insurance contracts which can drive choices and evaluation of financial paybacks.

To simplify, the expanded ecosystem approach we are fostering involves three main actors: science, industry, policy. In few words, decision-makers ask for scientific evidences to negotiate with the private citizen or enterprises in order to reach a reasonable compromise between environmental and economic sustainability.

In this scenario, some scientists do take advantage by addressing unanswered questions while maintaining their position of privilege in closed communities. Therefore solutions rarely will emerge.

This being said, the approach to achieve a knowledge-based evaluation of the impacts of anthropogenic activities on different ecosystems cannot to be restricted to an "affair" of scientific debates. It is not simply a challenge of scientific communication or knowledge transfer, but a lack of methodology which very rarely adopts the proper process for the involvement of agents and tools.

Global challenges address complex systems, where the ecosystem is composed by many different agents and the prefix "eco" should not be limited to the environmental aspects.

Understanding the interactions and roles in the functioning of ecosystems is still a scientific challenge. We propose to reflect on the meaning and appropriateness of approaches and processes promoted as appropriate to tackle global challenges. Many scientific results and clues from analogous approaches to complexity can definitely help.

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Sensing of mechanical stimuli in copepods

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Abstract

Copepods are ubiquitous millimetre-scale crustaceans, representing the most abundant component of the marine zooplankton assemblage. Despite their small size, copepods are equipped with an array of finely tuned sensors by which acquiring information from the surrounding environment. Such information is subsequently used by copepods to activate specific behavioural adaptive responses, for example escaping from an approaching predator or attacking a prey. In this contribution we focus on mechanical sensors used by copepod to detect fluid deformations. First, we summarise the types of sensors possessed by copepods, then we report on the behavioural responses of key target species upon solicitation. This report evidences the tight link between sensory array, neurophysiologic performance and behaviour, shedding light on the ability of these organisms to dynamically interact with their environment.

Context

Present in any aquatic habitat, copepods are the most abundant metazoans on Earth (Huys and Boxshall 1991). They show an amazing diversity of life forms, from free-living to parasitic, and exceptional colonising abilities (Huys and Boxshall 1991). They are typically small, with a characteristic body length mostly between 0.5 and 5.0 mm in the marine planktonic forms (Huys and Boxshall 1991). Copepods contribute up to 97% of the total marine zooplankton biomass, provide the link between lower (e.g., phytoplankton, detritus) and upper (e.g., carnivorous zooplankton, fish larvae) trophic levels, contribute to the vertical flux of carbon through the egestion of fecal pellets and sustain recycled production by ammonium excretion (as reviewed in Uttieri 2018).

Similarly to other crustaceans, the interaction of copepods with the surrounding environment is guaranteed by a complex array of sensors distributed over their body (Laverack 1968, Bush and Laverack 1982) which, using Laverack's (1968) words, "accept energy of one type (mechanical, light, chemical) and transform it to another (electrical)". The information collected is then converted into an electric stimulus that travels through the central nervous system of the organism, triggering appropriate responses. As reviewed in Heuschele and Selander (2014), copepods rely on light, chemical and light stimuli.

Changes in the light field are detected by means of naupliar eyes, located in the middle of the head and primarily used to drive diel vertical migrations (Heuschele and Selander 2014 and references therein).

Chemical and mechanical sensors are instead distributed over copepods' body, but mostly along their first antennae (A1) Chemoreception is mainly ascribed to aesthetascs, formed by the aggregation of a large number of sensors (Laverack 1988), and mostly distributed over the first segments of the A1s (Boxshall et al. 1997). Other chemical receptors can be distributed over the body, for example on the mouthparts or in proximity of the gonopore (Heuschele and Selander 2014 and references therein).

The detection of mechanical stimuli is primarily appointed to setae, the most widespread supracuticular mechanoreceptors found in crustaceans. They have a 9+0 basal body structure (Strickler and Bal 1973), with a whorl of nine peripheral microtubule doublets arranged in circle. Seat a thick cuticle and flexible base inside a socket articulation allowing them to bend in all or in specific directions depending on socket morphology and basal innervation (Bush and Laverack 1982). Setae with different morphologies are associated with specific functions (Yen et al. 1992), and some present an apical pore likely allowing the sensors to perform as both mechanical and chemical detectors (Jacques 1989). Additional supracuticular receptors, the so-called pegs, are articulated hair barely projecting over the cuticle (Bush and Laverack 1982). In addition, some sensors can perform at the same time as mechanical and chemical receptors, showing intermediate morphological and nervous features.

State of the art & Results

Mechanical stimuli are involved in the detection of prey and predators (e.g., Fields and Yen 2002), but also mates (Yen et al. 1998). The arrangement of setae along the A1 provide indications about the modality of cue detection and is linked to the swimming behaviour of the copepod and to its modality of prey capture (Uttieri et al. 2008a, Uttieri et al. 2008b). Notwithstanding its key role, the process of signal detection is understood only in part. Légier-Visser et al. (1986) assumed copepod mechanoreceptors acted as pressure detectors, but this view was subsequently criticised in favour of velocity-based (Kiørboe et al. 1999) and fluid-deformation (Yen and Okubo 2002) models.

Among crustaceans, copepod setae are the most responsive and sensitive (Weatherby and Lenz 2000, Fields and Yen 2002), responding to frequencies from 30 Hz up to 5 kHz with reaction times in the order of ms (Yen et al. 1992, Gassie et al. 1993, Fields and Weissburg 2004). Differently from other processes, neurophysiological responses have a low temperature dependance (Lenz et al. 2005). The distal tip of the A1 represents the most sensitive area to predator signals, bearing the longest setae and working as an early-warning system (Lenz and Yen 1993)

The primary role of A1s in the detection of mechanical stimuli was verified by ablation experiments (e.g., Gill and Crisp 1985), direct stimulation (e.g., Gill 1985) and electrophysiological recordings (e.g., Yen et al. 1992). In particular, electrophysiological experiments permit the quantification of the neurophysiologic responses and consequently of the nerve impulse traffic associated with a given signal. Typically, such trials are carried out by keeping the animal in insulating mineral oil leaving only one A1 protrude in sea water, applying standard extracellular recording techniques to measure the electric potential between two reference electrodes (Gassie et al. 1993, Fields et al. 2002). Most of the literature on this topic has been focused on large species for ease of handling (Yen et al. 1992, Lenz and Yen 1993, Fields et al. 2002, Fields and Weissburg 2004), permitting the recording from the entire animal but also from the detached A1.

Preliminary experiments were carried out at the Stazione Zoologica Anton Dohrn to measure the neurophysiologic performance of the copepod *Clausocalanus furcatus*, a species with a body length of approximately 1 mm. The integration of sensory structure mapping along the A1s (Uttieri et al. 2008a) with the analysis of swimming behaviour and prey capture area (Uttieri et al. 2008b) suggested that the first segments of the A1s (where setae were more densely aggregated) were the most responsive to mechanical stimulation from a food item (Figure 1). Standard neurophysiology techniques were thus used to evaluate the sensitivity of three different sectors (basal, median and distal) of *C. furcatus* A1s. Mechanoreceptive setae were stimulated using a pump system tuned to simulate the movement of a typical prey.

The results of these experiments demonstrated that the most intense responses were shown by the A1 sector with the highest abundance of setae. The distal tip, where setae were scarce, recorded the least intense response. These results were confirmed by the silencing of the nervous response in presence of TTX (tetrodotoxin, a potent non-protein marine neurotoxin), confirming that the nerve traffic recorded by the system was due to the active response of mechanoreceptors.

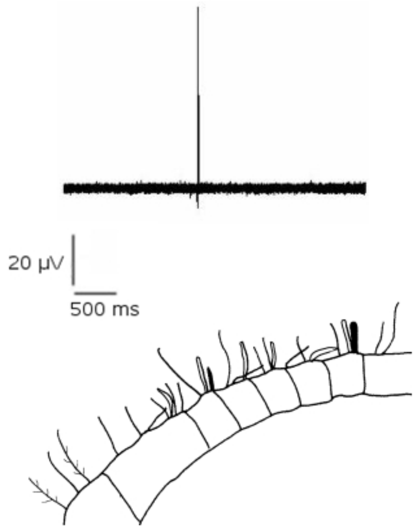

Figure 1: Traffic nerve impulse (upper panel) recorded in correspondence of the first segments of the A1 of *Clausocalanus furcatus* (lower panel). Along the outer edge of the A1 are aligned the different sensors of C. furcatus: mechanoreceptive seate (thin structures), chemoreceptors (aesthetascs; white cylindrical structures) and dual function sensors (mechanical and chemical; black cylinders).

Reflections

The analysis of the types and distribution of sensors present on copepod A1s provide fundamental information about the modalities by which these organisms interact with their surroundings. The literature data and the experiments here described unveil the close relationship between sensory array, neurophysiologic fitness and behaviour in copepods. Detailed microscopic investigations (SEM, TEM, LSCM) can be complemented with neurophysiologic recordings of the nerve impulse traffic upon solicitation by specific mechanical triggers mimicking predators, prey or mates. In the specific case of *C. furcatus*, the results presented here allows a more complete picture of the mechanisms utilised by this small calanoid to detect potential food items. In addition, the results discussed indicate a differential level of sensitivity of the three sectors of the A1.

This approach could be expanded by exposing copepods to different types of signals and by addressing the neurophysiologic responses of key target species. As a further perspective, it would be extremely instructive to evaluate if the response at the level of the individual can be used as a communication strategy among conspecifics. To date, no experiments have been carried out on this topic, but they would shed light on the possibility of a collective behavioural response of single individuals to a shared signal. The results that could be gathered would provide new insight not only in the biology of these small crustaceans, but also in the modalities by which these organisms react dynamically to an ever-changing environment.

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Sound SEAgnals

Tim Wyatt

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Abstract

The Cambrian radiation and the evolution of predation added animal noises (zoophony) to the geophysical sound environment (geophony). New sensory systems were needed to navigate this augmented soundscape. The widening panorama of signals, sounds amongst organisms, demanded more sophisticated hardware and software to extract meanings from it. Here it is suggested that pre-Cambrian sounds remained important for many marine organisms, i.e. inshore plankton.

Watch, find, attack, seize, eat, evade, escape, menace, hide, embrace – these intermittent activities have defined much behaviour in the animal world, at least since the Cambrian revolution. There is nothing ambiguous about body armour, or claws (Godfrey-Smith 2018), or about the grasping teeth (conodonts) of Protohertzina (an ancestral chaetognath, and perhaps one of the earliest planktonic predators), 'curved and scimitar-like', each 'with a wicked little point' (Brasier 2009). There was a quantum leap in the hostility of the world as evolution discovered new ways to obtain food. A co-evolutionary arms race had begun, fuelled by rising levels of oxygen in the ocean.

New life-styles introduced the need for new signals. All these activities needed sense organs and information processing systems with which to fashion appropriate behaviour. Recognizably modern sensors first appeared in the fossil record in the Palaeozoic, when it became progressively more necessary to decipher the signals of neighbours as well as those of the inanimate surroundings. To earlier, simpler systems responsive to light and chemicals, metazoan evolution added eyes and antennae, and sense organs to measure shear, acceleration, and to listen to the newly evolving acoustic signals of neighbours.

There are no sounds in a vacuum, nor in a world without hearing. The 'Cambrian information revolution' (Plotnick 2010) added animal sounds (zoophony) to the background geophysical noises (geophony), and perhaps in shallow waters to the high-pitched ping of photosynthesis in algal mats. Soundscapes became more diversified, sensory diets acquired extra dimensions, leading to a necessary diversification of detectors, signal processing, and response mechanisms. Alarms, beacons, cues, gestures, signs, were added to the grist of natural selection; phonotypes augmented phenotypes. The widening panorama of signals, sounds amongst them, demanded more sophisticated hardware and software to extract meanings from it.

But meanings are in the maps made from the scapes, not in the tools which construct them. Crude data, like crude oil, needs refineries, and outputs need consumers. Different species detect different signals, transduce them into different arrays of information and combine them with inside information. Each species has its own shorthand, its own unique reductionist tools, its own algorithms, to convert perception into cognition. We cannot follow the trails of ants, or hear the sonar of bats. But whalers of the past could listen for the songs of their prey through the wooden hulls of their ships!

It is supposed that hearing organs evolved from earlier mechanoreceptors able to detect subsonic vibrations or pressure changes. Vibrations are the physics outside, sound (the audible aspect of the pressure wave) is the biology inside. The fusion thresholds at which vibrational frequencies becomes sound are functions of sensory transduction, different for each species, hence distinctions between sounds, vibrations and water movements are not clearly separated.

Physics sets limits to the biological uses of sound. The efficiency of emission increases with frequency (high-pass filter), transmission decreases with frequency (low-pass filter). Thus the frequencies evolved and used by communicating animals suggest the intended ranges of recipients. Also, as sound signals propagate, they are attenuated by geometric spreading, and degraded and distorted by refraction, diffraction and reflection. Higher frequencies are not heard at great distances. All these physical constraints are site specific. The extent to which sound signals can provide useful information given these limitations measures the acuity of an auditory system and the proficiency of processing. In animals with nervous systems, sound intensity and direction, frequencies and temporal variations – and melody, harmony, rhythm, and punctuating silence – are transformed into patterns of neuronal activity.

A broad definition of hearing states that "an animal hears when it behaves as if it has located a sound source" (Pumphrey 1950). This definition can be broadened by replacing animal with organism, and expanding sound to include audible features and vibrations. Then a protist can hear too if a response can be elicited to the direction or quality (either or both) of a sound. Some recent experiments suggest that even a flowering plant (Oenothera) responds to the buzzing of bees by increasing the sugar content of its nectar (Veits et al 2019), hence telling the bees to come again and improve the dispersal of its pollen.

Protistan hearing abilities have not been explored, but the existence of protists including algae specialized for life in turbulent inshore environments, combined with their ability to avoid being thrown ashore by breaking waves, indicates that they have the means to avoid that fate, and the means to know when it threatens. The problem is classically referred to by the German term Uferflucht for "shore avoidance". Plankton may drift with the wind but it is rarely cast ashore. Yet planktonic larvae find the gaps in the shore and enter the mouths of the estuaries and lagoons which serve them as nursery areas, and others can settle within the surf zone itself. Ability to sense the proximity of the shore combined with downward migration may be part of the solution.

A simple 2-D model of the nearshore circulation describes an ingoing surface current generated by Stokes drift, an outgoing intermediate current, the undertow, and an ingoing bottom current. There are thus two intermediate depths where there is no net shoreward transport (Longuet-Higgins 1953). Rather more attention has been focused on the problem of how larval or postlarval stages of intertidal and subtidal organisms, having been dispersed into coastal waters, regain the shore where they must eventually settle.
Barnacle cyprids and crab megalopae move shoreward by remaining near the surface; this ensures that they are swept horizontally into the convergences generated by shoreward directed internal waves released from the thermocline as the ebb tide slackens (Shanks & Wright 1987). Stokes drift may amplify this process. Shoreward drift will be halted by moving downwards. We can thus imagine that appropriately timed vertical migration is in principle able to counteract the threat of being thrown ashore by the surface flow, either by finding the depth of no net transport, or by entering the undertow and standing off again (Squires 1996).

The question remains, what signals are used to detect the proximity of the shore. In placid lakes, the polarization of light is a possibility (Burckardt 1910; Siebeck 1968; Schwind 1999). But on windswept ocean shores, the directional content offered by polarization must be eliminated as light penetrates foam generated by breaking waves. In such circumstances, vibrational signals generated by breaking waves are a possible alternative (Wolterek 1928). In the absence of breakers, where the shore is interrupted by the entrances of lagoons or estuaries, the behavioural response is not evoked, and the larvae continue in the direction of their nursery/settling grounds. Then the sound of breaking waves must be an important cue for nearshore animals and even for inshore phytoplankton, and probably for epipelagic life more generally.

Despite the Cambrian revolution, the sounds of the pre-Cambrian ocean have not ceased, and still offer a source of information, even to morphologically simple organisms which lack the sophisticated sense organs and nervous systems of arthropods, molluscs and vertebrates. The blind man’s stick converts sound and touch into sight. The sound of surf pounding on reefs and banks, or beaches and cliffs, warns of their proximity to organisms able to hear it and interpret it. Very small organisms cannot contribute significantly to a soundscape since they cannot muster the energy needed to do so. Nevertheless, they must be sensitive to sound, and in some environments ability to respond to sound is essential. Action based on effective pattern recognition improves the likelihood of survival.

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Tuning communication for functioning: sounding in/the fluids

PROGRAMME

Remotely: 2 July - 8 July 2020

17 June 10:00 – 12:30 **SEA-GNALS**

Introduction

10:00 – 10:05 Welcome and rules for the workshop
10:05 – 10:20 Rationale and Goals: Stem Materials (Pier Francesco Moretti – CNR S&T Foresight)

Acoustic communication and disturbances in aquatic environment

10:20 – 10:40 Detecting underwater acoustic signals: what are we hearing? (Giorgio Riccobene - INFN)
10:40 – 11:00 Communication in marine environment: big sized animals. Use of animal data logger to link sound exposure to diving behavior and physiology (Walter M. X. Zimmer – Saclant centre)
11:00 – 11:20 Communication in marine environment: other organisms (Giusi Buscaino CNR-IAS)
Break
11:40 – 12:00 Sensing of mechanical stimuli in marine zooplanktonic organisms (Maria Grazia Mazzocchi & Marco Uttieri - SZN)
12:00 – 12:20 Impacts of underwater noise on physiology of marine organisms (Gianni Pavan – Università di Pavia)
Collection of questions in chat mode

24 June 10:00 – 12:00 **SYNC-NALS**

10:00 – 10:20 Neuronal communication (Silvia Battistoni CNR-IMEM)
10:20 – 10:40 Functioning of brain waves (Valentina Benfenati CNR-ISOF)
10:40 – 10:50 Micro-organisms frequencies (Gianni Longo & Marco Girasole – CNR-ISM)
10:50 – 11:10 Title to be decided (Gianluca Gagliardi, CNR-INO)
11:10 – 11:40 The role of synchronism (Silvano Buogo CNR-INM)
Collection of questions in chat mode

18:00 Electronic distribution of questions to the audience

8 Luglio 10:00 – 12:30 **COMMUN-NALS**

10:00 – 10:20 Reflections on the approaches (Alice Affatati)
10:20 – 10:30 Grouping of questions (Pier Francesco Moretti - CNR-UREI)
10:30 – 12:30 Discussion (Chaired by Maurizio Ribera Alcalà)

Expert Bio and keywords

<table>
<tr><td>Family Name</td><td>Affatati</td><td>Given Name</td><td>Alice</td><td>Sex</td><td>F</td><td rowspan="5"></td></tr>
<tr><td>Nationality</td><td colspan="2">Italian</td><td>Year of birth</td><td colspan="2">1984</td></tr>
<tr><td>Organization</td><td colspan="2">Istituto Nazionale di Oceanografia e di Geofisica Sperimentale –OGS (Italy) & Memorial University of Newfoundland (Canada)</td><td>Degrees</td><td colspan="2">Engineering</td></tr>
<tr><td>Your best professional achievement</td><td colspan="5">In the context of my work at GEOMAR my findings provided novel insights into the behaviour of silver nanoparticles in seawater, with much faster than anticipated removal of nanoparticles when entering the ocean. The fast removal is due to kinetically rapid coagulation processes, which have not been observed by others due to the improved and novel detection techniques employed. Experiments with high dilutions solutions of nanoparticles in seawater were developed for the first time ever. Hopefully, in the underwater acoustics field the best achievement is jet to come.</td></tr>
<tr><td>Your best personal achievement</td><td colspan="5">I was one of the members of the team that performed the longest ever great white shark (Carcharodon carcharias) continuous acoustic manual tracking (107hrs on a 4.2m female) with the largest ever recorded difference between a white shark stomach temperature and the surrounding waters (+13.5 degrees).</td></tr>
<tr><td>Short bio (max 300 words)</td><td colspan="6">I have a background in Engineering but I have tailored my studies to include Oceanography as well.
I worked in the acoustic telemetry and bioacoustics field in South Africa and Namibia focusing on studying the behavior of cetaceans and great white sharks (Carcharodon carcharias) also in relation to the physical properties of the ocean.
Furthermore, at GEOMAR (Kiel, Germany) I was involved in an interdisciplinary experimental oceanography project. At the National Institute of Oceanography and Applied Geophysics – OGS (Trieste, Italy) I have been researching underwater noise and its effects on marine fauna and at the Laboratory of Applied Bioacoustics (Vilanova I la Geltrú, Spain) cetacean bioacoustics.
Among other things, I am now working with a group of experts on the subject of the effects of underwater noise in the Arctic Ecosystem in the Framework on the United Nations Decade of Ocean Science for Sustainable Development in the Arctic Ocean.</td></tr>
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Expert Bio and keywords

Family Name	Basios	Given Name	Vasileios	Sex	M	Picture	
Nationality	Greek			Year of birth	1962		
Organization	Interdisciplinary Centre for Nonlinear Phenomena and Complex Systems (Cenoli-ULB) & Département de Physique des Systèmes Complexes et Mécanique Statistique.			Degree[s] in Physics	PhD, MSc, BSc		
Your best professional achievement	Participation in developing a new paradigm of crystallization/aggregation during ESA's complex matter initiative.						
Your best personal achievement	Developing a novel approach for biological information processing implementing inverse Bayesian inference.						
Short bio (max 300 words)	Vasileios Basios is a senior researcher in Complex Systems, interdisciplinary physics, with over 25 years in research and teaching. He is conducting research in the foundations of complexity science and nonlinear systems, in self-organization of complex matter. He serves as an adviser for research projects and as research faculty in international graduate schools on Complexity and Non-linear dynamics. He worked on self-organization and a new paradigm of nucleation/aggregation in nano-materials (proteins and zeolites) with the team of Prof. G. Nicolis at ULB sponsored by the European Space Agency (ESA). That was a biotechnology initiative for the, in orbit laboratory, of the 'ProMISS' and 'GCB' experiments on Thermodynamic and Statistical Mechanical Aspects of Protein Crystallization and Pattern Formation in Reaction-diffusion systems; "Complex Matter" initiative. Where he also organized and led panel discussions of ESA Topical Teams, workshops and conferences on Complexity science. The issue of collective dynamics in living and non-living matter is a focal interest of his current studies and in collaboration with the team of Prof. Yukio-Pegio Gunji at Tokyo's Waseda university, they have developed a novel approach to Bayesian inference, called 'Bayesian and Inverse Bayesian inference' (BiB), that has furnished novel insights especially on the area of biological information processing. He has extensive experience in large scale simulations and modelling of nonlinear and stochastic systems, neural networks, Monte-Carlo methods and, Bayesian inference, for nonlinear complex systems. In addition he is engaged in addressing varied and diverse audiences from learned specialists to laymen and students on the theme of complexity, philosophy of science and the history of scientific ideas, related to complex systems' science. During his formative years he was tutored by Ilya Prigogine (Nobel Laureate) and Grégoire Nicolis at ULB; where he got his PhD, after studying with John S. Nicolis 'cybernetics', at the University of Patras.						

Expert Bio and keywords

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<tr><td>Family Name</td><td>Buogo</td><td>Given Name</td><td>Silvano</td><td>Sex</td><td>M</td><td rowspan="5"></td></tr>
<tr><td>Nationality</td><td colspan="2">Italian</td><td>Year of birth</td><td colspan="2">1964</td></tr>
<tr><td>Organization</td><td colspan="2">National Research Council of Italy</td><td>Degree[s] in Physics</td><td colspan="2">Physics</td></tr>
<tr><td>Your best professional achievement</td><td colspan="5"></td></tr>
<tr><td>Your best personal achievement</td><td colspan="5"></td></tr>
<tr><td>Short bio (max 300 words)</td><td colspan="6">Working in underwater acoustics since the mid ‘90s mostly on metrology, bubble dynamics, and ultrasound for medicine and biology. Visiting scholar at the Johns Hopkins University, Baltimore, in 1993-1994. Research Assistant at the formerly SACLANTCEN, La Spezia (now CMRE) in 1996. Researcher at the Institute of Acoustics, Rome, from 1999 to 2017 and afterwards at the Institute of Marine Engineering.</td></tr>
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Expert Bio and keywords

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<tr><td>Family Name</td><td>Buscaino</td><td>Given Name</td><td colspan="2">Giuseppa</td><td>Sex</td><td>F</td><td rowspan="5"></td></tr>
<tr><td>Nationality</td><td colspan="2">Italian</td><td>Year of birth</td><td colspan="3">1973</td></tr>
<tr><td>Organization</td><td colspan="2">CNR</td><td>Degree[s] in</td><td colspan="3">Natural Science</td></tr>
<tr><td>Your best professional achievement</td><td colspan="6">To build, where there was none, and maintain a research line in marine and freshwater bioacoustics.</td></tr>
<tr><td>Your best personal achievement</td><td colspan="6">Becoming a kitesurfer.</td></tr>
<tr><td>Short bio</td><td colspan="7">Giuseppa Buscaino, MSc in Natural Science, PhD in Environmental Science. She is researcher at the Italian National Research Council. Her main research topics are: marine bioacoustics and acoustic ecology, evaluation of the impact of noise on marine organisms, temporal patterns of main components of marine soundscape and correlations with physical driven factors. She obtained the National Academic Qualification as Full Professor in Zoology (2018) and she is associate researcher at University of Palermo since 2017. She is the CNR representative at the EMSO Joint Research Unit. Author of 78 ISI publications, HI=21, number of citations=1207 (scopus, 11 November 2020).</td></tr>
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Expert Bio and keywords

<table>
<tr><td>Family Name</td><td>Fabbri</td><td>Given Name</td><td>Roberta</td><td>Sex</td><td>F</td><td rowspan="5"></td></tr>
<tr><td>Nationality</td><td colspan="2">Italy</td><td>Year of birth</td><td colspan="2">1989</td></tr>
<tr><td>Organization</td><td colspan="2">Consiglio Nazionale delle Ricerche, Istituto per la Sintesi Organica e la Fotoreattività</td><td>Degree[s] in</td><td colspan="2">BSc and MSc degrees in Biomedical Engineering, University of Bologna</td></tr>
<tr><td>Your best professional achievement</td><td colspan="5">Accepted review article “Graphene Glial-interfaces: challenges and perspectives” submitted on Nanoscale journal.</td></tr>
<tr><td>Your best personal achievement</td><td colspan="5">Received the opportunity to attend a PhD program, as research and formation experience.</td></tr>
<tr><td>Short bio
(max 300 words)</td><td colspan="6">Roberta Fabbri is a PhD student in in Biomedical, Electrical and Systems Engineering, curriculum Bioengineering, at the University of Bologna. She is currently working at CNR-ISOF, in the group coordinated by Dr. Valentina Benfenati, who supervised both BSc and MSc theses. During BSc thesis, she focused on the research advancements regarding the biomedical technologies for the study and rehabilitation of retinal functionality. In continuity with MSc thesis, her research activity is mainly devoted to the development and validation of carbon-based advanced biomedical interfaces and interpretative models for the study of brain glial cell. Particularly, she is interested in investigating the functional interaction of astrocytes with nanostructured graphene-based materials and devices, towards the engineering of graphene-glial interfaces aiming at selective modulation of glial functions. Her interdisciplinary research field ranges from material science and nanotechnology to biology and biomedical applications of innovative biomaterial interfaces in neuroscience and neurology.</td></tr>
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Expert Bio and keywords

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<tr><td>Family Name</td><td>LONGO</td><td>Given Name</td><td>GIOVANNI</td><td>Sex</td><td>M</td><td rowspan="5"></td></tr>
<tr><td>Nationality</td><td>ITALIAN</td><td colspan="2">Year of birth</td><td colspan="2">1975</td></tr>
<tr><td>Organization</td><td colspan="2">CONSIGLIO NAZIONALE DELLE RICERCHE – ISTITUTO DI STRUTTURA DELLA MATERIA</td><td>Degree[s] in (Phd please in the short bio text below)</td><td colspan="2">PHYSICS</td></tr>
<tr><td>Your best professional achievement</td><td colspan="5">The nanomotion sensor</td></tr>
<tr><td>Your best personal achievement</td><td colspan="5">20 years in Curva Sud.</td></tr>
<tr><td>Short bio (max 300 words)</td><td colspan="6">Dr. Longo has graduated in physics in 2000 at the Rome university La Sapienza, and has obtained his PhD in 2006 on the “Study of Oligonucleotide-Loaded Silicon Surfaces with AFM and Quantitative Fluorescence”.
Dr. Longo has focused on the characterization of nanostructures and of nanosized systems (mainly of biological and medical interest); particularly on scanning probe microscopies and high-resolution characterization techniques. By developing different kinds of nanomechanical sensors, he has applied them to the study of a wide range of scientific problems. These include the study of nanoscale contaminants in marine environments, the development and characterization of nanostructured coatings for implant osseointegration and the development and use of the nanomotion sensor in microbiology, biology and oncology applications.</td></tr>
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Expert Bio and keywords

<table>
<tr><td>Family Name</td><td>Moretti</td><td>Given Name</td><td colspan="2">Pier Francesco</td><td>Sex</td><td>M</td><td rowspan="5"></td></tr>
<tr><td>Nationality</td><td colspan="2">Italian</td><td>Year of birth</td><td colspan="3">1967</td></tr>
<tr><td>Organization</td><td colspan="2">CNR</td><td>Degree[s] in</td><td colspan="3">Physics</td></tr>
<tr><td>Your best professional achievement</td><td colspan="6">The interpretation of the reddening of some asteroids' spectra has allowed to develop a procedure for the analysis of hardening of steels, which has been patented.</td></tr>
<tr><td>Your best personal achievement</td><td colspan="6">A noir fiction book I wrote to transfer my dreams and fears.</td></tr>
<tr><td>Short bio</td><td colspan="7">Pier Francesco Moretti is a physicist with two PhDs, more than 80 international publications in astrophysics, material sciences, and a patent. His skills in research were mainly focused in spectroscopy, image analysis and innovative technologies. He worked in USA and Austria. He has been involved in many international research projects and has been responsible of the Office for the International Activities of the CNR Department of Earth and Environment. He has participated many European projects and Governmental Boards (JPI Oceans, Marine Board, Sherpa groups of G7-Science). He has been vice-chair of the Research Working Party of the EU Competitiveness Council during the Italian Presidency of the European Union.
He was a permanent professor in Physics at nautical and technical institutes.
He now works at the CNR Liaison Office in Brussels, mainly involved in support to policy, coordination of national programmes and foresight activities for technological development.
See also pierfrancescomoretti.eu</td></tr>
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Expert Bio and keywords

<table>
<tr><td>Family Name</td><td>Uttieri</td><td>Given Name</td><td>Marco</td><td>Sex</td><td>M</td><td rowspan="5"></td></tr>
<tr><td>Nationality</td><td colspan="2">Italian</td><td>Year of birth</td><td colspan="2">1978</td></tr>
<tr><td>Organization</td><td colspan="2">Stazione Zoologica Anton Dohrn, Naples, Italy</td><td>Degree[s] in (Phd please in the short bio text below)</td><td colspan="2">Environmental Sciences (Oct 2001)</td></tr>
<tr><td>Your best professional achievement</td><td colspan="5">I organised and are presently chairing the ICES Working Group entitled “Towards a EURopean OBservatory of the non-indigenous calanoid copepod Pseudodiaptomus marinUS”, coordinating more than 50 researchers from different European and extra-EU Countries.</td></tr>
<tr><td>Your best personal achievement</td><td colspan="5">I am father of two kids, the most challenging activity in my life.</td></tr>
<tr><td>Short bio (max 300 words)</td><td colspan="6">I graduated in Environmental Sciences (University of Naples “Parthenope”, I) in 2001 and I was awarded a PhD in “Dynamics of Marine Ecosystems” (Open University, UK, and Stazione Zoologica Anton Dohrn, I). Following post-doc experiences, I worked as Assistant Professor (non tenure) in Zoology at University of Naples “Parthenope” (2012-2015), and since 2017 I am working as research scientist at Stazione Zoologica Anton Dohrn. My research interests focus principally on different aspects of the biology and ecology of zooplanktonic organisms from both marine and freshwater environments, resort to different tools, building an integrated, multidisciplinary approach.
I was PI of the MOKA (Modelling and Observation of zooplankton orgAnisms) project, financed by Italian Ministry of Research and Education within the FIR-FIRB framework. I also acted as Responsible of two Research Units in the framework of the “Flagship Project RITMARE – The Italian Research for the Sea” project, coordinated by the Italian National Research Council and funded by the Italian Ministry of Education, University and Research within the National Research Program 2011-2013. Over the years I published more than 40 Scopus indexed contributions, and acted as Editor of the book “Trends in Copepod Studies”. I serve as Editorial Board Member for: Scientific Reports, Frontiers in Marine Science, Journal of Marine Science and Engineering.</td></tr>
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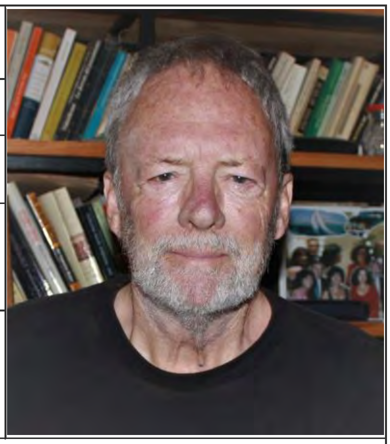
Expert Bio and keywords

<table>
<tr><td>Family Name</td><td>Wyatt</td><td>Given Name</td><td colspan="2">Timothy</td><td>Sex</td><td>M</td><td rowspan="5"></td></tr>
<tr><td>Nationality</td><td colspan="2">British</td><td>Year of birth</td><td colspan="3">1937</td></tr>
<tr><td>Organization</td><td colspan="2">none</td><td>Degree[s] in</td><td colspan="3">Medicine</td></tr>
<tr><td>Your best professional achievement</td><td colspan="6">for others to judge</td></tr>
<tr><td>Your best personal achievement</td><td colspan="6"></td></tr>
<tr><td>Short bio
(max 300 words)</td><td colspan="7">In 1967, Tim joined the British Scientific Civil Service, at the Ministry of Agriculture Fisheries and Food (MAFF, now CEFAS) laboratory in Lowestoft. There he worked closely with the renowned fisheries and plankton scientist, David Cushing, on zooplankton dynamics in the North Sea, and on larval fish problems. Cushing wrote several books on both fisheries and plankton, and in 1979 Cushing, as Editor-in-Chief, with Tim Wyatt, as Deputy Editor, founded the Journal of Plankton Research (JPR). When Cushing stepped down from JPR in 1999, Tim became Editor-in-Chief until 2002. He was also Editor of the UNESCO-IOC Harmful Algae News from 1991 to 2012.
Tim has published on fisheries cycles, zooplankton, phytoplankton, secreted organic matter and ecological engineering, as well as the significance of mythology, religion and ethics in science history and methods.
See also https://issha.org/publications-resources/hab-trailblazers/tim-wyatt/</td></tr>
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