A community perspective on the concept of marine holobionts: ~~state-of-the-art~~current status, challenges, and future directions

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[*] This working group gathers 31 scientists from ten different countries, with expertise covering different scientific disciplines including philosophy, evolution, computer sciences, marine biology, ecology, chemistry, and microbiology, who participated in a workshop on marine holobionts, organized at the Roscoff Biological Station in March 2018. Their aim was to exchange ideas regarding key concepts and opportunities in marine holobiont research, to start structuring the community, and to identify and tackle key challenges in the field.

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Abstract:

Host-microbe interactions play crucial roles in marine ecosystems, but we still have very little understanding of the mechanisms that govern these relationships, the evolutionary processes that shape them, and their ecological consequences. The holobiont concept is a renewed paradigm in biology that can help describe and understand these complex systems. It posits that a host and its associated microbiota, living together in a ~~long-lasting~~stable relationship, form the holobiont, and have to be studied together, as a coherent biological and functional unit, ~~in order~~ to understand ~~the~~its biology, ecology and evolution~~ of the organisms~~. Here we discuss critical concepts and opportunities in marine holobiont research and identify key challenges in the field. We highlight the potential economic, sociological, and environmental impacts of the holobiont concept in marine biological, evolutionary, and environmental sciences with comparisons to terrestrial science ~~whenever~~wherever appropriate. ~~A~~Given the connectivity and the unexplored biodiversity of marine ecosystems, a deeper understanding of such complex systems~~, however, will require~~ requires further technological and conceptual advances. ~~The~~For the marine scientific community, the most significant challenge ~~will be~~is to bridge functional research on ~~simple and~~ tractable and original model systems and global approaches~~. This will require scientists to work together as an (inter)active community in order to address, for instance,~~ addressing ecological and evolutionary questions~~ and~~. This will be crucial for establishing the roles of marine holobionts in biogeochemical cycles, but also developing concrete applications of the holobiont concept in aquaculture and marine ecosystem management projects.

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Commenté [C1]: These two aspects could be presented as specificities of marine ecosystems.

Commenté [C2]: This is too vague. Future research axes need to be specified.

Commenté [C3]: This can be removed to save space.

Commenté [C4]: Please give a few examples of relevant model systems and relevant approaches.

Commenté [C5]: Please give some examples of concrete applications.

Glossary

Anna Karenina principle – a number of factors can cause a system to fail, but only a narrow range of parameters characterizes a working system; based on the first sentence of Leo Tolstoy's "Anna Karenina": "Happy families are all alike; every unhappy family is unhappy in its own way."

Dysbiosis – microbial imbalance in a symbiotic community that affects the health of the host.

Ecosystem services – any direct or indirect benefits that humans can draw from an ecosystem; they include provisioning services (*e.g.*, food), regulating services (*e.g.*, climate), cultural services (*e.g.*, recreation), and supporting services (*e.g.*, habitat formation).

Ectosymbiosis – a symbiotic relationship in which symbionts live on the surface of a host. This includes, for instance, algal biofilms, the skin microbiome, but also extracellular symbionts on the digestive glands, such as gut bacteria.

Endosymbiosis – a symbiotic relationship in which a symbiont lives inside ~~a~~the host~~; a~~ cells; prominent ~~example~~examples are mitochondria, plastids/photosymbionts, or nitrogen fixing bacteria in plant root nodules. Compared to ectosymbiosis these relationships often exhibit a higher degree of interdependence and co-evolution.

Gnotobiosis – the condition in which all organisms present in a culture can be controlled.

Holobiont – an ecological (and evolutionary) unit of different species living together in ~~a long-lasting relationship~~symbiosis.

Horizontal transmission – acquisition of the ~~associated~~ microbiome ~~by a new generation of hosts~~ from the environment.

Host – the largest partner (in size) in a symbiotic community.

Infochemical – a usually diffusible chemical compound ~~used to exchange information between organisms~~that mediates inter- and intraspecific communication.

Microbial gardening – ~~behavior,~~the act of frequently ~~the release of~~releasing growth-enhancing or inhibiting chemicals or metabolites that ~~favors~~favor the development of a microbial community beneficial to the host.

Microbiome – the combined genetic information encoded by the microbiota; may also refer to the microbiota itself.

Microbiota – all microorganisms present in a particular environment or associated with a particular host.

Nested ecosystems – a view of ecosystems where each individual system can be decomposed into smaller systems and/or considered part of a larger system, all of which still qualify as ecosystems.

Phagocytosis – a process by which a eukaryotic cell ingests other cells or solid particles.

Phycosphere – the physical envelope surrounding a phytoplankton cell; usually rich in organic matter.

Phylosymbiosis – congruence in the phylogeny of different hosts and the composition of their associated microbiota.

Commenté [C6]: Please include one or two key references for every definition of the glossary.

Commenté [C7]: The reference of the book should be included.

Commenté [C8]: Please briefly explain how gnotobiotic systems are obtained. Explain the difference with "germ-free" systems.

Commenté [C9]: Why is evolutionary into brackets? Please include one sentence and one reference to mention the debate on this topic.

Commenté [C10]: To be replaced with holobiont?

Commenté [C11]: "Russian dolls" could be used to explain the concept of nestedness. This would make a link to Figure 2.

Commenté [C12]: Add one sentence to give examples of phagocyted cells.

Rasputin effect – the phenomenon that commensals and mutualists can become parasitic in certain conditions; after the Russian monk Rasputin who became the confidant of the Tsar of Russia, but later helped bring down the Tsar's empire during the Russian revolution.

Sponge loop – sponges efficiently recycle dissolved organic matter turning it into detritus that becomes food for other consumers.

Symbiont – an organism living in symbiosis; usually ~~refers~~used to refer to but not restricted to the smaller/microbial partners living in commensalistic or mutualistic relationships (see also host).

Symbiosis – a close and ~~long-~~lasting or recurrent (*e.g.* over generations) relationship between organisms living together; includes mutualistic, commensalistic, and parasitic relationships.

Vertical transmission – acquisition of the associated microbiome by a new generation of hosts from the parents (~~contrary~~as opposed to horizontal transmission).

Marine holobionts from their origins to the present

The history of ~~marine~~the holobiont concept

Current theory proposes a single origin for eukaryotic cells through the symbiotic assimilation of prokaryotes to form ~~cellular organelles such as plastids and~~ first mitochondria and later plastids through several independent symbiotic events (reviewed in Archibald 2015). These **ancestral and founding symbiotic events**, which prompted the metabolic and cellular complexity of eukaryotic life, most likely occurred in the ocean~~, where eukaryotic phagocytosis s widespread~~ (Martin *et al.* 2008).

Despite the general acceptance of this so-called endosymbiotic theory, **the term 'holobiont'** did not immediately enter the scientific vernacular. It was coined by Lynn Margulis in 1990, who proposed that evolution has worked mainly through symbiosis-driven leaps that merged organisms into new forms referred to as 'holobionts', and only secondarily through gradual mutational changes ~~(Margulis 1990; O'Malley 2017).~~(Margulis and Fester 1991; O'Malley 2017). However, the concept did not become widely used until it was co-opted by coral biologists over a decade later. Corals and dinoflagellate algae of the family Symbiodiniaceae are one of the most iconic examples of symbioses found in nature; most corals are incapable of long-term survival without the products of photosynthesis provided by their endosymbiotic algae. Rohwer *et al.* (2002) were the first to use the word "holobiont" to describe ~~corals, where the holobiont comprised the coral organism (host), Symbiodiniaceae, endolithic algae, prokaryotes, fungi, other unicellular eukaryotes, and viruses, together used to describe~~ a unit of selection *sensu* Margulis (Rosenberg *et al.* 2007b)~~.~~ for corals, where the holobiont comprised the cnidarian polyp (host), algae of the family Symbiodiniaceae, various ectosymbionts (endolithic algae, prokaryotes, fungi, other unicellular eukaryotes), and viruses.

Commenté [C13]: Parasitic relationships are not included in the definition of symbiont (above). The two definitions should be consistent.

Commenté [C14]: This section should be structured in chronological order. Dates and influential scientists in the field need to be made clearer.

Commenté [C15]: The terms "symbiont" and "symbiosis" are defined in the glossary. "Symbiotic"should be in bold, or you could change the sentence to use first "symbiont" or "symbiosis"

Commenté [C16]: The term phagocytosis (in the glossary) should be included in this paragraph.

Commenté [C17]: Why is it in bold? This is not in the glossary.

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Commenté [C18]: The name/date of the theory and the main protagonists should be mentioned in the previous paragraph.

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Commenté [C19]: This term should be bold, it is defined in the glossary. Please check that the terms defined in the glossary appear in bold the first time they are used.

Although initially driven by studies of marine organisms, much of the research on the emerging properties and significance of holobionts has since been carried out in other fields of research: the microbiota of the rhizosphere of plants or the animal gut became predominant models and have led to an ongoing paradigm change in agronomy and medical sciences (Bulgarelli *et al.* 2013; Shreiner *et al.* 2015; Faure *et al.* 2018). Holobionts occur in ~~all habitats,~~ **terrestrial and aquatic habitats**, and several analogies between these ecosystems can be made. For example, it is clear that interactions within and across holobionts are mediated by chemical cues and signals in the environment, ~~so-called~~dubbed infochemicals~~;~~ ~~(Loh *et al.* 2002; Rolland *et al.* 2016; Saha *et al.* 2019). The major differences across systems are notably due to the physical nature of water, which often ensures stronger physicochemical interconnections among niches and habitats.~~(Loh *et al.* 2002; Harder *et al.* 2012; Rolland *et al.* 2016; Saha *et al.* 2019). The major differences across terrestrial and aquatic systems are due to the physicochemical properties of water resulting in higher(?) chemical connectivity and signaling between macro- and micro-organisms in aquatic or moist environments. In marine ecosystems, carbon fluxes also appear to be swifter and trophic modes more flexible, leading to higher plasticity of functional interactions (Mitra *et al.* 2013). Moreover, dispersal barriers are usually lower, allowing for faster microbial shifts in marine holobionts (Kinlan and Gaines 2003; Martin-Platero *et al.* 2018). Finally, phylogenetic diversity at broad taxonomic scales (*i.e.*, supra-kingdom, kingdom~~, phyla~~ and phylum levels), is higher in ~~the sea~~aquatic realms than on land, with much of the ~~marine~~aquatic diversity yet to be uncovered (de Vargas *et al.* 2015; Thompson *et al.* 2017), ~~notably~~especially for marine viruses ~~(Middelboe and Brussaard 2017).~~(Middelboe and Brussaard 2017; Gregory *et al.* 2019). The recent discovery of this astonishing marine microbial diversity and the scarcity of marine holobiont research suggest a high potential for complex cross~~-~~ lineage interactions yet to be explored in marine holobiont systems (Figure 1).

~~Evolution of holobionts~~

These examples and the associated debate over how to define organisms or functional entities has led to the **revival of 'holism'**, ~~the~~a philosophical notion~~,~~ first proposed by Aristotle~~.~~ ~~Since~~ in the 4th century BC. However, a major shift happened during the Age of "Enlightenment ~~and~~" when the ~~shift toward~~dominant thought summarized as "dissection science~~", one dominant thought in sciences~~" was to focus on the smallest component of a system in order to understand it better. ~~Holistic~~By contrast, holistic thinking states that systems should be studied in their entirety, with a focus on the interconnections between their various components rather than on the individual parts ~~rather than the parts themselves~~ (Met. Z.17, 1041b11–33). Such systems have emergent properties that result from the irreducible behavior of a system that is 'larger than the sum of its parts'. ~~The boundaries of holobionts are usually delimited by a physical envelope, which corresponds to the area of local influence of the host. However, they may also be defined in a context-dependent way as a 'Russian Matryoshka doll' encompassing all the levels of host-microbiota associations up to the community and ecosystem level.~~In this context the boundaries of holobionts are usually delimited by a physical gradient, which corresponds to the area of local

Commenté [C20]: To be defined in the glossary

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Commenté [C21]: What is the nature of the interactions within holobionts and across holobionts? Please give some examples of interaction types.

Commenté [C22]: Within holobionts or across holobionts?

Commenté [C23]: What do we know about microbial fluxes across marine holobionts? Have they been measured?

Commenté [C24]: The whole paragraph should be re-structured to better highlight the main differences between marine and terrestrial holobionts. The differences are more clearly stated in the responses to comments:
"The two main differences between marine and terrestrial holobionts we have identified are the high level of connectedness of habitats and the diversity of phylogenetic lineages"

Commenté [C25]: To be defined in the glossary.

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influence of the host, *e.g.* in unicellular algae the so-called **phycosphere** (Seymour *et al.* 2017). However, they may also be defined in a context-dependent way as a 'Russian Matryoshka doll', encompassing all the levels of host-symbiont associations from intimate endosymbiosis with a high degree of co-evolution up to the community and ecosystem level; a concept referred to as “nested ecosystems” (Figure 2~~; McFall-Ngai *et al.* 2013; Pita *et al.* 2018)~~; McFall-Ngai *et al.* 2013; Pita *et al.* 2018).

Such a view raises fundamental questions for studies of the **evolution**~~,~~ **of holobionts**, especially regarding the relevant units of selection and the role of co-evolution. For instance, plant and animal evolution involves new functions co-constructed by members of the holobiont or elimination of functions redundant between them (Selosse *et al.* 2014). Rosenberg and Zilber-Rosenberg (2018) have argued that all animals and plants can be considered ~~as~~ holobionts, and thus ~~advocated~~advocate the hologenome theory of evolution. It proposes that natural selection acts at the level of the holobiont and the hologenome (*i.e.*, the combined genomes of the host and all members of its microbiota; Rosenberg *et al.* 2007a; Zilber-Rosenberg and Rosenberg 2008). This interpretation of Margulis' definition of a 'holobiont' considerably broadened fundamental concepts in evolution and speciation and has not been free of criticism (Douglas and Werren 2016)~~. It is, however, generally recognized that, although it should not be accepted as a default for explaining features of host-symbiont associations~~, especially when applied on a community or ecosystem level (Moran and Sloan 2015)~~, the holobiont needs to be at least~~. More recently, it has been shown that species that interact indirectly with the host can also be important in shaping coevolution within mutualistic multi-partner assemblages (Guimarães *et al.* 2017). Thus, the holobiont concept and its complexity should be further considered when addressing evolutionary and ecological questions.

Marine holobiont models

Today, an increasing number of marine model organisms, both unicellular and multicellular, are being used in holobiont research, often with different emphasis and levels of experimental control, but altogether covering a large range of scientific topics. Here, we provide several illustrative examples of this diversity and some of the insights they have provided.

Environmental or “semi-controlled” models: Radiolarians and foraminiferans (both heterotrophic protists dwellers harboring endosymbiotic microalgae) are emerging as critical ecological models for unicellular photosymbiosis due to their ubiquitous presence in the world's oceans (Decelle *et al.* 2015; Not *et al.* 2016). The discovery of deep-sea hydrothermal vents revealed symbioses of animals with chemosynthetic bacteria that have later been found in many other marine ecosystems (Dubilier *et al.* 2008; Rubin-Blum *et al.* 2019) and frequently exhibited high levels of metabolic and taxonomic diversity ~~organisms are being used as holobiont model systems in research, often with a different emphasis, but altogether covering a large range of scientific topics. Examples of this diversity and related insights are provided in this section. Sponge-bacteria interactions are particularly promising for the discovery of novel bacterial lineages or new drugs (Blunt *et al.* 2016; Bibi *et al.* 2017).~~(Duperron *et al.* 2008; Petersen *et al.*

Commenté [C26]: Where are the limits of the holobiont under this definition? This should be explained.

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Commenté [C27]: This definition should be moved to the glossary.

Commenté [C28]: What is complex? The concept of holobiont or the holobiont? This sentence is too vague and should be rephrased.

Commenté [C29]: A few pictures of the main marine holobiont models could be included.

Commenté [C30]: Please explain what “environmental model” and “semi-controlled model” mean.

2016; Ponnudurai *et al.* 2017). The cosmopolitan haptophyte *Emiliania huxleyi*, promoted by associated bacteria (Seyedsayamdost *et al.* 2011; Segev *et al.* 2016), produces key intermediates in the carbon and sulfur biogeochemical cycles making it an important model phytoplankton species.

Controlled bi- or trilateral associations: Only a few models, covering a small part of the overall marine biodiversity, are currently being cultivated *ex-situ* and can be used in fully controlled experiments, where they can be cultured aposymbiotically (*i.e.*, without symbionts). The flatworm *Symsagittifera* (= *Convoluta*) *roscoffensis* (Arboleda *et al.* 2018), the sea anemone *Exaiptasia* (Baumgarten *et al.* 2015; Wolfowicz *et al.* 2016), the upside-down jellyfish *Cassiopea* (Ohdera *et al.* 2018), and their respective intracellular green and dinoflagellate algae have, in addition to corals, become models for fundamental research on evolution of metazoan-algal photosymbiosis. In particular the sea anemone *Exaiptasia* has been used to explore photobiology disruption and restoration of cnidarian symbioses (Lehnert *et al.* 2012). ~~Similarly, radiolarians~~The *Vibrio*-squid model provides insights into the effect of microbiota ~~and foraminiferans (both heterotrophic protists dwellers harboring endosymbiotic microalgae) are emerging as critical ecological models for unicellular photosymbiosis due to their ubiquitous presence in the world's oceans (Decelle *et al.* 2015; Not *et al.* 2016). The discovery of deep-sea hydrothermal vents revealed symbioses of animals with chemosynthetic bacteria that have later been found in many other marine ecosystems (Dubilier *et al.* 2008; Rubin-Blum *et al.* 2019) and frequently exhibited high levels of metabolic and taxonomic diversity (Duperron *et al.* 2008; Petersen *et al.* 2016; Ponnudurai *et al.* 2017).The *Vibrio*-squid model provides insights into the effect of microbiotas~~ on animal development, circadian rhythms, and immune systems (McFall-Ngai 2014). ~~The cosmopolitan haptophyte *Emiliania huxleyi*, promoted by associated bacteria (Seyedsayamdost *et al.* 2011; Segev *et al.* 2016), produces important intermediates in the carbon and sulfur biogeochemical cycles making it an important model phytoplankton species. The green alga *Ostreococcus*, also an important player in marine primary production, has been shown to exchange vitamins with its microbiota~~The unicellular green alga *Ostreococcus*, an important marine primary producer, has been shown to exchange vitamins with specific associated bacteria (Cooper *et al.* 2019). ~~The recent sequencing of several host genomes and their associated microorganisms, as well as improved experimental protocols, have led to new insights in many of these model systems, yet most experiments are still carried out in environmental or "semi-controlled" conditions. Only a few models, covering a small part of the overall marine biodiversity, are currently being cultivated *ex-situ* and can be used in fully controlled experiments, where they can be cultured aposymbiotically (*i.e.*, without symbionts). This is the case *e.g.* for the green macroalga *Ulva mutabilis*, which~~The green macroalga *Ulva mutabilis* has enabled the exploration of bacteria-mediated growth and morphogenesis including the identification of original chemical interactions in the holobiont (Wichard 2015; Kessler *et al.* 2018)~~ or for zooxanthellate sea anemones of the genus *Exaiptasia*, which have been used to explore photobiology disruption and restauration~~. Although the culture conditions in these highly-controlled model systems differ from the natural environment, these systems are essential

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Commenté [C31]: This definition could be moved to the glossary. The difference with "gnotobiosis" should be explained.

Mis en forme : Couleur de police : Automatique

Mis en forme : Couleur de police : Texte 1, to gain elementary mechanistic understanding of the functioning and thus also the evolution ~~of cnidarian symbioses (Lehnert *et al.* 2012). Although the culture conditions in these highly controlled model systems are less realistic, we believe that such systems are essential to gain elementary mechanistic understanding of the functioning~~ of marine holobionts.

Marine holobionts as drivers of ecological processes

~~Marine~~Motile and macroscopic marine holobionts can act as **dissemination vectors** for geographically restricted microbial taxa. For instance, pelagic mollusks or vertebrates have a high capacity for ~~dispersion~~dispersal (*e.g.*, against currents and through stratified water layers). It has been estimated that fish and marine mammals may enhance the original dispersion rate of their microbiota by a factor of 200 to 200,000 (Troussellier *et al.* 2017) and marine birds may even act as bio-vectors across ecosystem boundaries (Bouchard Marmen *et al.* 2017). This ~~holobiont~~host-driven dispersal of microbes can include non-native or invasive species as well as pathogens (Troussellier *et al.* 2017).

A related ecological function of holobionts is their potential to sustain rare species. Hosts provide an environment that favors the growth of specific microbial communities ~~different~~distinct from the surrounding environment (including rare microbes). They may, for instance, provide a nutrient-rich niche in the otherwise nutrient-poor seawater (Smriga *et al.* 2010; Webster *et al.* 2010; Burke *et al.* 2011; Chiarello *et al.* 2018), and the interaction between host and microbiota can allow both partners to cross biotope boundaries (*e.g.*, Woyke 2006) and colonize extreme environments (Bang *et al.* 2018). Holobionts thus contribute to marine microbial diversity and possibly resilience in the context of environmental change (Troussellier *et al.* 2017).

Microbially regulated biological processes are important drivers of **global biogeochemical cycles** (Falkowski *et al.* 2008; Madsen 2011; Anantharaman *et al.* 2016). ~~These cycles describe the diverse global biogeochemical cycling are still sparse.~~ In the open ocean, it is estimated that symbioses with the cyanobacterium UCYN-A contribute ~20% to ~~the~~ total N_2 fixation (Thompson *et al.* 2012; Martínez-Pérez *et al.* 2016). In benthic systems, sponges and corals may support entire ecosystems *via* their involvement in nutrient cycling thanks to their microbial partners (Raina *et al.* 2009; Fiore *et al.* 2010; Cardini *et al.* 2015; Pita *et al.* 2018), functioning as sinks~~/~~ and sources of nutrients. In particular the “sponge loop” recycles dissolved organic matter and makes it available to higher trophic levels in the form of detritus (de Goeij *et al.* 2013; Rix *et al.* 2017). In coastal sediments, bivalves hosting methanogenic archaea have been shown to increase the benthic methane efflux by a factor of up to eight, potentially accounting for 9.5% of total methane emissions from the Baltic Sea (Bonaglia *et al.* 2017).

Such impressive metabolic versatility is accomplished because of the simultaneous occurrence of disparate biochemical machineries (*e.g.*, aerobic and anaerobic pathways) in ~~the~~ individual symbionts, providing new metabolic abilities to the holobiont, such as the synthesis of specific essential amino acids, photosynthesis, or chemosynthesis (Venn *et al.* 2008; Dubilier *et al.* 2008). These metabolic capabilities have the potential to extend the ecological niche of the

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Commenté [C32]: To be defined in the glossary. The section could then be structured according to the main processes. The link to the previous section on model holobionts should be clarified.

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holobiont as well as its resilience to climate and environmental changes (Berkelmans and van Oppen 2006; Gilbert *et al.* 2010; Dittami *et al.* 2016; Shapira 2016; Godoy *et al.* 2018). It is therefore paramount to include the holobiont concept in predictive models that investigate the consequences of human impacts on the marine realm and its biogeochemical cycles.

Challenges and opportunities in marine holobiont research

~~Deciphering marine~~Marine holobiont ~~functioning~~assembly and regulation

Commenté [C33]: The term "assembly processes" should be defined in the glossary. Vellend's four processes for community assembly could be used to structure this section.

Two critical challenges that can be partially addressed by using model systems are 1) to decipher the factors determining holobiont composition; and 2) to elucidate the impacts and roles of the different partners in these complex systems over time. Some marine invertebrates, such as ~~clams~~bivalves transmit part of the~~ core~~ microbiota maternally (Bright and Bulgheresi 2010; Funkhouser and Bordenstein 2013). In other marine holobionts, ~~however,~~ vertical transmission may be weak and inconsistent~~ and~~, whereas mixed **modes of transmission** (vertical and horizontal) or intermediate modes (pseudo-vertical, where horizontal acquisition frequently involves symbionts of parental origin) are ~~the most~~more common (Bjork *et al.* 2018, preprint). ~~Better understanding~~ Identifying the factors ~~that shape the~~shaping holobiont composition ~~of holobionts~~and understanding their evolution is highly relevant for marine organisms given that~~,~~ ~~despite a highly connected and microbe-rich environment,~~ most marine hosts display a high specificity for their microbiota and even patterns of phylosymbiosis ~~for some associations~~ (Kazamia *et al.* 2016; Brooks *et al.* 2016; Pollock *et al.* 2018)~~.~~, despite a highly connected and microbe-rich environment,.

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The immune system of the host is one way to regulate the microbial composition of ~~the holobiont~~both marine and terrestrial holobionts. Perturbations in this system can lead to dysbiosis, and eventually microbial infections (Selosse *et al.* 2014; de Lorgeril *et al.* 2018). Dysbiotic individuals frequently display higher variability in their microbial community composition than healthy individuals, an observation in line with the "Anna Karenina principle" (Zaneveld *et al.* 2017), although there are exceptions to this rule (*e.g.*, Marzinelli *et al.* 2015). A specific case of dysbiosis is the so-called "Rasputin effect" ~~when~~where benign endosymbionts opportunistically become detrimental to the host due to processes such as reduction in immune response under food deprivation, coinfections, or environmental pressure (Overstreet and Lotz 2016). Many diseases are now interpreted as the result of a microbial imbalance and the rise of opportunistic or polymicrobial infections upon host stress (Egan and Gardiner 2016). ~~In~~For instance in reef-building corals~~, for example~~, warming destabilizes cnidarian-dinoflagellate associations, and some beneficial *Symbiodiniacea* strains switch their physiology and sequester more resources for their own growth at the expense of the coral host (Baker *et al.* 2018).

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Another factor regulating holobiont composition is **chemically mediated microbial gardening.** This concept has already been demonstrated for land plants, where root exudates are used by plants to manipulate microbiome composition (Lebeis *et al.* 2015). ~~In marine environments, comparable studies are only starting to emerge. For instance, seaweeds can chemically garden beneficial microbes aiding normal morphogenesis *via* exuded metabolites (Kessler *et al.* 2018), and corals of the genera *Acropora* and *Platygyra* structure their surface-associated microbiome by producing chemo-attractants and anti-bacterial compounds (Ochsenkühn *et al.* 2018). In the context of ongoing global change, an understanding of how the community and functional structure of resident microbes are resilient to perturbations remains critical to predict and assure the health of their host and the ecosystem.~~In marine environments, the phylogenetic diversity of hosts and symbionts suggests both conserved and marine-specific chemical interactions, but comparable studies are only starting to emerge. For instance, seaweeds can chemically garden beneficial microbes facilitating normal morphogenesis and increasing disease resistance (Kessler *et al.* 2018; Saha and Weinberger 2019), and seaweeds and corals structure their surface-associated microbiome by producing chemo-attractants and anti-bacterial compounds (Harder *et al.* 2012; Ochsenkühn *et al.* 2018). There are fewer examples of chemical gardening in unicellular hosts, but it seems highly likely that similar processes are in place (Gribben *et al.* 2017; Cirri and Pohnert 2019). In the context of ongoing global change, an understanding of how the community and functional structure of resident microbes are resilient to perturbations remains critical to predict and promote the health of their host and the ecosystem, yet it is still missing in most mathematical models, or additional information on biological interactions would be required to make the former more accurate (Bell *et al.* 2018).

Integrating marine model systems with large-scale studies

By compiling what ~~scientists~~a sample of researchers today consider the most important trends and challenges in the field of marine holobiont research (Figure 3), we identified two distinct clusters: mechanistic understanding and predictive modeling. This illustrates that, on the one hand, the scientific community is focusing on **the establishment of models** for the identification of specific molecular interactions between marine organisms at a given point in space and time, up to the point of synthesizing functional mutualistic communities~~ constituting functional marine holobionts~~ *in vitro* (Kubo *et al.* 2013). On the other hand, another part of the community is moving towards **global environmental sampling schemes** such as the *TARA* Oceans expedition (Pesant *et al.* 2015) or the Ocean Sampling Day (Kopf *et al.* 2015), and towards long-term data series (*e.g.*, Wiltshire *et al.* 2010; Harris 2010). What emerges as both lines of research progress is the understanding that small-scale functional studies in the laboratory are inconsequential unless they are applicable to ecologically-relevant complex systems. At the same time, large scale-studies remain descriptive and with little predictive power unless we understand the mechanisms driving the observed processes. We illustrate the importance of integrating both approaches in Figure 3, where the node related to potential

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Commenté [C34]: In Figure 3, predictive modelling is associated to large-scale studies, while mechanistic understanding is associated to the study of model holobionts. This should be stated in the text.

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applications was perceived as a central hub at the interface between mechanistic understanding and predictive modeling.

A successful example **allying both** ~~ends of the spectrum in terrestrial environments~~**functional and large-scale approaches** are the root nodules of legumes, which harbor nitrogen-fixing bacteria. In this system with a reduced number of symbionts involved, the functioning, distribution, and to some extent the evolution of these nodules, are now well understood (Epihov *et al.* 2017). The integration of this knowledge into agricultural practices has led to substantial yield improvements (*e.g.*, Kavimandan 1985; Alam *et al.* 2015). In the more diffuse and partner-rich system of mycorrhizal symbioses between plant roots and soil fungi, a better understanding of the interactions has also been achieved *via* the investigation of environmental diversity patterns in combination with experimental culture systems with reduced diversity (van der Heijden *et al.* 2015).

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We consider it essential to implement comparable efforts in marine sciences through interdisciplinary research combining ~~biology, ecology, and mathematical modeling.~~physiology, biochemistry, ecology, and mathematical modeling. A key factor here will be **the identification and development of new model systems** for **keystone holobionts** that will allow the hypotheses generated by large-scale data sets to be tested in controlled experiments. Such approaches will enable the identification of common interaction patterns between organisms within holobionts and nested ecosystems. In addition to answering fundamental questions, they will help address the ecological, societal, and ethical issues that arise from attempting to actively manipulate holobionts (*e.g.*, in aquaculture) in order to enhance their resilience and protect them from the impacts of global change (Llewellyn *et al.* 2014).

Emerging methodologies to approach the complexity of holobiont partnerships

Commenté [C35]: A figure linking the emerging methodologies and the future research axes would be useful.

As our conceptual understanding ~~on~~of the different levels of ~~the~~holobiont organization ~~of holobionts~~ evolves, so does the need for multidisciplinary approaches and the development of tools and technologies to handle the unprecedented amount of data and their integration into dedicated ecological and evolutionary models. Here, progress is often fast-paced and provides exciting opportunities to address some of the challenges in holobiont research.

Notably, a giant technological stride has been the explosion of **affordable '–omics' technologies** allowing molecular ecologists to move from metabarcoding (*i.e.*, sequencing of a taxonomic marker) to ~~meta-~~metagenomics or single-cell genomics in the case of unicellular hosts, metatranscriptomics, and metaproteomics, thus advancing our understanding from phylogenetic to functional analyses of the holobiont (Bowers *et al.* 2017; Meng *et al.* 2018). ~~For instance, metaproteomics~~These approaches are equally useful in marine and in terrestrial environments, but the existence of numerous poorly studied lineages in the former make the generation of good annotations and reference databases an additional challenge for marine biologists. Metaproteomics combined with stable isotope fingerprinting can help study the

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metabolism of single species within the holobiont (Kleiner *et al.* 2018). In parallel, meta-metabolomics approaches have advanced over the last decades, and can be used to unravel the chemical interactions between partners. One current limitation here, especially in marine systems, is that many compounds are still undescribed in databases and are present in low quantities in natural environments, although recent technological advances such as molecular networking and meta-mass shift chemical profiling to identify relatives of known molecules promise significant ~~improvements~~advancement (Hartmann *et al.* 2017).

~~Additionally, it~~A further challenge in holobiont research is ~~highly challenging~~ to identify the origin of ~~a compound~~compounds among the different partners of the holobionts and to determine ~~its degree of~~their involvement in the maintenance and performance of the holobiont system. Well-designed experimental setups may help answer some of these questions (*e.g.*, Quinn *et al.* 2016), but they will also require high levels of replication due to extensive intra-species variability. Recently developed *in vivo* and *in situ* **imaging techniques** combined with 'omics' approaches can provide spatial and qualitative information (origin, distribution, and concentration of a molecule or nutrient), shedding new light on the role of each partner of the holobiont system at the subcellular level. The combination ~~and~~of stable isotope labelling and chemical imaging (mass spectrometry imaging such as secondary ion mass spectrometry and matrix-assisted laser desorption ionization, and synchrotron X-ray fluorescence~~),~~,) is particularly valuable in this context, as it enables the investigation of metabolic exchange between the different components of a holobiont (Musat *et al.* 2016; Raina *et al.* 2017). ~~Finally, three-dimensional electron microscopy may help evaluate to what extent different components of a holobiont are physically integrated (Colin *et al.* 2017; Decelle *et al.* 2019)~~Finally, three-dimensional electron microscopy may help evaluate to what extent different components of a holobiont are physically integrated (Colin *et al.* 2017; Decelle *et al.* 2019), where high integration is one indication of highly specific interactions. All of these techniques can be employed in both marine and terrestrial systems, but in marine systems the high phylogenetic diversity of organisms adds to the complexity of adapting and optimizing the techniques.

One consequence of the development of such new methods is the ~~intellectual~~ feedback they provide to improve existing **models** and to develop entirely new ones, for example by conceptualizing holobionts as ~~mass balance~~the sum of ~~elements~~the interactions between the host and its microbiota ~~and its host~~ (Skillings 2016; Berry and Loy 2018), or by redefining boundaries between the holobiont and the ecosystem (Zengler and Palsson 2012). Such models may incorporate metabolic complementarity between different components of the holobiont (Dittami *et al.* 2014; Bordron *et al.* 2016), simulate microbial communities starting from different cohorts of randomly generated microbes for comparison with actual metatranscriptomics and/or metagenomics data (Coles *et al.* 2017), or even employ machine learning techniques to predict host-associated microbial communities (Moitinho-Silva *et al.* 2017).

A side-effect of these recent developments has been to shift the focus of holobiont research away from laboratory culture-based experiments. ~~Here we~~We argue that maintaining **cultivation efforts** to capture ~~as much as possible of~~the maximum holobiont biodiversity

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possible remains essential in order to experimentally test hypotheses and investigate physiological mechanisms. A striking example of the importance of laboratory experimentation is the way germ-free mice re-inoculated with cultivated bacteria (the so-called gnotobiotic mice) have contributed to the understanding of interactions within the holobiont in animal health and physiology (*e.g.*, Faith *et al.* 2014; Selosse *et al.* 2014). Innovations in cultivation techniques for axenic (or germ-free) hosts (*e.g.*, Spoerner *et al.* 2012) or in microbial cultivation such as microfluidic systems (*e.g.*, Pan *et al.* 2011) and cultivation chips (Nichols *et al.* 2010) may provide a way to obtain pure cultures. Yet, bringing individual components of holobionts into cultivation can still be a daunting challenge due to the strong interdependencies between organisms as well as the existence of yet unknown metabolic processes that may create specific requirements. In this context, single-cell omics analyses can provide critical information on some of the growth requirements of the organisms, and can complement approaches of high-throughput culturing (Gutleben *et al.* 2018). Established cultures can then be developed into model systems to move towards mechanistic understanding and experimental testing of hypothetical processes within the holobiont~~.~~ derived from environmental meta'-omics' approaches. A few such model systems have already been mentioned above, but omics techniques can broaden the range of available models, enabling generalizations about the functioning of marine holobionts and their interactions in marine environments (Wichard and Beemelmanns 2018).

Ecosystem services and holobionts in natural and managed systems

A better understanding of marine holobionts ~~can~~will likely have ~~straightforward~~direct **socioeconomic consequences** for coastal marine ecosystems, which have been estimated to provide services worth almost 50 trillion (10^{12}) US$ per year (Costanza *et al.* 2014). Most of the management practices ~~of these~~in marine systems have so far been based exclusively on the biology and ecology of macro-organisms. A multidisciplinary approach that provides mechanistic understanding of habitat-forming organisms as holobionts will ultimately improve the predictability and management of coastal ecosystems. For example, host-associated ~~microbiotas~~microbiota could be integrated into the proxies used to assess the health of ecosystems. Microbial shifts and dysbiosis constitute early warning signals that may allow managers to predict potential impacts and intervene more rapidly and effectively (van Oppen *et al.* 2017; Marzinelli *et al.* 2018).

One form of intervention could be to promote positive changes of host-associated microbiotas, in ways analogous ~~ways~~ to the use of **pre- and/or probiotics** in humans (Singh *et al.* 2013) or inoculation of beneficial microbes in plant farming (Berruti *et al.* 2015; van der Heijden *et al.* 2015). In macroalgae, beneficial bacteria identified from healthy seaweed holobionts could be applied to diseased plantlets in order to suppress the growth of detrimental ones and/or to prevent disease outbreaks in aquaculture settings. In addition to bacteria, these macroalgae frequently host endophytic fungi that may have protective functions for the algae (Porras-Alfaro and Bayman 2011; Vallet *et al.* 2018). Host-associated microbiota could also be

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Commenté [C36]: The term "biomonitoring" could be introduced here and defined in the glossary.

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Commenté [C37]: The term "biocontrol" could be introduced here and defined in the glossary.

manipulated to shape key phenotypes in cultured marine organisms. For example, specific bacteria associated with microalgae may enhance ~~their~~algal growth (Amin *et al.* 2009; Kazamia *et al.* 2012; Le Chevanton *et al.* 2013), increase ~~their~~ lipid content (Cho *et al.* 2015), and participate in the bioprocessing of algal biomass (Lenneman *et al.* 2014). More recently, the active modification of the coral microbiota has even been advocated as a means to boost the resilience of the holobiont to climate change (van Oppen *et al.* 2015; Peixoto *et al.* 2017), an approach which would, however, bear a high risk of unanticipated and unintended ecological consequences.

Commenté [C38]: The term "biostimulation" could be introduced here and defined in the glossary.

Code de champ modifié

Finally, one could implement holistic approaches in the framework of **fish farms**. Recent developments including integrated multi-trophic aquaculture, recirculating aquaculture, offshore aquaculture, ~~and~~ species selection, and breeding increase yields and reduce the resource constraints and environmental impacts of intensive aquaculture (Klinger and Naylor 2012). However, the intensification of aquaculture often goes hand in hand with increased disease outbreaks both in industry and wild stocks. A holistic microbial management approach may provide an efficient solution to these latter problems (De Schryver and Vadstein 2014).

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Nevertheless, when considering their biotechnological potential, it should also be ~~envisaged~~noted that marine microbiota ~~may~~are likely to be vulnerable to anthropogenic influences and that their deliberate engineering, introduction from exotic regions, or inadvertent perturbations may have profound, and yet entirely **unknown, consequences ~~on~~for marine ecosystems**. Terrestrial environments provide numerous examples of unwanted plant expansions or ecosystem perturbations linked to microbiota (*e.g.*, Dickie *et al.* 2017), and ~~examples~~cases where holobionts manipulated by human ~~result~~resulted in pests (*e.g.*, Clay and Holah 1999)~~,~~ ~~calling~~ call for a cautious and ecologically-informed evaluation of holobiont-based technologies.

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Conclusions

Commenté [C39]: A list of priority research questions, indicating the biological models and emerging tools that are appropriate to address them, seems to me essential for the paper to become a reference in the field.

Marine ecosystems represent highly connected reservoirs of largely unexplored biodiversity. They are of critical importance to feed the ever-growing world population, constitute ~~a~~ significant ~~player~~players in global biogeochemical cycles but are also threatened by human activities and global change. In order to ~~unveil~~unravel some of the basic principles of life and its evolution, and to protect and sustainably exploit marine natural resources, it is paramount to consider the complex biotic interactions that shape the marine communities and their environment. The scope of these interactions ranges from simple molecular signals between two partners ~~*via*~~to complex assemblies of eukaryotes, prokaryotes, and viruses with one or several hosts, to entire ecosystems. We believe that the concept of holobionts will be most useful and heuristic if used with a degree of malleability. It ~~does~~ not only ~~represent~~represents the fundamental understanding that all living organisms have intimate connections with their immediate neighbors ~~that~~, which may impact all aspects of their biology, but also enables us to define units of interacting organisms that are most suitable to answer specific scientific, societal, and economic questions. The consideration of the holobiont concept marks a ~~real~~ paradigm shift in biological and environmental sciences, and a successful response to the underlying challenges will largely depend on the capacity of but only if scientists to work together as an (inter)active and transdisciplinary community bringing together holistic and mechanistic views. This will result in tangible outcomes including a better understanding of evolutionary and adaptive processes, improved modeling of habitats and biogeochemical cycles, and application of the holobiont concept in aquaculture and ecosystem management projects.

Acknowledgments

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602 # Figures

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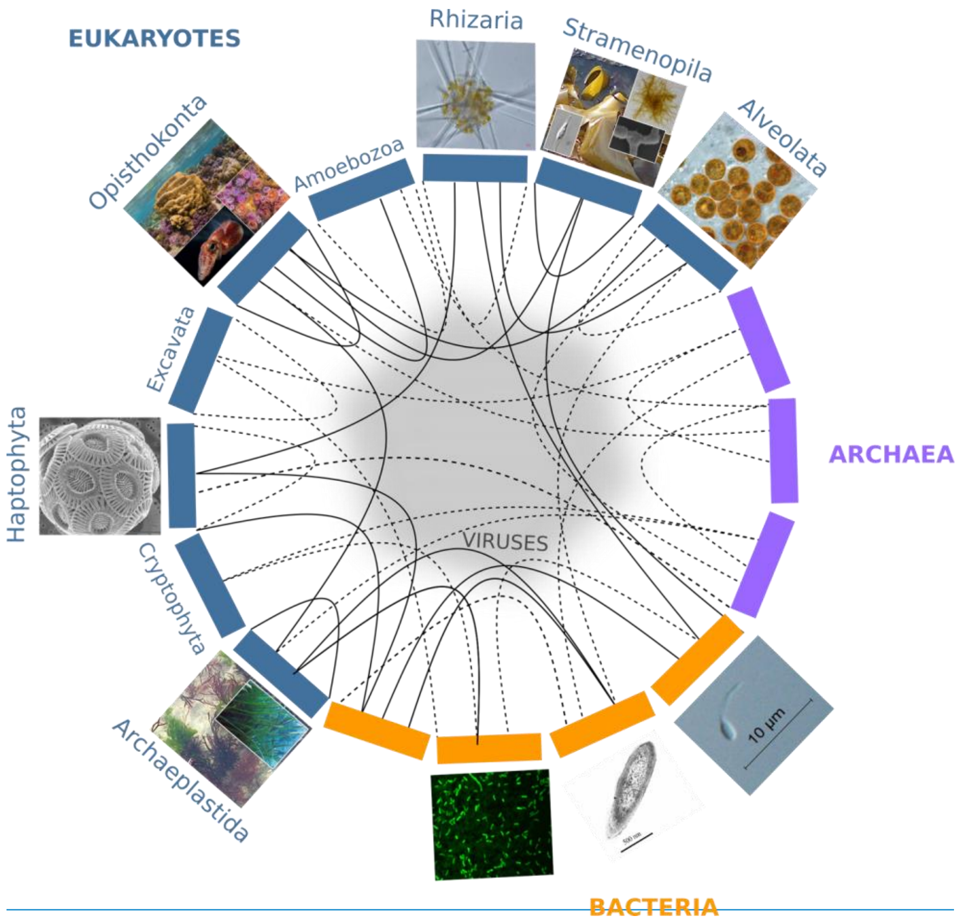

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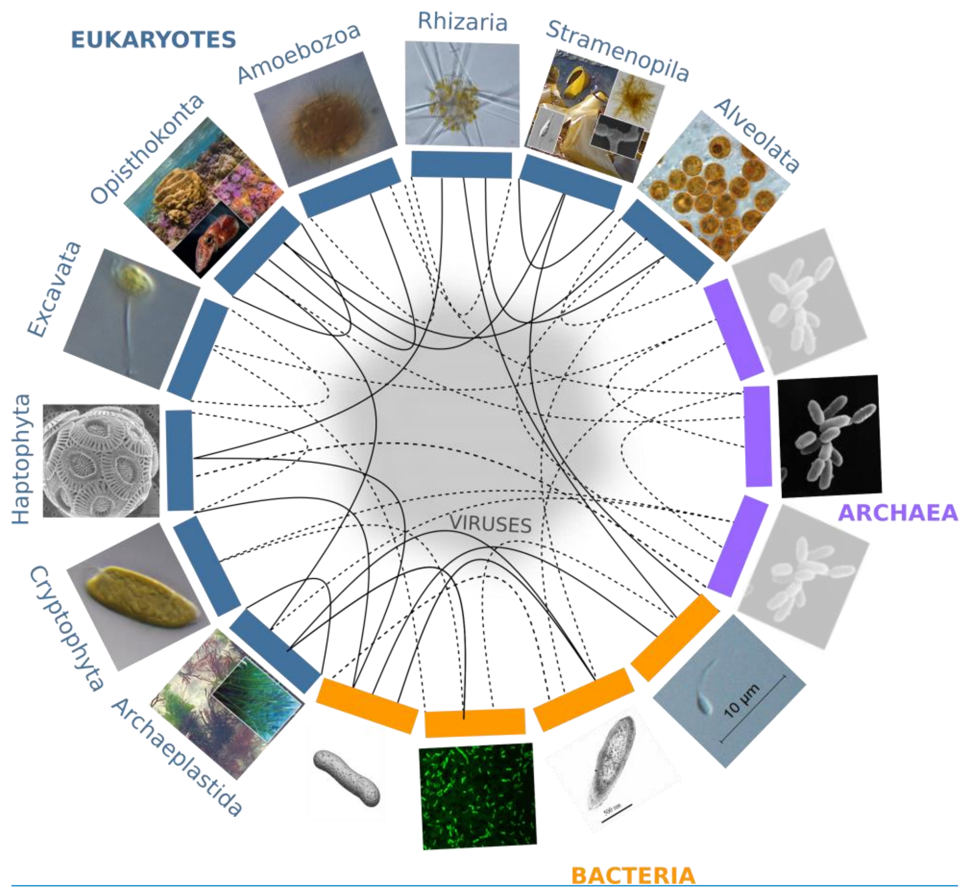

Figure 1. Partners forming marine holobionts are widespread across the tree of life including all kingdoms (eukaryotes, bacteria, archaea, viruses), and represent a large diversity of potential models for exploring complex biotic interactions across lineages. Plain lines correspond to holobionts referred to in the present manuscript. Dashed lines are examples of potential interactions. Photo credits: Archaeplastida - C. Leblanc, U Cardini; Cryptophyta, Excavata, Amoebozoa – Roscoff Culture Collection; Stramenopila – C. Leblanc, S. M. Dittami, H. KleinJan; Alveolata – A. M. Lewis; Rhizaria – F. Not; Haptophyta – A. R. Taylor; Opisthonkonta – C. Frazee, M. McFall-Ngai, W. Thomas, L. Thiault; Bacteria - E Nelson, L Sycuro, S. M. Dittami, S. ~~Le Panse~~Le Panse, Planktomania; Archaea – National Space Science Data Center.

Commenté [C40]: What are the differences between the three pictures of archaea? I can't see them.
I think that all these small pictures could be replaced by a few bigger pictures of model holobionts.

Commenté [C41]: The position of viruses in the middle of the circle give the impression that they lack host-specificity.

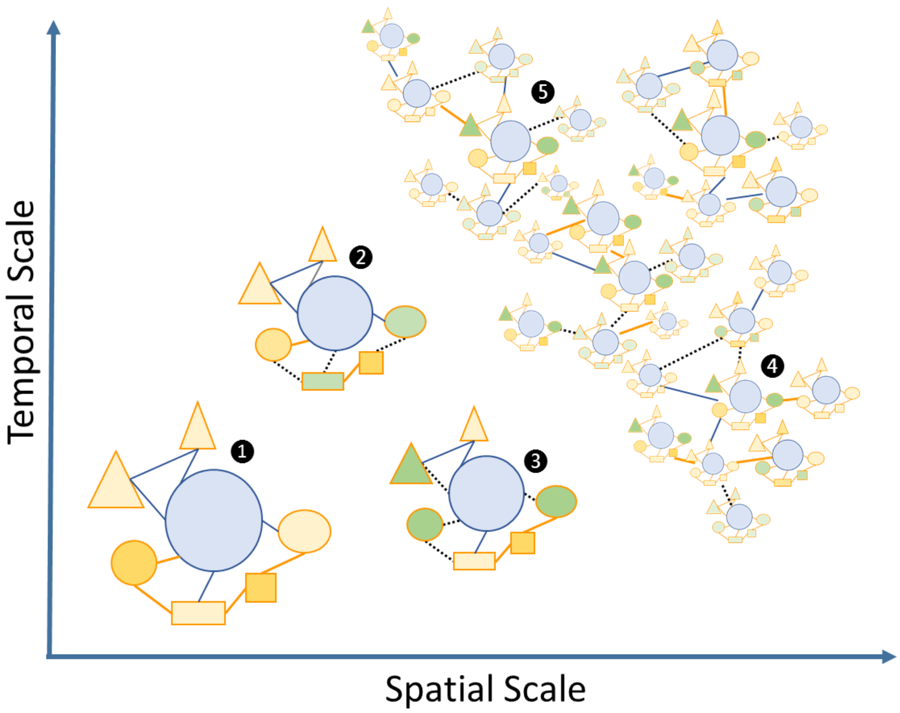

Figure 2. Schematic view of the "Russian Doll" complexity and dynamics of holobionts, according to diverse spatiotemporal scales. The host (blue circles), and associated microbes (all other shapes) including bacteria and eukaryotes that may be inside (*i.e.*, endosymbiotic or outside the host, *i.e.* ectosymbiotic, are connected by either beneficial (solid orange lines), neutral (solid blue lines) or pathogenic (dashed black lines) interactions respectively. The different clusters can be illustrated by the following examples: 1, a model holobiont in ~~one~~a stable physiological condition (*e.g.*, in controlled laboratory condition); 2 and 3, holobionts changing during their life cycle or submitted to stress conditions; 4 and 5, marine holobionts in the context of global sampling ~~campaign~~campaigns or long-term time series.

Commenté [C42]: The nature of the interactions should change when considering larger spatial and temporal scales.

Commenté [C43]: The term "dysbiosis" could be introduced in the figure.

Commenté [C44]: Horizontal (4) and vertical (5) transmission of microorganisms could be represented as network links.


Novel physiology
Experimental verification
Evolutionary scale
(New) model systems
Co-adaptability
Phycosphere
Functional response
Integrating scales
Mechanistic understanding
Spatial scale
Loss of infochemical properties
Functional redundancy
Development of new technologies
Biogeochemical cycles
Consequences of global change
Large data set analysis
Gardening of holobionts
Networks
Predictive models
Biosecurity
Diversity
Potential use, management
Risk assessment
New species (OTUs)
Harmful algae
Ecosystem services
Diseases

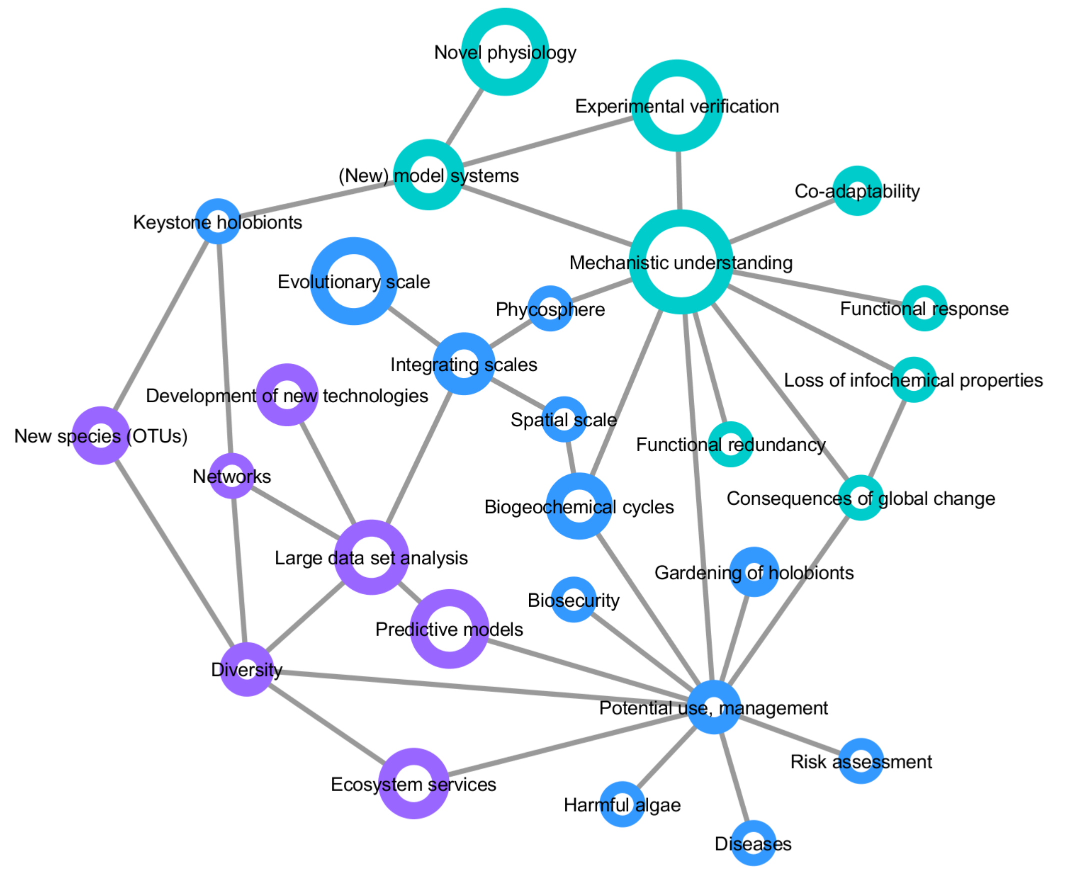

Figure 3: Mind map of key concepts, techniques, and challenges related to marine holobionts. ~~This~~The basis of this map was generated during the Holomarine workshop held in Roscoff in 2018 (https://www.euromarinenetwork.eu/activities/HoloMarine). The size of the nodes reflects the number of votes each keyword received from the participants of the workshop (total of 120 votes from 30 participants). The two main clusters corresponding to predictive modeling and mechanistic modeling, are displayed in purple and turquoise, respectively. Among the intermediate nodes linking these disciplines (blue) "potential use, management" was the most connected.

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Commenté [C45]: Predictive modelling is associated to large-scale studies, while mechanistic understanding is associated to the study of model holobionts. You could circle each cluster and give a title to each approach, and better highlight the node "potential use, management" that corresponds to the common goal.

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