A community perspective on the concept of marine holobionts: 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 to describe and understand these complex systems. It posits that a host and its associated microbiota, living together in a stable relationship, form the holobiont, and have to be studied together~~,~~ as a coherent biological and functional unit~~,~~ to understand its biology, ecology, and evolution. 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 wherever~~sciences where appropriate. Given the connectivity and the unexplored biodiversity ~~of~~specific to marine ecosystems, a deeper understanding of such complex systems requires further technological and conceptual advances~~. For~~, *e.g.* the ~~marine scientific community, the~~development of controlled experimental model systems for holobionts from all major lineages and the modeling of (info)chemical-mediated interactions between organisms. The most significant challenge is to bridge ~~functional~~cross-disciplinary research on tractable ~~and original~~ model systems ~~and global approaches addressing~~in order to address key ecological and evolutionary questions. This will be crucial ~~for establishing~~to decipher the roles of marine holobionts in biogeochemical cycles, but also developing concrete applications of the holobiont concept ~~in aquaculture and marine ecosystem~~*e.g.* to increase yield or disease resistance in aquacultures or to protect and restore marine ecosystems through management projects.

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Commenté [SAH1]: I am unsure this is really useful, considering the change in the next sentence that encompass the major differences

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Commenté [SAH2]: Here we propose that one of the first significant challenge

(some very iomportant ones are also required)

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Commenté [SAH3]: The distance still seems large between understanding the chemical meduiated interactions between a handful of paired host-symbionts systems and the role of holobionths in biochemical cycles

I suggest rephrasing focusing on what such focuse on well understood model systems and experimental approach will represent as a first (yet very important) step.

Also, developing concrete applications is vague, whereas the proposed applications for aquaculture aznd restorations are narrow (ie not accounting for conservation, fight against invasions etc...). What about

"This first step is crucial to decipher the main drivers of the dynamics and evolution of the holobionth, and to account for the holobiont concept in applied area such as the conservation or exploitation of marine ecosystems and resources"?

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Glossary[1]

~~**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.~~

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" (1878): "Happy families are all alike; every unhappy family is unhappy in its own way" (Zaneveld *et al.* 2017).

Aposymbiotic culture – a culture of a host or a symbiont without its main symbiotic partner(s) (*e.g.* Kelty and Cook 1976). In contrast to gnotobiotic cultures, aposymbiotic cultures are usually not germ-free.

Biological control (biocontrol) – methods ~~of~~for controlling diseases or pests by introducing or supporting natural enemies of the former (see *e.g.* Hoitink and Boehm 1999).

Biomonitoring – the use of living organisms as indicator for the health of an environment or ecosystem.

Community assembly process – the accumulation of species in a novel habitat, according to Vellend the four main forces relevant for microbial community assembly are evolutionary diversification, dispersal, selection, and ecological drift (Vellend 2010; Nemergut *et al.* 2013).

Dysbiosis – microbial imbalance in a symbiotic community that affects the health of the host (Egan and Gardiner 2016).

Ecological process – the processes responsible for the functioning and dynamics of ecosystems including biogeochemical cycles, community assembly processes, interactions between organisms, and climatic processes (see *e.g.* Bennett *et al.* 2009).

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~~).~~.) (Millennium Ecosystem Assessment Panel 2005).

~~**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**~~**Ectosymbiosis** – a symbiotic relationship in which symbionts live on the surface of a host. This includes, for instance, algal biofilms or the skin microbiome (Nardon and Charles 2001).

Emergent property – a property of complex systems (e.g. holobionts), which arises from interactions between the components and that is not the sum of the component properties (see *e.g.* Theis 2018).

Endosymbiosis (sometimes also referred to more precisely as endocytobiosis; Nardon and Charles 2001) – a symbiotic relationship in which a symbiont lives inside the host cells; prominent examples are mitochondria, plastids/photosymbionts, or nitrogen fixing

Commenté [C4]: A reference should be included here

Commenté [C5]: This definition should be improved. Community assembly is a continuous process, it does not only occur in novel habitats. Moreover the species scale is not the most appropriate for microorganisms.

Commenté [C6]: I would replace it with Nemergut. Vellend used the term « speciation » ; Nemergut replaced it with « diversification »

[1] If no other examples of the use of each term are cited below, the definition was based on the online version of the Merriam-Webster dictionary (2019): https://www.merriam-webster.com/

bacteria in plant root nodules. ~~Compared to ectosymbiosis these relationships often exhibit a higher degree of interdependence and co-evolution.~~See also **ectosymbiosis**.

Gnotobiosis – the condition in which all organisms present in a culture can be controlled~~.~~, *i.e.* germ-free (axenic) organisms or organisms with a controlled community of symbionts. Gnotobiotic individuals may be obtained *e.g.* by surgical removal from the mother (vertebrates) or by surface sterilization of seeds (plants) and subsequent handling in a sterile environment and possible inoculation with selected microbes (Hale *et al.* 1973; Williams 2014).

~~**Holobiont** – an ecological (and evolutionary) unit of different species living together in symbiosis.~~

~~**Horizontal transmission** – acquisition of the associated microbiome from the environment.~~

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

~~**Infochemical** – a usually diffusible chemical compound that mediates inter- and intraspecific communication.~~

~~**Microbial gardening** – the act of frequently releasing growth-enhancing or inhibiting chemicals or metabolites that 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.~~

Holism – a theory that organisms are best viewed as intimately interacting parts of a whole, which is more than the sum of the parts.

Holobiont – an ecological unit of different species living together in symbiosis. Whether or to what extent holobionts are also a unit of evolution is still a matter of debate (Douglas and Werren 2016).

Hologenome – the combined genomes of the host and all members of its microbiota~~;~~ (Rosenberg *et al.* 2007a; Zilber-Rosenberg and Rosenberg 2008)

Horizontal transmission – acquisition of the associated microbiome from the environment (*e.g.* Roughgarden 2019, preprint).

Host – the largest or dominant partner in a holobiont.

Infochemical – a chemical compound, usually diffusible, that carries information on the environment, such as the presence of other organisms, and can be used to mediate inter- and intraspecific communication (Dicke and Sabelis 1988).

Microbial gardening – the act of frequently releasing growth-enhancing or inhibiting chemicals or metabolites that favor the development of a microbial community beneficial to the host (see *e.g.* Saha and Weinberger 2019).

Commenté [SAH7]: The word organism is restruictive when it comes to ot the definition of a term as large as holism (i.e. beyond ecology). The definition in the early part of ther introduction fits better. In order to avoid repetition, maybe the Oxford dictionnary definition would be better?

"the theory that parts of a whole are in intimate interconnection, such that they cannot exist independently of the whole, or cannot be understood without reference to the whole, which is thus regarded as greater than the sum of its parts. Holism is often applied to mental states, language, and ecology."

Commenté [C8]: This definition is much larger than the one given in the summary : « It posits that a host and its associated microbiota, living together in a stable relationship, form the holobiont". Microorganisms are usually central in holobiont definitions.

Commenté [SAH9]: Or Myers & Rothman, 1995 in TREE ?

Microbiome – the combined genetic information encoded by the microbiota; may also refer to the microbiota itself or the microbiota and its environment (see Marchesi and Ravel 2015).

Microbiota – all microorganisms present in a particular environment or associated with a particular host (see Marchesi and Ravel 2015).

Nested ecosystems – a view of ecosystems where each individual system, like a "Russian doll", can be decomposed into smaller systems and/or considered part of a larger system (Figure 2), all of which still qualify as ecosystems (*e.g.* McFall-Ngai *et al.* 2013).

Phagocytosis – a process by which a eukaryotic cell ingests other cells or solid particles, *e.g.* the uptake of bacteria by sponges (Leys *et al.* 2018).

Phycosphere – the physical envelope surrounding a phytoplankton cell; usually rich in organic matter (see Amin *et al.* 2012).

Phylosymbiosis – congruence in the phylogeny of different hosts and the composition of their associated microbiota (Brooks *et al.* 2016).

Rasputin effect – the phenomenon that commensals and mutualists can become parasitic in certain conditions~~;~~ (Overstreet and Lotz 2016); 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.~~

Sponge loop – sponges efficiently recycle dissolved organic matter turning it into detritus that becomes food for other consumers (de Goeij *et al.* 2013).

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

Symbiosis – a close and lasting or recurrent (*e.g.* over generations) relationship between organisms living together; ~~includes~~ usually refers to mutualistic~~, commensalistic, and parasitic~~ relationships, but also includes commensalism and parasitism.

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

Vertical transmission – acquisition of the associated microbiome by a new generation of hosts from the parents (as opposed to horizontal transmission; *e.g.* Roughgarden 2019, preprint).

Marine holobionts from their origins to the present

The history of the holobiont concept

~~Current theory proposes~~**Holism** is a philosophical notion first proposed by Aristotle in the 4th century BC. It, 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 (Met. Z.17, 1041b11–33). Such systems have **emergent properties** that result from the behavior of a system that is 'larger than the sum of its parts'. However, a major shift away from holism occurred during the Age of "Enlightenment" when the dominant thought summarized as

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"dissection science" was to focus on the smallest component of a system as a means of understanding it.

The idea of holism started to regain popularity when the endosymbiosis theory was first proposed by Mereschkowski (1905) and further developed by Wallin (1925). Still accepted today, this theory posits a single origin for eukaryotic cells through the **symbiotic** assimilation of prokaryotes to form first mitochondria and later plastids (the latter through several independent symbiotic events) via **phagocytosis** (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 (Martin *et al.* 2008).

Despite the general acceptance of this so-called endosymbioticthe endosymbiosis 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 and Fester 1991; O'Malley 2017). However, the concept didwas 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 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.

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 changeshift in agronomy and medical sciences (Bulgarelli *et al.* 2013; Shreiner *et al.* 2015; Faure *et al.* 2018). Holobionts occur in terrestrial and aquatic habitats alike, and several analogies between these ecosystems can be made. For example, it is clear thatin all of these habitats, interactions within and across holobionts such as induction of chemical defenses, nutrient acquisition, or biofilm formation are mediated by chemical cues and signals in the environment, dubbed **infochemicals** (Loh *et al.* 2002; Harder *et al.* 2012; Rolland *et al.* 2016; Saha *et al.* 2019). TheNevertheless, we can identify two major differences acrossbetween terrestrial and aquatic systems are due to. First, the physicochemical properties of water resultingresult 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 across holobionts (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). FinallySecondly, phylogenetic diversity at broad taxonomic scales (*i.e.*,. supra-kingdom, kingdom and phylum levels), is higher in aquatic realms than oncompared to land, with much of the aquatic diversity yet to be uncovered (de Vargas *et al.* 2015; Thompson *et al.* 2017), especially for marine viruses (Middelboe and Brussaard 2017; Gregory *et al.* 2019). The recent discovery of thissuch astonishing marine microbial diversity andin parallel with the scarcity of marine holobiont research suggests a high potential for complex cross-lineage interactions yet to be explored in marine holobiont systemsholobionts (Figure 1).

Commenté [SAH10]: be ùmore specific on the field this happened: "in biology" ?

Commenté [C11]: Do you mean « microbial community shifts » ?

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These examples and the associated debate over how to define organisms or functional entities has led to the **revival of ‘holism’**, a philosophical notion first proposed by Aristotle in the 4th century BC. However, a major shift happened during the Age of "Enlightenment" when the dominant thought summarized as “dissection science” was to focus on the smallest component of a system in order to understand it better. By contrast, holistic thinkingThe 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 (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’. In this context the boundaries of holobionts are usually delimited by a physical gradient, which corresponds to the area of local 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’, encompassingsetting the boundaries of the holobiont depending on the interactions and biological functions that are being considered. Thus holobionts may encompass 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).

Such a viewconceptual perspective raises fundamental questions for studies ofwhen studying 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*et al.* (2018) have(2010) and Rosenberg and Zilber-Rosenberg (2018) argued that all animals and plants can be considered holobionts, and thus 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)., suggesting that natural selection acts at the level of the holobiont and its hologenome. 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), especially when applied on aat the community or ecosystem level (Moran and Sloan 2015). 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 itsthe underlying complexity of holobiont systems 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, (Figure 1), 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, *i.e.* holobiont systems in which microbiome composition is not or only partially controlled: radiolarians and foraminiferans (both heterotrophic protistsprotist 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

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Commenté [C12]: The paragraph is expected to set out a list of questions

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Commenté [C13]: Is it a question or a statement ?

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Commenté [SAH14]: "better defined and further considered" or "refined and further considered" ?

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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)~~(Dubilier *et al.* 2008; Rubin-Blum *et al.* 2019) and frequently ~~exhibited~~exhibit high levels of metabolic and taxonomic diversity (Duperron *et al.* 2008; Petersen *et al.* 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). The *Vibrio*-squid model provides insights into the effect of microbiota on animal development, circadian rhythms, and immune systems (McFall-Ngai 2014). 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 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). Although the culture conditions in these highly-controlled model systems differ from the natural environment, these systems are essential to gain elementary mechanistic understanding of the functioning, the roles, and ~~thus also~~ the evolution of marine holobionts.

Marine holobionts as drivers of **ecological processes**

~~Motile~~Work on model systems has demonstrated that motile and macroscopic marine holobionts can act as dissemination vectors for geographically restricted microbial taxa. ~~For instance, pelagic~~Pelagic mollusks or vertebrates ~~have a~~are textbook examples of high ~~capacity for~~ dispersal capacity organisms (*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 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 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.*,~~They may, for instance, provide a nutrient-rich niche in the otherwise nutrient-poor surroundings (Smriga *et al.* 2010; Webster *et al.* 2010; Burke, Thomas, *et al.* 2011; Chiarello *et al.* 2018). ~~Woyke 2006) and colonize extreme~~

Commenté [SAH15]: See referencves on green algae as well fitting the desvcription here such as articles by Hollants et al

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Commenté [SAH16]: The influence of marine holobiont on ecological processes

Commenté [SAH17R16]: All can be considered as drivers, here the difference is the way one can obtain a correct/better appraisal of ecological processes at stake by scaling up to the holobiont. This should better reflect in the title. The suggestion above is not perfect

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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 Lastly, biological processes regulated by microbes are important drivers of global biogeochemical cycles (Falkowski *et al.* 2008; Madsen 2011; Anantharaman *et al.* 2016). In the open ocean, it is estimated that symbioses with the cyanobacterium UCYN-A contribute ~20% to 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 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 Furthermore, the interaction between host and microbiota can potentially extend the metabolic capabilities have the potential to extend the ecological niche of thea holobiont as well asin a way that augments 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), or allows it to cross biotope boundaries (*e.g.*, Woyke 2006) and colonize extreme environments (Bang *et al.* 2018). . It is thereforeHolobionts thus contribute to marine microbial diversity and possibly resilience in the context of global environmental changes (Troussellier *et al.* 2017) and it is paramount to include the holobiont concept in predictive models that investigate the consequences of human impacts on the marine realm and its biogeochemical cycles.

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Challenges and opportunities in marine holobiont research

Marine **holobiont assembly** and regulation

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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,organisms such as bivalves transmit part of the microbiota maternally (Bright and Bulgheresi 2010; Funkhouser and Bordenstein 2013). In other marine holobionts, vertical transmission may be weak and inconsistent, whereas mixed **modes of transmission (vertical and horizontal)** or intermediate modes (pseudo-vertical, where horizontal acquisition frequently involves symbionts of parental origin) are more common (Bjork *et al.* 2018, preprint).(Björk *et al.* 2019). Identifying the factors shaping holobiont composition and understanding their evolution is highly relevant for marine organisms given that most marine hosts display a high specificity for their microbiota and even patterns of **phylosymbiosis** (Kazamia *et al.* 2016;

Brooks *et al.* 2016; Pollock *et al.* 2018), despite a highly connected and microbe-rich environment,..

During microbiota transmission (whether vertical or horizontal), "*selection*" by the host (as opposed to "*drift*") is a key process in establishing or maintaining a holobiont microbial community that is distinct from the environment. The immune system of the host is one way to regulate the microbial composition ofof performing this selection in both marine and terrestrial holobionts. Perturbations in this system, and perturbations 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)(*e.g.* Marzinelli *et al.* 2015). A specific case of dysbiosis is the so-called "**Rasputin effect**" 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). For instance in reef-building corals, 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, leading to coral bleaching and even death (Baker *et al.* 2018).

Another factor regulatingway of selecting a holobiont compositionmicrobial community is by 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, the phylogenetic diversity of hosts and symbionts suggests both conserved and marine-specific chemical interactions, but comparable studies are only starting to emerge.still in their infancy. For instance, seaweeds can chemically garden beneficial microbes, facilitating normal morphogenesis and increasing disease resistance (Kessler *et al.* 2018; Saha and Weinberger 2019)(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)(Gribben *et al.* 2017; Cirri and Pohnert 2019). In

In addition to selection and ecological drift, "dispersal" and evolutionary "diversification" have been proposed as key processes in community assembly. Both these processes are, however, difficult to quantify in microbial communities (Nemergut *et al.* 2013). The only data currently at our disposal to studyquantify these processes are the diversity and distribution of microbes. Considering the high connectivity of aquatic environments, differences in marine microbial communities are frequently attributed to a combination of selection and drift (e.g. Burke, Steinberg, *et al.* 2011), a conclusion that still requires validation. Diversification is mainly considered in the sense of coevolution or adaptation to host selection, which may also be driven by the horizontal acquisition of genes, but to our knowledge, unlike in primates (Moeller *et al.* 2016), no information exists on the co-speciation of host-associated microbes in marine holobionts to date.

Increasing our knowledge on the contribution of these processes to holobiont community assembly in marine systems is a key challenge, especially in the context of ongoing global

Commenté [C18]: Selection can also be exterted by other microorganisms. This is what triggers priority effects. This should be mentionned somewhere in this paragraph.

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Commenté [C19]: This sentence suggests that drift homogenizes communities associated with the host and communities associated with its environment. I'm not sure of it, drift is a random process.

Commenté [SAH20]: Examples of drift mediated compositioon are not given here (drift is only mentioned as opposed to selection here above)

Commenté [C21]: Several frameworks were developped to quantify processes of community assembly
See for instance
Stegen et al. 2013. Quantifying community assembly processes and identifying features that impose them. ISME J
Dini-Andreote et al. 2015 Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. PNAS

Commenté [SAH22]: , rather than limited dispersal
I suppose this is what you mean?

Commenté [SAH23]: Except for primates ?

Commenté [SAH24]: Not sure this can be stated as is, or this has to be better specified. Indeed, see

Peek, A. S., Feldman, R. A., Lutz, R. A., & Vrijenhoek, R. C. (1998). Cospeciation of chemoautotrophic bacteria and deep-sea clams. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 9962–9966.
Or
Lanterbecq D, Rouse GW, Eeckhaut I. 2010. Evidence for cospeciation events in the host–symbiont system involving crinoids (Echinodermata) and their obligate associates, the myzostomids (Myzostomida, Annelida). *Molecular Phylogenetics and Evolution 54: 357–371.*
Or

Yet to be discussed under the light of
de, Vienne, D.M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M.E. and Giraud, T. (2013), Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. New Phytol, 198: 347-385. doi:10.1111/nph.12150

and Moran et al 2015 PloS biol cited here aboe for the reserves to interpret congruent phylogenetic patterns as co speciation indices change~~, an~~. Moreover, 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~~. Yet, this notion is still missing in most mathematical or formal models, or additional information on biological interactions would be required to make the former more accurate ~~(Bell *et al.* 2018)~~(Bell *et al.* 2018).

Integrating marine model systems with large-scale studies

By compiling ~~what a sample of researchers today considera~~ a survey of the most important trends and challenges in the field of marine holobiont research (Figure 3), we identified two distinct opinion clusters: one focused on mechanistic understanding and work with model systems whereas another targets large-scale and heterogeneous data set analyses and predictive modeling. This illustrates that, on the one hand, the scientific community is ~~focusing on~~interested in 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 *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)~~(*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~~made applicable to ecologically-relevant ~~complex~~ systems. At the same time, large scale-studies remain mostly descriptive and ~~with~~bear 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 applications was perceived as a central hub at the interface between mechanistic understanding and predictive modeling.

A successful example ~~**allying**~~merging both 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)~~(*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).

~~We consider it essential to implement~~We advocate the implementation of comparable efforts in marine sciences through interdisciplinary research combining physiology, biochemistry, ecology, and ~~mathematical~~computational modeling. A key factor ~~here~~ will be the identification and development of ~~**new**~~tractable 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~~organismal 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, conservation) in

Commenté [SAH25]: why here ?

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Commenté [C26]: This is not clear, what notion is missing and what kind of mathematical models do you refer to ?

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Commenté [C27]: Recent advances in community (predictive) modelling could be cited here Ovaskainen et al. 2017. How to make more out of community data? A conceptual framework and its implementation as models and software. Ecology Letters

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Commenté [SAH28]: invasion

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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

As our conceptual understanding of the different levels of holobiont organization 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~~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 metagenomics or single-cell genomics~~ in the case of unicellular hosts~~, metatranscriptomics, and metaproteomics, thus advancing our ~~understanding~~research from phylogenetic to functional analyses of the holobiont ~~(Bowers *et al.* 2017; Meng *et al.* 2018). 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.~~(Bowers *et al.* 2017; Meng *et al.* 2018; Figure 4). These approaches are equally useful in marine and in terrestrial environments, but the scarcity of well-studied lineages in the former makes the generation of good annotations and reference databases challenging for marine biologists. Metaproteomics combined with stable isotope fingerprinting can help study the 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~~ particularly relevant to marine systems~~,~~ is that many compounds are ~~still undescribed in~~often not referenced in the mostly terrestrial-based 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 advancement~~may help to overcome this challenge (Hartmann *et al.* 2017).

A further challenge in holobiont research is to identify the origin of compounds among the different partners of the holobionts and to determine 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.~~(*e.g.* Quinn *et al.* 2016), but they will also require high levels of replication in order to represent the extensive intra-species variability found in marine systems. 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~~molecular level. The combination 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

Commenté [SAH29]: lineage ?
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Commenté [SAH30]: contribution ?
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Commenté [SAH31]: compartments ?
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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~~these techniques.

One consequence of the development of such new methods is the feedback they provide to improve existing models ~~and~~or to develop entirely new ones, ~~for example~~*e.g.* by conceptualizing holobionts as the ~~sum~~combination of the interactions between the host and its microbiota (Skillings 2016; Berry and Loy 2018), or by redefining boundaries between the holobiont and ~~the ecosystem~~its environment (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~~move holobiont research away from laboratory culture-based experiments. We argue that maintaining cultivation efforts to capture the maximum holobiont biodiversity 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, and behavior ~~(*e.g.*, Faith *et al.* 2014; Selosse *et al.* 2014)~~(*e.g.* Neufeld *et al.* 2011; Faith *et al.* 2014; Selosse *et al.* 2014). Innovations in cultivation techniques for axenic (or germ-free) hosts ~~(*e.g.*, Spoerner *et al.* 2012)~~(*e.g.* Spoerner *et al.* 2012) or in microbial cultivation such as microfluidic systems ~~(*e.g.*, Pan *et al.* 2011)~~(*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~~have specific requirements. In this context, single-cell ~~omics~~'-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, *e.g.* by genome sequencing and the development of genetic tools, in order 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~~'-omics' techniques ~~can~~have the potential to broaden the range of available models, enabling ~~generalizations about~~a better understanding of 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 will likely have 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 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

Commenté [SAH32]: Unclear. In this part we were not in the accuracy of the description/prediction of host-microbial communities any more but in the distribution of interactions among them? Or do you mean the strength of the association between host and the different components of associated microbial communities

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Commenté [C33]: The new field of « culturomics » could be briefly described here (Lagier et al., 2012, CMI)

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Commenté [C34]: This is an important statement, it could be part of the summary

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habitat-forming organisms as holobionts will ultimately improve the predictability and management of coastal ecosystems. For example, host-associated microbiota could be integrated ~~into the~~in **biomonitoring** programs as 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).~~(van Oppen *et al.* 2017; Marzinelli *et al.* 2018).

One form of intervention could be to promote positive changes of host-associated ~~microbiotas~~microbiota, in ways analogous 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 used as **biological control** agents and 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 manipulated to shape key phenotypes in cultured marine organisms. For example, specific bacteria associated with microalgae may enhance algal growth (Amin *et al.* 2009; Kazamia *et al.* 2012; Le Chevanton *et al.* 2013), increase 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.

Finally, one could implement holistic approaches in the framework of fish farms. Recent developments including integrated multi-trophic aquaculture, recirculating aquaculture, offshore aquaculture, 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).

~~Nevertheless, when considering their biotechnological potential, it should also be noted that marine microbiota 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 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 cases where holobionts manipulated by human resulted in pests (*e.g.*, Clay and Holah 1999) call for a cautious and ecologically-informed evaluation of holobiont-based technologies.~~

Nevertheless, when considering their biotechnological potential, it should also be noted that marine microbiota are likely vulnerable to anthropogenic influences and that their deliberate engineering, introduction from exotic regions, or inadvertent perturbations may have profound, and yet entirely unknown, consequences 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 cases where holobionts manipulated by human resulted in pests (*e.g.* Clay and Holah 1999) call for a cautious and ecologically-informed evaluation of holobiont-based technologies in marine systems.

Commenté [C35]: Here you describe curative treatments. Biocontrol treatments can also be preventive.

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Commenté [SAH36]: I don't understand this sentence what is detrimental? Do you mean unhealthy hosts? Or non-target/ undesired species?

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Commenté [SAH37]: Side consequences ?

Mis en forme : Anglais (États-Unis)

Commenté [SAH38]: Also (the list is far from exhaustive)

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Commenté [SAH39]: Amount of

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Commenté [SAH40]: How so ? it may be worth detailing in one sentenced the idea deve"lopped in this article?

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Commenté [SAH41]: Often hidden in their hosts, several cases reported in the marine realm as well

Conclusions

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Marine ecosystems represent highly connected reservoirs of largely unexplored biodiversity. They are of critical importance to feed the ever-growing world population, constitute significant players in global biogeochemical cycles but are also threatened by human activities and global change. In order to 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 ~~to~~, to complex ~~assemblies~~ assemblages of eukaryotes, prokaryotes, and viruses with one or several hosts, or even entire ecosystems. Accordingly, current key questions in marine holobiont research cover a wide range of topics: What are the exchanges that occur between different partners of the holobiont, and what are the cues and signals driving these exchanges? What are the relevant units of selection in marine holobionts? How do holobiont systems and the interactions within them change over time and in different conditions? How do such changes impact ecological processes? How can this knowledge be applied to our benefit and where do we need to draw limits? Identifying and consolidating key model systems while adapting emerging "-omics", imaging, and culturing technologies to them will be critical to the development of "holobiont-aware" ecosystem models.

Commenté [SAH42]: And how do they condition the survival, dynamics and evolution of the different partners? What are the cues…

Commenté [SAH43]: And dispersal

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 the fundamental fact that all living organisms have intimate connections with their immediate neighbors, 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 paradigm shift in biological and environmental sciences, but only if scientists 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~~as well as application of the holobiont concept in aquaculture and ecosystem management projects.

Commenté [SAH44]: Undertsanding of biochemical cycles?

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Conflict of interest

The authors of this preprint declare that they have no financial conflict of interest with the content of this article. FN is one of the PCI Ecology recommenders.

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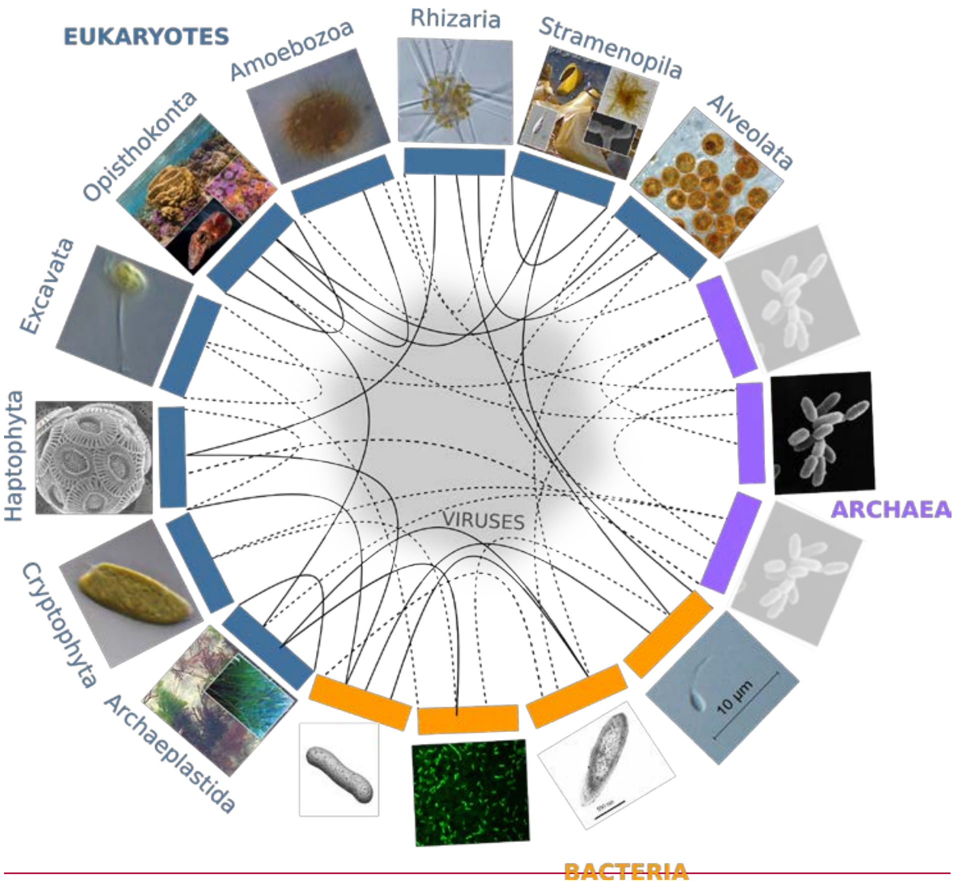
Figures



EUKARYOTES
Amoebozoa
Rhizaria
Stramenopila
Alveolata
Opisthokonta
Excavata
Haptophyta
Cryptophyta
Archaeplastida
VIRUSES
ARCHAEA
10 µm
BACTERIA

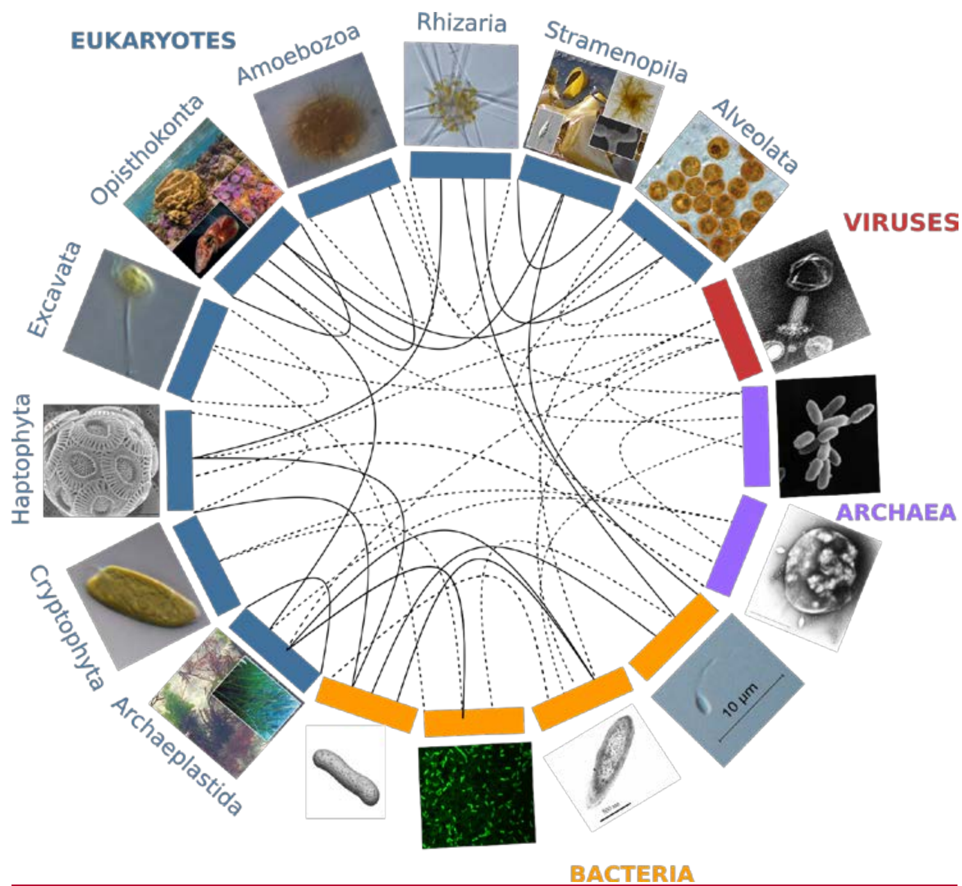
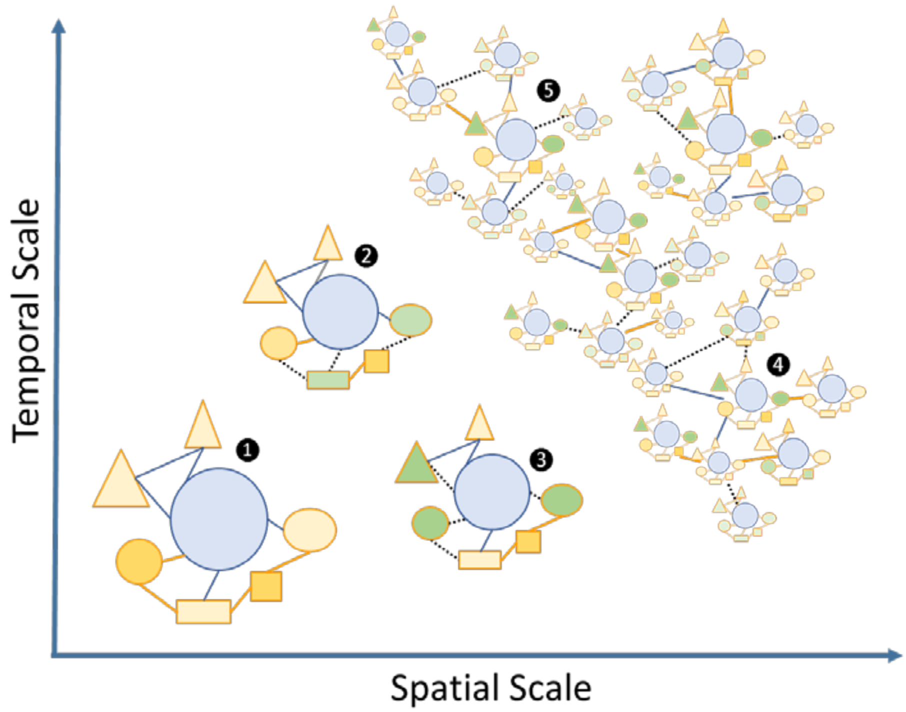


Figure 1. Partners forming marine holobionts are widespread across the tree of life including all kingdoms (eukaryotes, bacteria, archaea, viruses), and represent a large diversity of potential models for exploring complex biotic interactions across lineages. Plain lines correspond to holobionts referred 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, Planktomania; Archaea – National Space Science Data Center., Xiaoyu Xiang; Viruses M. B. Sullivan et al..



Temporal Scale
Spatial Scale

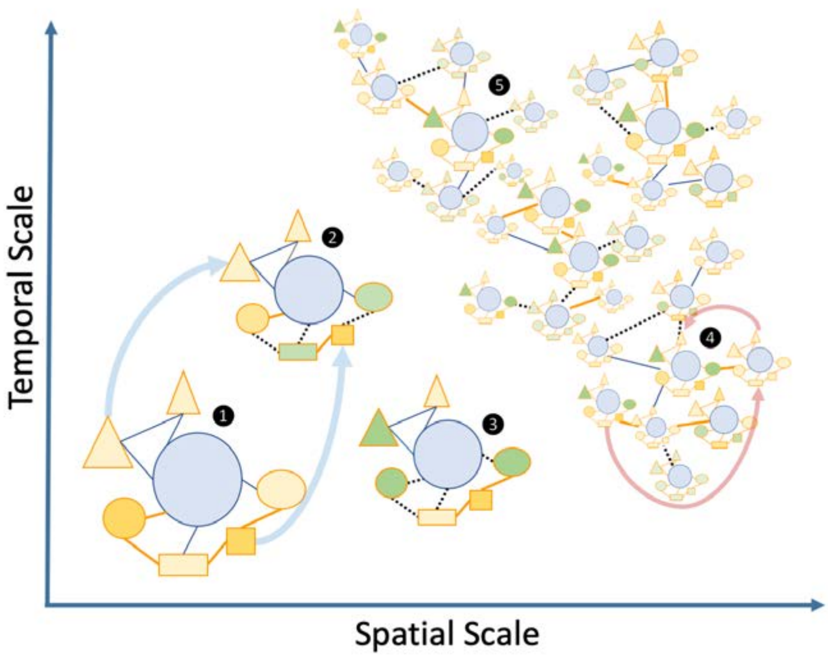
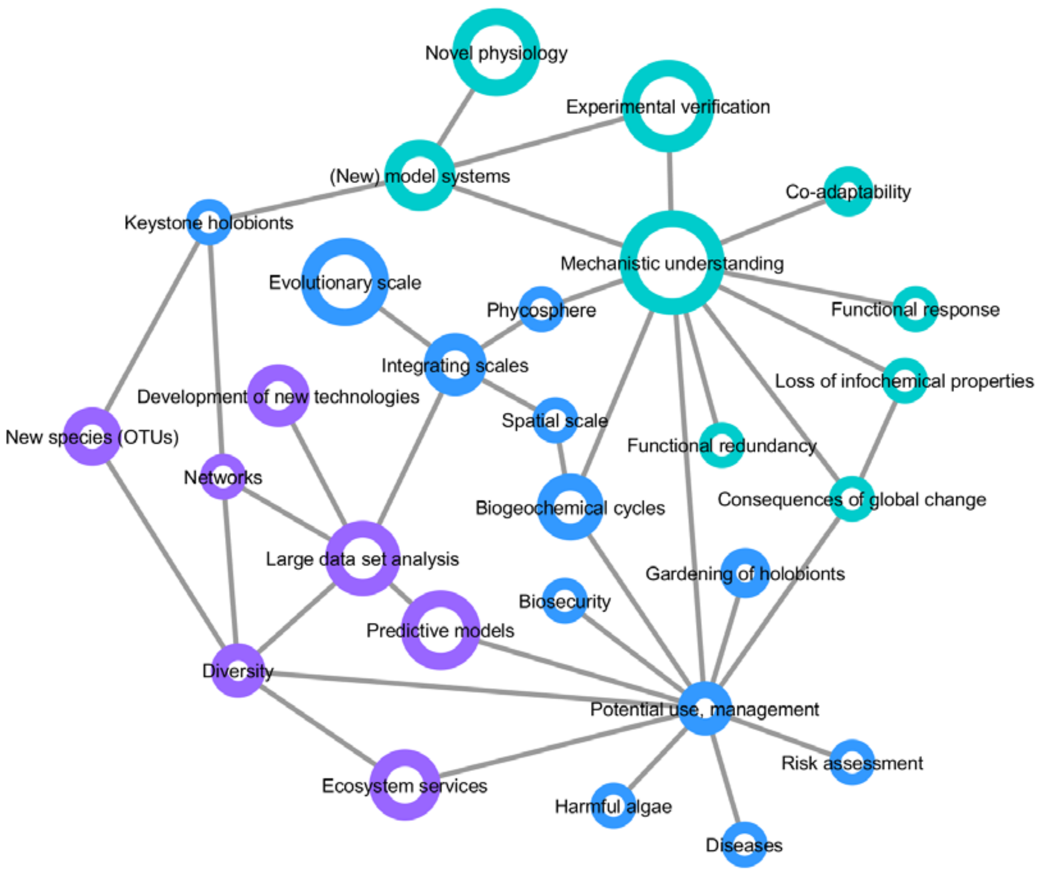


Figure 2. Schematic view of the "Russian Doll" complexity and dynamics of holobionts, according to diverse spatiotemporal scales. The host (blue circles), and associated microbes (all other shapes) including bacteria and eukaryotes that may be inside (*i.e.*~~,~~. endosymbiotic or outside the host, *i.e.*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


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

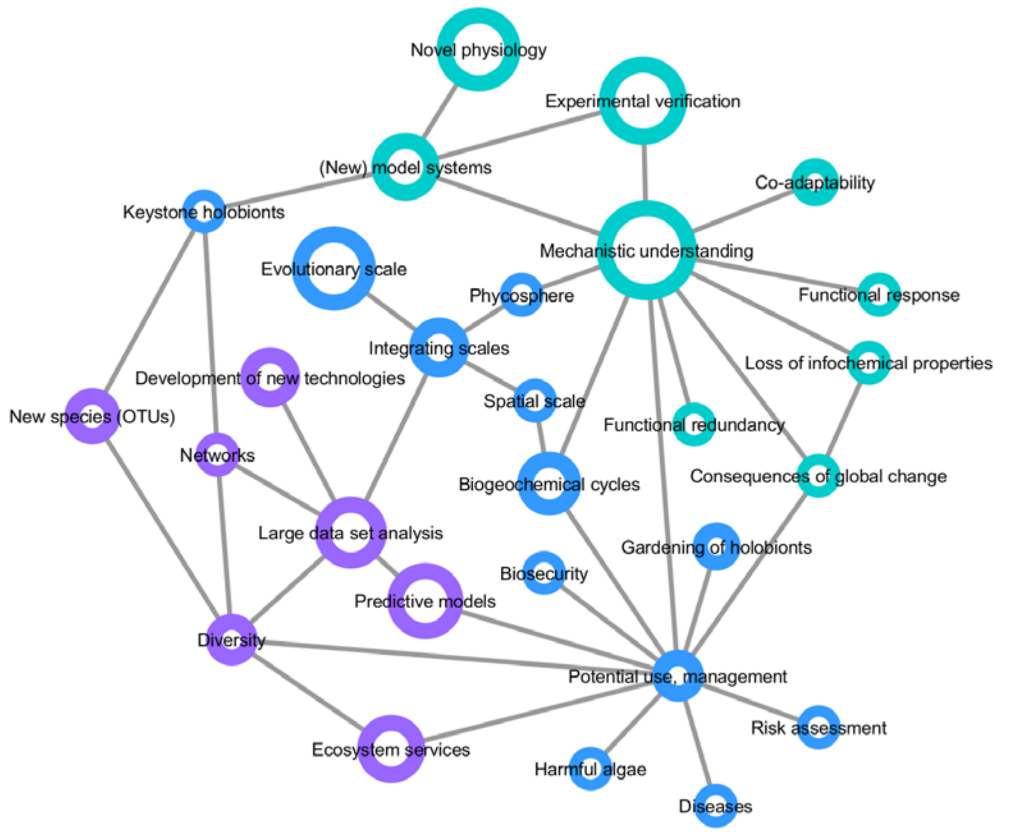


Figure 3: Mind map of key concepts, techniques, and challenges related to marine holobionts. The basis of this map was generated during the Holomarine workshop held in Roscoff in 2018 (https://www.euromarinenetwork.eu/activities/HoloMarine). The size of the nodes reflects the number of votes each keyword 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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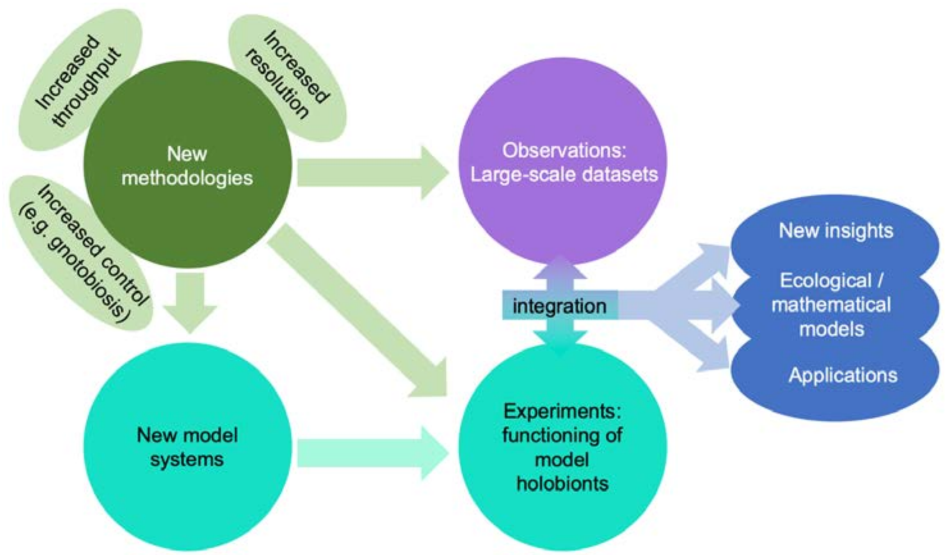

Figure 4: Impact of emerging methodologies (green) on the main challenges in marine holobiont research identified in this paper.

Commenté [C45]: There are three shades of green, which one corresponds to emerging methodologies ?

Commenté [C46]: What is the color for the main challenges ?

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