

1 *A community perspective on the concept of*  
2 *marine holobionts: current status, challenges,*  
3 *and future directions*

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## 89 Abstract:

90 Host-microbe interactions play crucial roles in marine ecosystems, but we still have very  
91 little understanding of the mechanisms that govern these relationships, the evolutionary  
92 processes that shape them, and their ecological consequences. The holobiont concept is a  
93 renewed paradigm in biology that can help to describe and understand these complex systems. It  
94 posits that a host and its associated microbiota, living together in a stable relationship, form the  
95 holobiont, and have to be studied together as a coherent biological and functional unit to  
96 understand its biology, ecology, and evolution. Here we discuss critical concepts and  
97 opportunities in marine holobiont research and identify key challenges in the field. We highlight  
98 the potential economic, sociological, and environmental impacts of the holobiont concept in  
99 marine biological, evolutionary, and environmental sciences with comparisons to terrestrial  
100 sciences where appropriate. Given the connectivity and the unexplored biodiversity specific to  
101 marine ecosystems, a deeper understanding of such complex systems requires further  
102 technological and conceptual advances, *e.g.* the development of controlled experimental model  
103 systems for holobionts from all major lineages and the modeling of (info)chemical-mediated  
104 interactions between organisms. The most significant challenge is to bridge cross-disciplinary  
105 research on tractable model systems in order to address key ecological and evolutionary  
106 questions. This will be crucial to decipher the roles of marine holobionts in biogeochemical  
107 cycles, but also developing concrete applications of the holobiont concept *e.g.* to increase yield  
108 or disease resistance in aquacultures or to protect and restore marine ecosystems through  
109 management projects.  
110

111 Glossary<sup>1</sup>

- 112 **Anna Karenina principle** – a number of factors can cause a system to fail, but only a narrow  
113 range of parameters characterizes a working system; based on the first sentence of Leo  
114 Tolstoy’s “Anna Karenina” (1878): “Happy families are all alike; every unhappy family  
115 is unhappy in its own way” (Zaneveld *et al.* 2017).
- 116 **Aposymbiotic culture** – a culture of a host or a symbiont without its main symbiotic partner(s)  
117 (*e.g.* Kelty and Cook 1976). In contrast to gnotobiotic cultures, aposymbiotic cultures are  
118 usually not germ-free.
- 119 **Biological control (biocontrol)** – methods of controlling diseases or pests by introducing or  
120 supporting natural enemies of the former (see *e.g.* Hoitink and Boehm 1999).
- 121 **Biomonitoring** – the use of living organisms as indicator for the health of an environment or  
122 ecosystem.
- 123 **Community assembly process** – the accumulation of species in a novel habitat, according to  
124 Vellend the four main forces relevant for community assembly are diversification,  
125 dispersal, selection, and drift (Vellend 2010; Nemergut *et al.* 2013).
- 126 **Dysbiosis** – microbial imbalance in a symbiotic community that affects the health of the host  
127 (Egan and Gardiner 2016).
- 128 **Ecological process** – the processes responsible for the functioning and dynamics of ecosystems  
129 including biogeochemical cycles, community assembly processes, interactions between  
130 organisms, and climatic processes (see *e.g.* Bennett *et al.* 2009).
- 131 **Ecosystem services** – any direct or indirect benefits that humans can draw from an ecosystem;  
132 they include provisioning services (*e.g.* food), regulating services (*e.g.* climate), cultural  
133 services (*e.g.* recreation), and supporting services (*e.g.* habitat formation) (Millennium  
134 Ecosystem Assessment Panel 2005).
- 135 **Ectosymbiosis** – a symbiotic relationship in which symbionts live on the surface of a host. This  
136 includes, for instance, algal biofilms or the skin microbiome (Nardon and Charles 2001).
- 137 **Emergent property** – a property of complex systems (*e.g.* holobionts), which arises from  
138 interactions between the components and that is not the sum of the component properties  
139 (see *e.g.* Theis 2018).
- 140 **Endosymbiosis** (sometimes also referred to more precisely as endocytobiosis; Nardon and  
141 Charles 2001) – a symbiotic relationship in which a symbiont lives inside the host cells;  
142 prominent examples are mitochondria, plastids/photosymbionts, or nitrogen fixing  
143 bacteria in plant root nodules. See also **ectosymbiosis**.
- 144 **Gnotobiosis** – the condition in which all organisms present in a culture can be controlled, *i.e.*  
145 germ-free (axenic) organisms or organisms with a controlled community of symbionts.  
146 Gnotobiotic individuals may be obtained *e.g.* by surgical removal from the mother  
147 (vertebrates) or by surface sterilization of seeds (plants) and subsequent handling in a  
148 sterile environment and possible inoculation with selected microbes (Hale *et al.* 1973;  
149 Williams 2014).
- 150 **Holism** – a theory that organisms are best viewed as intimately interacting parts of a whole,  
151 which is more than the sum of the parts.

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<sup>1</sup> 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/>

- 152 **Holobiont** – an ecological unit of different species living together in symbiosis. Whether or to  
153 what extent holobionts are also a unit of evolution is still a matter of debate (Douglas and  
154 Werren 2016).
- 155 **Hologenome** – the combined genomes of the host and all members of its microbiota; Rosenberg  
156 *et al.* 2007a; Zilber-Rosenberg and Rosenberg 2008)
- 157 **Horizontal transmission** – acquisition of the associated microbiome from the environment (*e.g.*  
158 Roughgarden 2019, preprint).
- 159 **Host** – the largest or dominant partner in a holobiont.
- 160 **Infochemical** – a chemical compound, usually diffusible, that carries information on the  
161 environment, such as the presence of other organisms, and can be used to mediate inter-  
162 and intraspecific communication (Dicke and Sabelis 1988).
- 163 **Microbial gardening** – the act of frequently releasing growth-enhancing or inhibiting chemicals  
164 or metabolites that favor the development of a microbial community beneficial to the host  
165 (see *e.g.* Saha and Weinberger 2019).
- 166 **Microbiome** – the combined genetic information encoded by the microbiota; may also refer to  
167 the microbiota itself or the microbiota and its environment (see Marchesi and Ravel  
168 2015).
- 169 **Microbiota** – all microorganisms present in a particular environment or associated with a  
170 particular host (see Marchesi and Ravel 2015).
- 171 **Nested ecosystems** – a view of ecosystems where each individual system, like a “Russian doll”,  
172 can be decomposed into smaller systems and/or considered part of a larger system  
173 (Figure 2), all of which still qualify as ecosystems (*e.g.* McFall-Ngai *et al.* 2013).
- 174 **Phagocytosis** – a process by which a eukaryotic cell ingests other cells or solid particles, *e.g.* the  
175 uptake of bacteria by sponges (Leys *et al.* 2018).
- 176 **Phycosphere** – the physical envelope surrounding a phytoplankton cell; usually rich in organic  
177 matter (see Amin *et al.* 2012).
- 178 **Phylosymbiosis** – congruence in the phylogeny of different hosts and the composition of their  
179 associated microbiota (Brooks *et al.* 2016).
- 180 **Rasputin effect** – the phenomenon that commensals and mutualists can become parasitic in  
181 certain conditions (Overstreet and Lotz 2016); after the Russian monk Rasputin who  
182 became the confidant of the Tsar of Russia, but later helped bring down the Tsar’s empire  
183 during the Russian revolution.
- 184 **Sponge loop** – sponges efficiently recycle dissolved organic matter turning it into detritus that  
185 becomes food for other consumers (de Goeij *et al.* 2013).
- 186 **Symbiont** – an organism living in symbiosis; usually refers to the smaller/microbial partners  
187 living in mutualistic relationships (see also host), but also includes organisms in  
188 commensalistic and parasitic relationships.
- 189 **Symbiosis** – a close and lasting or recurrent (*e.g.* over generations) relationship between  
190 organisms living together; usually refers to mutualistic relationships, but also includes  
191 commensalism and parasitism.
- 192 **Vertical transmission** – acquisition of the associated microbiome by a new generation of hosts  
193 from the parents (as opposed to horizontal transmission; *e.g.* Roughgarden 2019,  
194 preprint).

## 195 Marine holobionts from their origins to the present

### 196 The history of the holobiont concept

197 **Holism** is a philosophical notion first proposed by Aristotle in the 4<sup>th</sup> century BC. It  
198 states that systems should be studied in their entirety, with a focus on the interconnections  
199 between their various components rather than on the individual parts (Met. Z.17, 1041b11–33).  
200 Such systems have **emergent properties** that result from the behavior of a system that is ‘larger  
201 than the sum of its parts’. However, a major shift away from holism occurred during the Age of  
202 "Enlightenment" when the dominant thought summarized as “dissection science” was to focus on  
203 the smallest component of a system as a means of understanding it.

204 The idea of holism started to regain popularity when the endosymbiosis theory was first  
205 proposed by Mereschkowski (1905) and further developed by Wallin (1925). Still accepted  
206 today, this theory posits a single origin for eukaryotic cells through the **symbiotic** assimilation of  
207 prokaryotes to form first mitochondria and later plastids (the latter through several independent  
208 symbiotic events) via **phagocytosis** (reviewed in Archibald 2015). These ancestral and founding  
209 symbiotic events, which prompted the metabolic and cellular complexity of eukaryotic life, most  
210 likely occurred in the ocean (Martin *et al.* 2008).

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

224 Although initially driven by studies of marine organisms, much of the research on the  
225 emerging properties and significance of holobionts has since been carried out in other fields of  
226 research: the **microbiota** of the rhizosphere of plants or the animal gut became predominant  
227 models and have led to an ongoing paradigm shift in agronomy and medical sciences (Bulgarelli  
228 *et al.* 2013; Shreiner *et al.* 2015; Faure *et al.* 2018). Holobionts occur in terrestrial and aquatic  
229 habitats alike, and several analogies between these ecosystems can be made. For example, in all  
230 of these habitats, interactions within and across holobionts such as induction of chemical  
231 defenses, nutrient acquisition, or biofilm formation are mediated by chemical cues and signals in  
232 the environment, dubbed **infochemicals** (Loh *et al.* 2002; Harder *et al.* 2012; Rolland *et al.*  
233 2016; Saha *et al.* 2019). Nevertheless, we can identify two major differences between terrestrial  
234 and aquatic systems. First, the physicochemical properties of water result in higher chemical  
235 connectivity and signaling between macro- and micro-organisms in aquatic or moist  
236 environments. In marine ecosystems, carbon fluxes also appear to be swifter and trophic modes  
237 more flexible, leading to higher plasticity of functional interactions across holobionts (Mitra *et al.*  
238 *et al.* 2013). Moreover, dispersal barriers are usually lower, allowing for faster microbial shifts in

239 marine holobionts (Kinlan and Gaines 2003; Martin-Platero *et al.* 2018). Secondly, phylogenetic  
240 diversity at broad taxonomic scales (*i.e.* supra-kingdom, kingdom and phylum levels), is higher  
241 in aquatic realms compared to land, with much of the aquatic diversity yet to be uncovered (de  
242 Vargas *et al.* 2015; Thompson *et al.* 2017), especially marine viruses (Middelboe and Brussaard  
243 2017; Gregory *et al.* 2019). The recent discovery of such astonishing marine microbial diversity  
244 in parallel with the scarcity of marine holobiont research suggest a high potential for complex  
245 cross-lineage interactions yet to be explored in marine holobionts (Figure 1).

246 The boundaries of holobionts are usually delimited by a physical gradient, which  
247 corresponds to the area of local influence of the host, *e.g.* in unicellular algae the so-called  
248 **phycosphere** (Seymour *et al.* 2017). However, they may also be defined in a context-dependent  
249 way as a ‘Russian Matryoshka doll’, setting the boundaries of the holobiont depending on the  
250 interactions and biological functions that are being considered. Thus holobionts may encompass  
251 all levels of host-symbiont associations from intimate **endosymbiosis** with a high degree of co-  
252 evolution up to the community and ecosystem level; a concept referred to as “**nested**  
253 **ecosystems**” (Figure 2; McFall-Ngai *et al.* 2013; Pita *et al.* 2018).

254 Such a conceptual perspective raises fundamental questions when studying the evolution  
255 of holobionts, especially regarding relevant units of selection and the role of co-evolution. For  
256 instance, plant and animal evolution involves new functions co-constructed by members of the  
257 holobiont or elimination of functions redundant between them (Selosse *et al.* 2014). Rosenberg  
258 *et al.* (2010) and Rosenberg and Zilber-Rosenberg (2018) argued that all animals and plants can  
259 be considered holobionts, and thus advocate the **hologenome** theory of evolution, suggesting that  
260 natural selection acts at the level of the holobiont and its hologenome. This interpretation of  
261 Margulis’ definition of a ‘holobiont’ considerably broadened fundamental concepts in evolution  
262 and speciation and has not been free of criticism (Douglas and Werren 2016), especially when  
263 applied at the community or ecosystem level (Moran and Sloan 2015). More recently, it has been  
264 shown that species that interact indirectly with the host can also be important in shaping  
265 coevolution within mutualistic multi-partner assemblages (Guimarães *et al.* 2017). Thus, the  
266 holobiont concept and the underlying complexity of holobiont systems should be further  
267 considered when addressing evolutionary and ecological questions.

## 268 Marine holobiont models

269 Today, an increasing number of marine model organisms, both unicellular and multicellular, are  
270 being used in holobiont research (Figure 1), often with different emphasis and levels of  
271 experimental control, but altogether covering a large range of scientific topics. Here, we provide  
272 several illustrative examples of this diversity and some of the insights they have provided.

273 Environmental or “semi-controlled” models, *i.e.* holobiont systems in which microbiome  
274 composition is not or only partially controlled: radiolarians and foraminiferans (both  
275 heterotrophic protist dwellers harboring endosymbiotic microalgae) are emerging as ecological  
276 models for unicellular photosymbiosis due to their ubiquitous presence in the world’s oceans  
277 (Decelle *et al.* 2015; Not *et al.* 2016). The discovery of deep-sea hydrothermal vents revealed  
278 symbioses of animals with chemosynthetic bacteria that have later been found in many other  
279 marine ecosystems (Dubilier *et al.* 2008; Rubin-Blum *et al.* 2019) and frequently exhibit high  
280 levels of metabolic and taxonomic diversity (Duperron *et al.* 2008; Petersen *et al.* 2016;  
281 Ponnudurai *et al.* 2017). The cosmopolitan haptophyte *Emiliania huxleyi*, promoted by  
282 associated bacteria (Seyedsayamdost *et al.* 2011; Segev *et al.* 2016), produces key intermediates

283 in the carbon and sulfur biogeochemical cycles, making it an important model phytoplankton  
284 species.

285 Controlled bi- or trilateral associations: Only a few models, covering a small part of the  
286 overall marine biodiversity, are currently being cultivated *ex-situ* and can be used in fully  
287 controlled experiments, where they can be cultured **aprosymbiotically**. The flatworm  
288 *Symsagittifera* (= *Convoluta*) *roscoffensis* (Arboleda *et al.* 2018), the sea anemone *Exaiptasia*  
289 (Baumgarten *et al.* 2015; Wolfowicz *et al.* 2016), the upside-down jellyfish *Cassiopea* (Ohdera  
290 *et al.* 2018), and their respective intracellular green and dinoflagellate algae have, in addition to  
291 corals, become models for fundamental research on evolution of metazoan-algal photosymbiosis.  
292 In particular, *Exaiptasia* has been used to explore photobiology disruption and restoration of  
293 cnidarian symbioses (Lehnert *et al.* 2012). The *Vibrio*-squid model provides insights into the  
294 effect of microbiota on animal development, circadian rhythms, and immune systems (McFall-  
295 Ngai 2014). The unicellular green alga *Ostreococcus*, an important marine primary producer, has  
296 been shown to exchange vitamins with specific associated bacteria (Cooper *et al.* 2019). The  
297 green macroalga *Ulva mutabilis* has enabled the exploration of bacteria-mediated growth and  
298 morphogenesis including the identification of original chemical interactions in the holobiont  
299 (Wichard 2015; Kessler *et al.* 2018). Although the culture conditions in these highly-controlled  
300 model systems differ from the natural environment, these systems are essential to gain  
301 elementary mechanistic understanding of the functioning, the roles, and the evolution of marine  
302 holobionts.

### 303 Marine holobionts as drivers of **ecological processes**

304 Work on model systems has demonstrated that motile and macroscopic marine holobionts  
305 can act as dissemination vectors for geographically restricted microbial taxa. Pelagic mollusks or  
306 vertebrates are textbook examples of high dispersal capacity organisms (*e.g.* against currents and  
307 through stratified water layers). It has been estimated that fish and marine mammals may  
308 enhance the original dispersion rate of their microbiota by a factor of 200 to 200,000  
309 (Troussellier *et al.* 2017) and marine birds may even act as bio-vectors across ecosystem  
310 boundaries (Bouchard Marmen *et al.* 2017). This host-driven dispersal of microbes can include  
311 non-native or invasive species as well as pathogens (Troussellier *et al.* 2017).

312 A related ecological function of holobionts is their potential to sustain rare species. Hosts  
313 provide an environment that favors the growth of specific microbial communities distinct from  
314 the surrounding environment (including rare microbes). They may, for instance, provide a  
315 nutrient-rich niche in the otherwise nutrient-poor surroundings (Smriga *et al.* 2010; Webster *et*  
316 *al.* 2010; Burke, Thomas, *et al.* 2011; Chiarello *et al.* 2018).

317 Lastly, biological processes regulated by microbes are important drivers of global  
318 biogeochemical cycles (Falkowski *et al.* 2008; Madsen 2011; Anantharaman *et al.* 2016). In the  
319 open ocean, it is estimated that symbioses with the cyanobacterium UCYN-A contribute ~20% to  
320 total N<sub>2</sub> fixation (Thompson *et al.* 2012; Martínez-Pérez *et al.* 2016). In benthic systems, sponges  
321 and corals may support entire ecosystems *via* their involvement in nutrient cycling thanks to their  
322 microbial partners (Raina *et al.* 2009; Fiore *et al.* 2010; Cardini *et al.* 2015; Pita *et al.* 2018),  
323 functioning as sinks and sources of nutrients. In particular the “**sponge loop**” recycles dissolved  
324 organic matter and makes it available to higher trophic levels in the form of detritus (de Goeij *et*  
325 *al.* 2013; Rix *et al.* 2017). In coastal sediments, bivalves hosting methanogenic archaea have  
326 been shown to increase the benthic methane efflux by a factor of up to eight, potentially



327 accounting for 9.5% of total methane emissions from the Baltic Sea (Bonaglia *et al.* 2017). Such  
328 impressive metabolic versatility is accomplished because of the simultaneous occurrence of  
329 disparate biochemical machineries (*e.g.* aerobic and anaerobic pathways) in individual  
330 symbionts, providing new metabolic abilities to the holobiont, such as the synthesis of specific  
331 essential amino acids, photosynthesis, or chemosynthesis (Venn *et al.* 2008; Dubilier *et al.*  
332 2008). Furthermore, the interaction between host and microbiota can potentially extend the  
333 metabolic capabilities of a holobiont in a way that augments its resilience to environmental  
334 changes (Berkelmans and van Oppen 2006; Gilbert *et al.* 2010; Dittami *et al.* 2016; Shapira  
335 2016; Godoy *et al.* 2018), or allow it to cross biotope boundaries (*e.g.* Woyke 2006) and colonize  
336 extreme environments (Bang *et al.* 2018). Holobionts thus contribute to marine microbial  
337 diversity and possibly resilience in the context of global environmental changes (Troussellier *et al.*  
338 2017) and it is paramount to include the holobiont concept in predictive models that  
339 investigate the consequences of human impacts on the marine realm and its biogeochemical  
340 cycles.  
341

## 342 Challenges and opportunities in marine holobiont research

### 343 Marine holobiont assembly and regulation

344 Two critical challenges partially addressed by using model systems are 1) to decipher the  
345 factors determining holobiont composition; and 2) to elucidate the impacts and roles of the  
346 different partners in these complex systems over time. Some marine organisms such as bivalves  
347 transmit part of the microbiota maternally (Bright and Bulgheresi 2010; Funkhouser and  
348 Bordenstein 2013). In other marine holobionts, vertical transmission may be weak and  
349 inconsistent, whereas mixed **modes of transmission (vertical and horizontal)** or intermediate  
350 modes (pseudo-vertical, where horizontal acquisition frequently involves symbionts of parental  
351 origin) are more common (Björk *et al.* 2019). Identifying the factors shaping holobiont  
352 composition and understanding their evolution is highly relevant for marine organisms given that  
353 most marine hosts display a high specificity for their microbiota and even patterns of  
354 **phylosymbiosis** (Kazamia *et al.* 2016; Brooks *et al.* 2016; Pollock *et al.* 2018), despite a highly  
355 connected and microbe-rich environment.

356 During microbiota transmission (whether vertical or horizontal), "*selection*" (as opposed  
357 to "*drift*") is a key process in establishing or maintaining a holobiont microbial community that is  
358 distinct from the environment. The immune system of the host is one way of performing this  
359 selection in both marine and terrestrial holobionts, and perturbations can lead to **dysbiosis**, and  
360 eventually microbial infections (Selosse *et al.* 2014; de Lorgeril *et al.* 2018). Dysbiotic  
361 individuals frequently display higher variability in their microbial community composition than  
362 healthy individuals, an observation in line with the "**Anna Karenina principle**" (Zaneveld *et al.*  
363 2017), although there are exceptions to this rule (*e.g.* Marzinelli *et al.* 2015). A specific case of  
364 dysbiosis is the so-called "**Rasputin effect**" where benign endosymbionts opportunistically  
365 become detrimental to the host due to processes such as reduction in immune response under  
366 food deprivation, coinfections, or environmental pressure (Overstreet and Lotz 2016). Many  
367 diseases are now interpreted as the result of a microbial imbalance and the rise of opportunistic  
368 or polymicrobial infections upon host stress (Egan and Gardiner 2016). For instance in reef-

369 building corals, warming destabilizes cnidarian-dinoflagellate associations, and some beneficial  
370 *Symbiodiniacea* strains switch their physiology and sequester more resources for their own  
371 growth at the expense of the coral host, leading to coral bleaching and even death (Baker *et al.*  
372 2018).

373 Another way of selecting a holobiont microbial community is by chemically mediated  
374 **microbial gardening**. This concept has been demonstrated for land plants, where root exudates  
375 manipulate **microbiome** composition (Lebeis *et al.* 2015). In marine environments, the  
376 phylogenetic diversity of hosts and symbionts suggests both conserved and marine-specific  
377 chemical interactions, but studies are still in their infancy. For instance, seaweeds can chemically  
378 garden beneficial microbes, facilitating normal morphogenesis and increasing disease resistance  
379 (Kessler *et al.* 2018; Saha and Weinberger 2019), and seaweeds and corals structure their  
380 surface-associated microbiome by producing chemo-attractants and anti-bacterial compounds  
381 (Harder *et al.* 2012; Ochsenkühn *et al.* 2018). There are fewer examples of chemical gardening  
382 in unicellular hosts, but it seems highly likely that similar processes are in place (Gribben *et al.*  
383 2017; Cirri and Pohnert 2019).

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

394 Increasing our knowledge on the contribution of these processes to holobiont community  
395 assembly in marine systems is a key challenge, especially in the context of ongoing global  
396 change. Moreover, understanding how the community and functional structure of resident  
397 microbes are resilient to perturbations remains critical to predict and promote the health of their  
398 host and the ecosystem. Yet, this notion is still missing in most mathematical or formal models,  
399 or additional information on biological interactions would be required to make the former more  
400 accurate (Bell *et al.* 2018).

## 401 Integrating marine model systems with large-scale studies

402 By compiling a survey of the most important trends and challenges in the field of marine  
403 holobiont research (Figure 3), we identified two distinct opinion clusters: one focused on  
404 mechanistic understanding and work with model systems whereas another targets large-scale and  
405 heterogeneous data set analyses and predictive modeling. This illustrates that, on the one hand,  
406 the scientific community is interested in the establishment of models for the identification of  
407 specific molecular interactions between marine organisms at a given point in space and time, up  
408 to the point of synthesizing functional mutualistic communities *in vitro* (Kubo *et al.* 2013). On  
409 the other hand, another part of the community is moving towards global environmental sampling  
410 schemes such as the *TARA* Oceans expedition (Pesant *et al.* 2015) or the Ocean Sampling Day  
411 (Kopf *et al.* 2015), and towards long-term data series (e.g. Wiltshire *et al.* 2010; Harris 2010).  
412 What emerges as both lines of research progress is the understanding that small-scale functional

413 studies in the laboratory are inconsequential unless made applicable to ecologically-relevant  
414 systems. At the same time, large scale-studies remain mostly descriptive and bear little predictive  
415 power unless we understand the mechanisms driving the observed processes. We illustrate the  
416 importance of integrating both approaches in Figure 3, where the node related to potential  
417 applications was perceived as a central hub at the interface between mechanistic understanding  
418 and predictive modeling.

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

428 We advocate the implementation of comparable efforts in marine sciences through  
429 interdisciplinary research combining physiology, biochemistry, ecology, and computational  
430 modeling. A key factor will be the identification and development of tractable model systems for  
431 keystone holobionts that allow hypotheses generated by large-scale data sets to be tested in  
432 controlled experiments. Such approaches will enable the identification of organismal interaction  
433 patterns within holobionts and nested ecosystems. In addition to answering fundamental  
434 questions, they will help address the ecological, societal, and ethical issues that arise from  
435 attempting to actively manipulate holobionts (*e.g.* in aquaculture, conservation) in order to  
436 enhance their resilience and protect them from the impacts of global change (Llewellyn *et al.*  
437 2014).

## 438 Emerging methodologies to approach the complexity of holobiont 439 partnerships

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

445 A giant technological stride has been the explosion of affordable ‘-omics’ technologies  
446 allowing molecular ecologists to move from metabarcoding (*i.e.* sequencing of a taxonomic  
447 marker) to metagenomics or single-cell genomics, metatranscriptomics, and metaproteomics,  
448 thus advancing our research from phylogenetic to functional analyses of the holobiont (Bowers  
449 *et al.* 2017; Meng *et al.* 2018; Figure 4). These approaches are equally useful in marine and in  
450 terrestrial environments, but the scarcity of well-studied lineages in the former makes the  
451 generation of good annotations and reference databases challenging for marine biologists.  
452 Metaproteomics combined with stable isotope fingerprinting can help study the metabolism of  
453 single species within the holobiont (Kleiner *et al.* 2018). In parallel, meta-metabolomics  
454 approaches have advanced over the last decades, and can be used to unravel the chemical  
455 interactions between partners. One limitation particularly relevant to marine systems is that many  
456 compounds are often not referenced in the mostly terrestrial-based databases, although recent

457 technological advances such as molecular networking and meta-mass shift chemical profiling to  
458 identify relatives of known molecules may help to overcome this challenge (Hartmann *et al.*  
459 2017).

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

478 One consequence of the development of such new methods is the feedback they provide  
479 to improve existing models or to develop entirely new ones, *e.g.* by conceptualizing holobionts  
480 as the combination of the interactions between the host and its microbiota (Skillings 2016; Berry  
481 and Loy 2018), or by redefining boundaries between the holobiont and its environment (Zengler  
482 and Pálsson 2012). Such models may incorporate metabolic complementarity between different  
483 components of the holobiont (Dittami *et al.* 2014; Bordron *et al.* 2016), simulate microbial  
484 communities starting from different cohorts of randomly generated microbes for comparison  
485 with actual metatranscriptomics and/or metagenomics data (Coles *et al.* 2017), or even employ  
486 machine learning techniques to predict host-associated microbial communities (Moitinho-Silva  
487 *et al.* 2017).

488 A side-effect of these recent developments has been to move holobiont research away  
489 from laboratory culture-based experiments. We argue that maintaining cultivation efforts to  
490 capture the maximum holobiont biodiversity possible remains essential to experimentally test  
491 hypotheses and investigate physiological mechanisms. A striking example of the importance of  
492 laboratory experimentation is the way germ-free mice re-inoculated with cultivated bacteria (the  
493 so-called **gnotobiotic** mice) have contributed to the understanding of interactions within the  
494 holobiont in animal health, physiology, and behavior (*e.g.* Neufeld *et al.* 2011; Faith *et al.* 2014;  
495 Selosse *et al.* 2014). Innovations in cultivation techniques for axenic (or germ-free) hosts (*e.g.*  
496 Spoerner *et al.* 2012) or in microbial cultivation such as microfluidic systems (*e.g.* Pan *et al.*  
497 2011) and cultivation chips (Nichols *et al.* 2010) may provide a way to obtain pure cultures. Yet,  
498 bringing individual components of holobionts into cultivation can still be a daunting challenge  
499 due to the strong interdependencies between organisms as well as the existence of yet unknown  
500 metabolic processes that may have specific requirements. In this context, single-cell ‘-omics’  
501 analyses can provide critical information on some of the growth requirements of the organisms,  
502 and complement approaches of high-throughput culturing (Gutleben *et al.* 2018). Established

503 cultures can then be developed into model systems, *e.g.* by genome sequencing and the  
504 development of genetic tools, in order to move towards mechanistic understanding and  
505 experimental testing of hypothetical processes within the holobiont derived from environmental  
506 meta'-omics' approaches. A few such model systems have already been mentioned above, but '-  
507 omics' techniques have the potential to broaden the range of available models, enabling a better  
508 understanding of the functioning of marine holobionts and their interactions in marine  
509 environments (Wichard and Beemelmanns 2018).

## 510 **Ecosystem services and holobionts in natural and managed systems**

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

521 One form of intervention could be to promote positive changes of host-associated  
522 microbiota, in ways analogous to the use of pre- and/or probiotics in humans (Singh *et al.* 2013)  
523 or inoculation of beneficial microbes in plant farming (Berruti *et al.* 2015; van der Heijden *et al.*  
524 2015). In macroalgae, beneficial bacteria identified from healthy seaweed holobionts could be  
525 used as **biological control** agents and applied to diseased plantlets in order to suppress the  
526 growth of detrimental ones and/or to prevent disease outbreaks in aquaculture settings. In  
527 addition to bacteria, these macroalgae frequently host endophytic fungi that may have protective  
528 functions for the algae (Porrás-Alfaro and Bayman 2011; Vallet *et al.* 2018). Host-associated  
529 microbiota could also be manipulated to shape key phenotypes in cultured marine organisms. For  
530 example, specific bacteria associated with microalgae may enhance algal growth (Amin *et al.*  
531 2009; Kazamia *et al.* 2012; Le Chevanton *et al.* 2013), increase lipid content (Cho *et al.* 2015),  
532 and participate in the bioprocessing of algal biomass (Lenneman *et al.* 2014). More recently, the  
533 active modification of the coral microbiota has even been advocated as a means to boost the  
534 resilience of the holobiont to climate change (van Oppen *et al.* 2015; Peixoto *et al.* 2017), an  
535 approach which would, however, bear a high risk of unanticipated and unintended ecological  
536 consequences.

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

544 Nevertheless, when considering their biotechnological potential, it should also be noted  
545 that marine microbiota are likely vulnerable to anthropogenic influences and that their deliberate  
546 engineering, introduction from exotic regions, or inadvertent perturbations may have profound,

547 and yet entirely unknown, consequences for marine ecosystems. Terrestrial environments  
548 provide numerous examples of unwanted plant expansions or ecosystem perturbations linked to  
549 microbiota (*e.g.* Dickie *et al.* 2017), and cases where holobionts manipulated by human resulted  
550 in pests (*e.g.* Clay and Holah 1999) call for a cautious and ecologically-informed evaluation of  
551 holobiont-based technologies in marine systems.

## 552 Conclusions

553 Marine ecosystems represent highly connected reservoirs of largely unexplored  
554 biodiversity. They are of critical importance to feed the ever-growing world population,  
555 constitute significant players in global biogeochemical cycles but are also threatened by human  
556 activities and global change. In order to unravel some of the basic principles of life and its  
557 evolution, and to protect and sustainably exploit marine natural resources, it is paramount to  
558 consider the complex biotic interactions that shape the marine communities and their  
559 environment. The scope of these interactions ranges from simple molecular signals between two  
560 partners, via complex assemblies of eukaryotes, prokaryotes, and viruses with one or several  
561 hosts, to entire ecosystems. Accordingly, current key questions in marine holobiont research  
562 cover a wide range of topics: What are the exchanges that occur between different partners of the  
563 holobiont, and what are the cues and signals driving these exchanges? What are the relevant units  
564 of selection in marine holobionts? How do holobiont systems and the interactions within them  
565 change over time and in different conditions? How do such changes impact ecological  
566 processes? How can this knowledge be applied to our benefit and where do we need to draw  
567 limits? Identifying and consolidating key model systems while adapting emerging “-omics”,  
568 imaging, and culturing technologies to them will be critical to the development of “holobiont-  
569 aware” ecosystem models.

570 We believe that the concept of holobionts will be most useful and heuristic if used with a  
571 degree of malleability. It not only represents the fundamental understanding that all living  
572 organisms have intimate connections with their immediate neighbors, which may impact all  
573 aspects of their biology, but also enables us to define units of interacting organisms that are most  
574 suitable to answer specific scientific, societal, and economic questions. The consideration of the  
575 holobiont concept marks a paradigm shift in biological and environmental sciences, but only if  
576 scientists work together as an (inter)active and transdisciplinary community bringing together  
577 holistic and mechanistic views. This will result in tangible outcomes including a better  
578 understanding of evolutionary and adaptive processes, improved modeling of habitats and  
579 biogeochemical cycles, as well as application of the holobiont concept in aquaculture and  
580 ecosystem management projects.

581

## 582 Conflict of interest

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

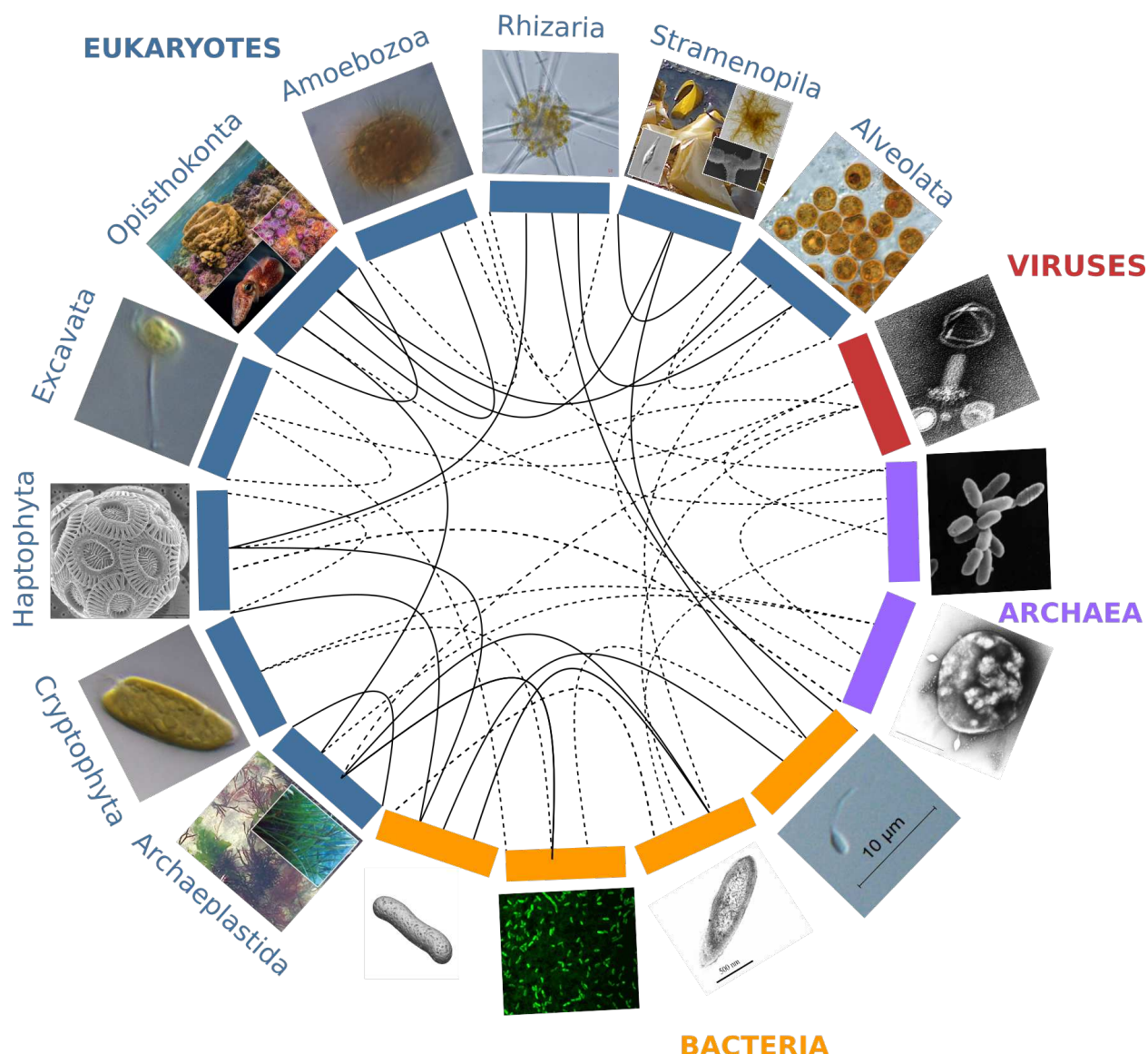
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## 608 Figures

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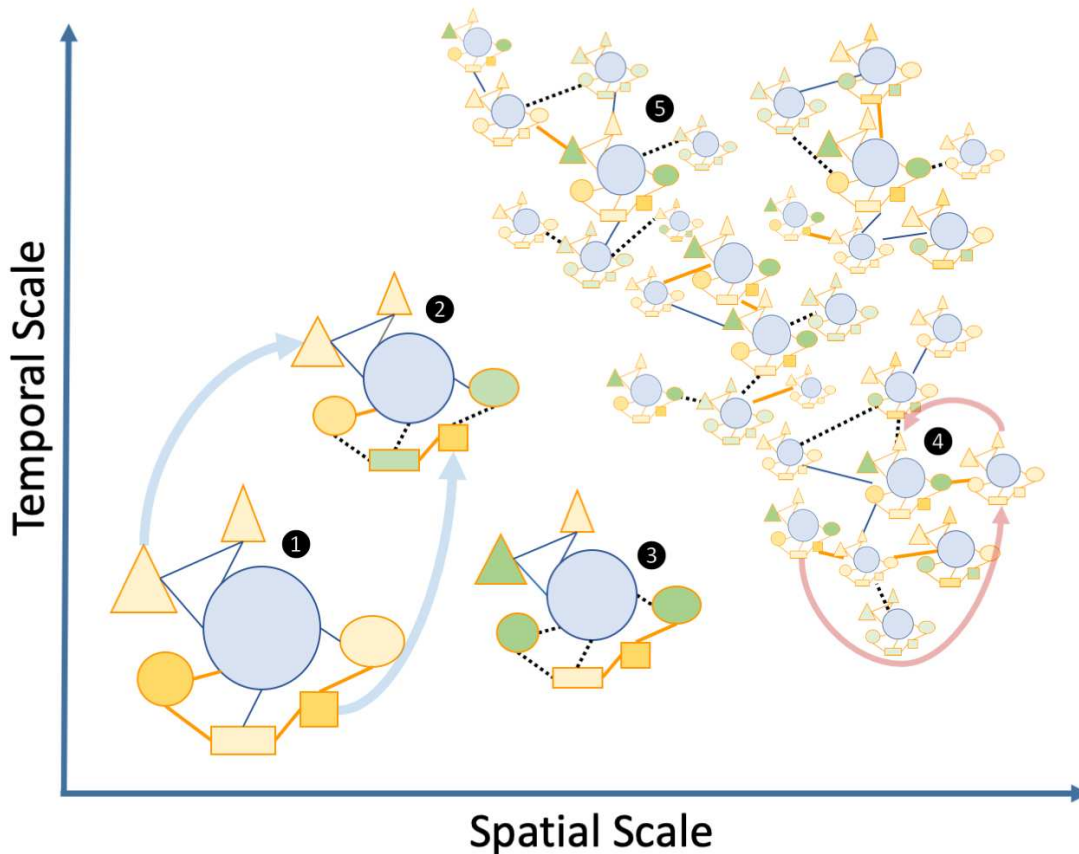


610 **Figure 1.** Partners forming marine holobionts are widespread across the tree of life including all  
 611 kingdoms (eukaryotes, bacteria, archaea, viruses), and represent a large diversity of potential  
 612 models for exploring complex biotic interactions across lineages. Plain lines correspond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

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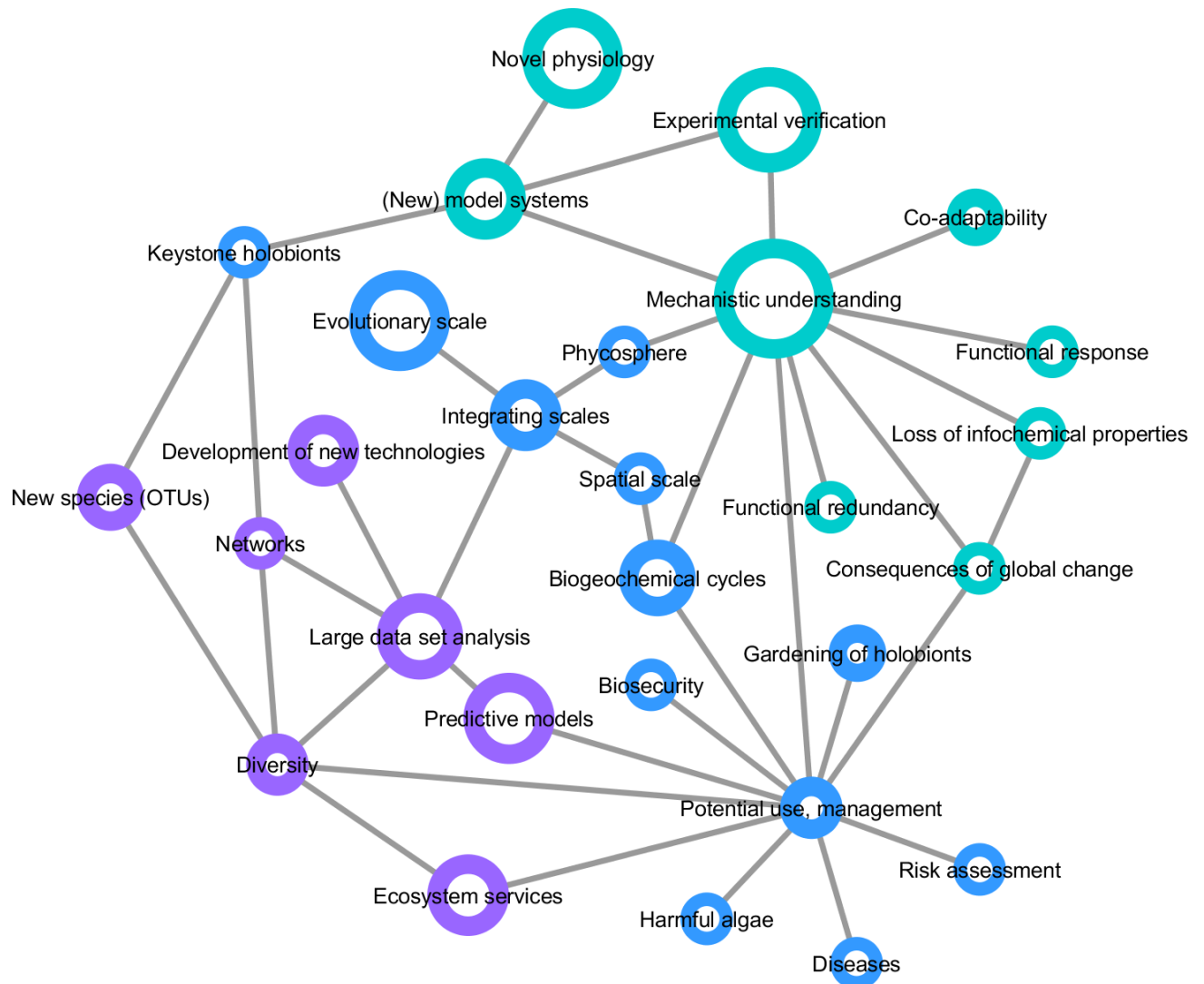


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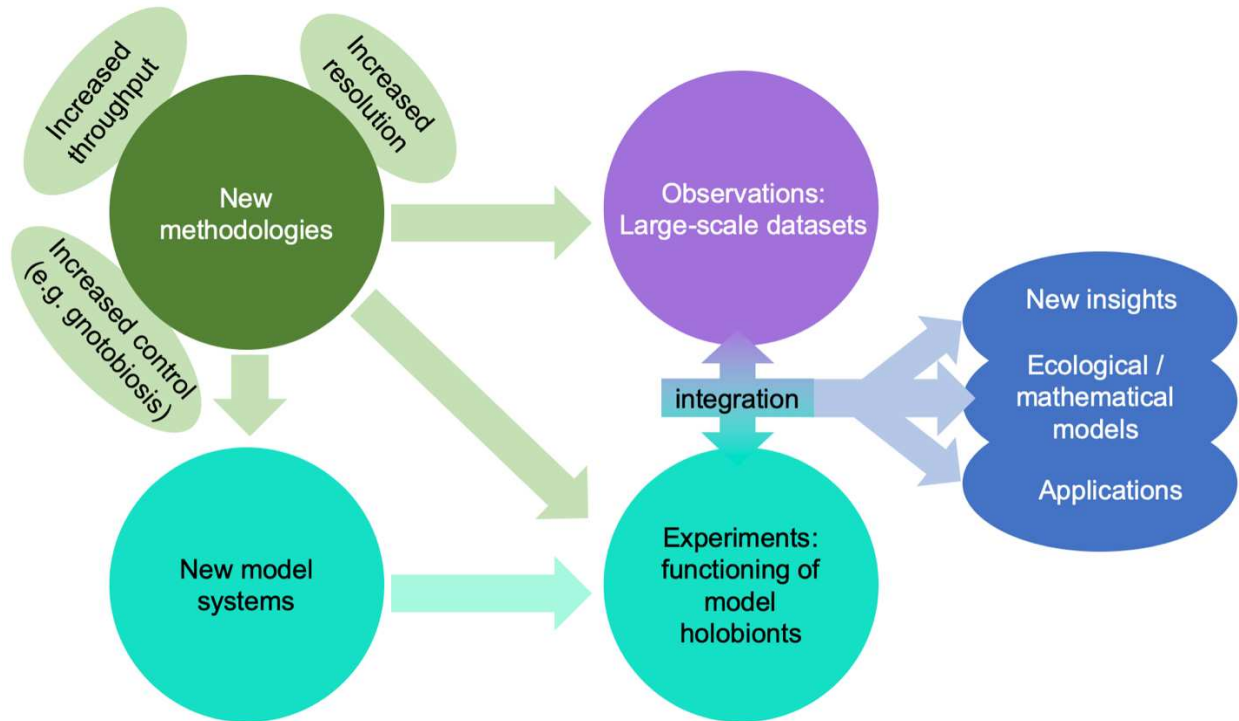
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**Figure 2.** Schematic view of the “Russian Doll” complexity and dynamics of holobionts, according to diverse spatiotemporal scales. The host (blue circles), and associated microbes (all other shapes) including bacteria and eukaryotes that may be 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 vertical transmissions 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.



644  
645

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



654  
655 **Figure 4:** Impact of emerging methodologies (green) on the main challenges in marine holobiont  
656 research identified in this paper.

## 657 References

- 658 **Alam F, Kim TY, Kim SY, et al. 2015.** Effect of molybdenum on nodulation, plant yield and nitrogen uptake in  
659 hairy vetch (*Vicia villosa* Roth). *Soil Science and Plant Nutrition* **61**: 664–675.
- 660 **Amin SA, Green DH, Hart MC, Küpper FC, Sunda WG, Carrano CJ. 2009.** Photolysis of iron-siderophore  
661 chelates promotes bacterial-algal mutualism. *Proceedings of the National Academy of Sciences of the United States*  
662 *of America* **106**: 17071–6.
- 663 **Amin SA, Parker MS, Armbrust EV. 2012.** Interactions between diatoms and bacteria. *Microbiology and*  
664 *Molecular Biology Reviews* **76**: 667–84.
- 665 **Anantharaman K, Brown CT, Hug LA, et al. 2016.** Thousands of microbial genomes shed light on interconnected  
666 biogeochemical processes in an aquifer system. *Nature Communications* **7**: 13219.
- 667 **Arboleda E, Hartenstein V, Martinez P, et al. 2018.** An emerging system to study photosymbiosis, brain  
668 regeneration, chronobiology, and behavior: the marine acoel *Symsagittifera roscoffensis*. *BioEssays* **40**: 1800107.
- 669 **Archibald JM. 2015.** Endosymbiosis and eukaryotic cell evolution. *Current Biology* **25**: R911–R921.
- 670 **Baker DM, Freeman CJ, Wong JCY, Fogel ML, Knowlton N. 2018.** Climate change promotes parasitism in a  
671 coral symbiosis. *ISME Journal* **12**: 921–930.
- 672 **Bang C, Dagan T, Deines P, et al. 2018.** Metaorganisms in extreme environments: do microbes play a role in  
673 organismal adaptation? *Zoology* **127**: 1–19.
- 674 **Baumgarten S, Simakov O, Esherick LY, et al. 2015.** The genome of *Aiptasia*, a sea anemone model for coral  
675 symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 11893–8.
- 676 **Bell JJ, Rovellini A, Davy SK, et al. 2018.** Climate change alterations to ecosystem dominance: how might sponge-  
677 dominated reefs function? *Ecology* **99**: 1920–1931.
- 678 **Bennett AF, Haslem A, Cheal DC, et al. 2009.** Ecological processes: a key element in strategies for nature  
679 conservation. *Ecological Management & Restoration* **10**: 192–199.
- 680 **Berkelmans R, van Oppen MJH. 2006.** The role of zooxanthellae in the thermal tolerance of corals: a “nugget of  
681 hope” for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences* **273**:  
682 2305–12.

- 683 **Berruti A, Lumini E, Balestrini R, Bianciotto V. 2015.** Arbuscular mycorrhizal fungi as natural biofertilizers:  
684 let's benefit from past successes. *Frontiers in Microbiology* **6**: 1559.
- 685 **Berry D, Loy A. 2018.** Stable-isotope probing of human and animal microbiome function. *Trends in Microbiology*  
686 **26**: 999–1007.
- 687 **Björk JR, Díez-Vives C, Astudillo-García C, Archie EA, Montoya JM. 2019.** Vertical transmission of sponge  
688 microbiota is inconsistent and unfaithful. *Nature Ecology & Evolution* **3**: 1172–1183.
- 689 **Bonaglia S, Brüchert V, Callac N, Vicenzi A, Chi Fru E, Nascimento FJA. 2017.** Methane fluxes from coastal  
690 sediments are enhanced by macrofauna. *Scientific Reports* **7**: 13145.
- 691 **Bordron P, Latorre M, Cortés M-P, et al. 2016.** Putative bacterial interactions from metagenomic knowledge with  
692 an integrative systems ecology approach. *MicrobiologyOpen* **5**: 106–117.
- 693 **Bouchard Marmen M, Kenchington E, Ardyna M, Archambault P. 2017.** Influence of seabird colonies and  
694 other environmental variables on benthic community structure, Lancaster Sound region, Canadian Arctic. *Journal of*  
695 *Marine Systems* **167**: 105–117.
- 696 **Bowers RM, Doud DFR, Woyke T. 2017.** Analysis of single-cell genome sequences of bacteria and archaea.  
697 *Emerging Topics in Life Sciences* **1**: 249–255.
- 698 **Bright M, Bulgheresi S. 2010.** A complex journey: transmission of microbial symbionts. *Nature Reviews.*  
699 *Microbiology* **8**: 218–30.
- 700 **Brooks AW, Kohl KD, Brucker RM, van Opstal EJ, Bordenstein SR. 2016.** Phyllosymbiosis: relationships and  
701 functional effects of microbial communities across host evolutionary history. *PLOS Biology* **14**: e2000225.
- 702 **Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P. 2013.** Structure and  
703 functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* **64**: 807–38.
- 704 **Burke C, Steinberg P, Rusch D, Kjelleberg S, Thomas T. 2011.** Bacterial community assembly based on  
705 functional genes rather than species. *Proceedings of the National Academy of Sciences of the United States of*  
706 *America* **108**: 14288–93.
- 707 **Burke C, Thomas T, Lewis M, Steinberg P, Kjelleberg S. 2011.** Composition, uniqueness and variability of the  
708 epiphytic bacterial community of the green alga *Ulva australis*. *ISME journal* **5**: 590–600.
- 709 **Cardini U, Bednarz VN, Naumann MS, et al. 2015.** Functional significance of dinitrogen fixation in sustaining  
710 coral productivity under oligotrophic conditions. *Proceedings of the Royal Society B: Biological Sciences* **282**:  
711 20152257.
- 712 **Le Chevanton M, Garnier M, Bougaran G, et al. 2013.** Screening and selection of growth-promoting bacteria for  
713 *Dunaliella* cultures. *Algal Research* **2**: 212–222.
- 714 **Chiarello M, Auguet J-C, Bettarel Y, et al. 2018.** Skin microbiome of coral reef fish is highly variable and driven  
715 by host phylogeny and diet. *Microbiome* **6**: 147.
- 716 **Cho D-H, Ramanan R, Heo J, et al. 2015.** Enhancing microalgal biomass productivity by engineering a  
717 microalgal–bacterial community. *Bioresource Technology* **175**: 578–585.
- 718 **Cirri E, Pohnert G. 2019.** Algae–bacteria interactions that balance the planktonic microbiome. *New Phytologist*  
719 **223**: 100–106.
- 720 **Clay K, Holah J. 1999.** Fungal endophyte symbiosis and plant diversity in successional fields. *Science* **285**: 1742–  
721 1744.
- 722 **Coles VJ, Stukel MR, Brooks MT, et al. 2017.** Ocean biogeochemistry modeled with emergent trait-based  
723 genomics. *Science* **358**: 1149–1154.
- 724 **Colin S, Coelho LP, Sunagawa S, et al. 2017.** Quantitative 3D-imaging for cell biology and ecology of  
725 environmental microbial eukaryotes. *eLife* **6**: e26066.
- 726 **Cooper MB, Kazamia E, Helliwell KE, et al. 2019.** Cross-exchange of B-vitamins underpins a mutualistic  
727 interaction between *Ostreococcus tauri* and *Dinoroseobacter shibae*. *ISME Journal* **13**: 334–345.
- 728 **Costanza R, de Groot R, Sutton P, et al. 2014.** Changes in the global value of ecosystem services. *Global*  
729 *Environmental Change* **26**: 152–158.
- 730 **Decelle J, Colin S, Foster RA. 2015.** Marine protists In: Ohtsuka S, Suzaki T, Horiguchi T, Suzuki N, Not F, eds.  
731 *Marine Protists*. Tokyo: Springer Japan, 465–500.
- 732 **Decelle J, Stryhanyuk H, Gallet B, et al. 2019.** Algal remodeling in a ubiquitous planktonic photosymbiosis.  
733 *Current Biology* in press.
- 734 **Dicke M, Sabelis MW. 1988.** Infochemical terminology: based on cost-benefit analysis rather than origin of  
735 compounds? *Functional Ecology* **2**: 131.
- 736 **Dickie IA, Bufford JL, Cobb RC, et al. 2017.** The emerging science of linked plant–fungal invasions. *New*  
737 *Phytologist* **215**: 1314–1332.
- 738 **Dittami SM, Duboscq-Bidot LL, Perennou M, et al. 2016.** Host–microbe interactions as a driver of acclimation to

- 739 salinity gradients in brown algal cultures. *ISME Journal* **10**: 51–63.
- 740 **Dittami SM, Eveillard D, Tonon T. 2014.** A metabolic approach to study algal–bacterial interactions in changing  
741 environments. *Molecular Ecology* **23**: 1656–1660.
- 742 **Douglas AE, Werren JH. 2016.** Holes in the hologenome: why host-microbe symbioses are not holobionts. *mBio* **7**:  
743 e02099.
- 744 **Dubilier N, Bergin C, Lott C. 2008.** Symbiotic diversity in marine animals: the art of harnessing chemosynthesis.  
745 *Nature Reviews Microbiology* **6**: 725–740.
- 746 **Duperron S, Halary S, Lorion J, Sibuet M, Gaill F. 2008.** Unexpected co-occurrence of six bacterial symbionts in  
747 the gills of the cold seep mussel *Idas* sp. (Bivalvia: Mytilidae). *Environmental Microbiology* **10**: 433–445.
- 748 **Egan S, Gardiner M. 2016.** Microbial dysbiosis: rethinking disease in marine ecosystems. *Frontiers in*  
749 *Microbiology* **7**: 991.
- 750 **Epihov DZ, Batterman SA, Hedin LO, Leake JR, Smith LM, Beerling DJ. 2017.** N<sub>2</sub>-fixing tropical legume  
751 evolution: a contributor to enhanced weathering through the Cenozoic? *Proceedings of the Royal Society B*:  
752 *Biological Sciences* **284**: 20170370.
- 753 **Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JJ. 2014.** Identifying gut microbe-host phenotype  
754 relationships using combinatorial communities in gnotobiotic mice. *Science Translational Medicine* **6**: 220ra11.
- 755 **Falkowski PG, Fenchel T, Delong EF. 2008.** The microbial engines that drive Earth’s biogeochemical cycles.  
756 *Science* **320**: 1034–1039.
- 757 **Faure D, Simon J-C, Heulin T. 2018.** Holobiont: a conceptual framework to explore the eco-evolutionary and  
758 functional implications of host-microbiota interactions in all ecosystems. *New Phytologist* **218**: 1321–1324.
- 759 **Fiore CL, Jarett JK, Olson ND, Lesser MP. 2010.** Nitrogen fixation and nitrogen transformations in marine  
760 symbioses. *Trends in Microbiology* **18**: 455–463.
- 761 **Funkhouser LJ, Bordenstein SR. 2013.** Mom knows best: the universality of maternal microbial transmission.  
762 *PLoS Biology* **11**: e1001631.
- 763 **Gilbert SF, McDonald E, Boyle N, et al. 2010.** Symbiosis as a source of selectable epigenetic variation: taking the  
764 heat for the big guy. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 671–678.
- 765 **Godoy O, Bartomeus I, Rohr RP, Saavedra S. 2018.** Towards the integration of niche and network theories.  
766 *Trends in Ecology & Evolution* **33**: 287–300.
- 767 **de Goeij JM, van Oevelen D, Vermeij MJA, et al. 2013.** Surviving in a marine desert: the sponge loop retains  
768 resources within coral reefs. *Science* **342**: 108–110.
- 769 **Gregory AC, Zayed AA, Conceição-Neto N, et al. 2019.** Marine DNA viral macro- and microdiversity from pole  
770 to pole. *Cell* **177**: 1109–1123.e14.
- 771 **Gribben PE, Nielsen S, Seymour JR, Bradley DJ, West MN, Thomas T. 2017.** Microbial communities in marine  
772 sediments modify success of an invasive macrophyte. *Scientific Reports* **7**: 9845.
- 773 **Guimarães PR, Pires MM, Jordano P, Bascompte J, Thompson JN. 2017.** Indirect effects drive coevolution in  
774 mutualistic networks. *Nature* **550**: 511–514.
- 775 **Gutleben J, Chaib De Mares M, van Elsas JD, Smidt H, Overmann J, Sippkema D. 2018.** The multi-omics  
776 promise in context: from sequence to microbial isolate. *Critical Reviews in Microbiology* **44**: 212–229.
- 777 **Hale MG, Lindsey DL, Hameed KM. 1973.** Gnotobiotic culture of plants and related research. *The Botanical*  
778 *Review* **39**: 261–273.
- 779 **Harder T, Campbell AH, Egan S, Steinberg PD. 2012.** Chemical mediation of ternary interactions between  
780 marine holobionts and their environment as exemplified by the red alga *Delisea pulchra*. *Journal of Chemical*  
781 *Ecology* **38**: 442–450.
- 782 **Harris R. 2010.** The L4 time-series: the first 20 years. *Journal of Plankton Research* **32**: 577–583.
- 783 **Hartmann AC, Petras D, Quinn RA, et al. 2017.** Meta-mass shift chemical profiling of metabolomes from coral  
784 reefs. *Proceedings of the National Academy of Sciences of the United States of America* **114**: 11685–11690.
- 785 **van der Heijden MGA, Martin FM, Selosse M-A, Sanders IR. 2015.** Mycorrhizal ecology and evolution: the  
786 past, the present, and the future. *New Phytologist* **205**: 1406–1423.
- 787 **Hoitink H, Boehm M. 1999.** Biocontrol within the context of soil microbial communities : a substrate-dependent  
788 phenomenon. *Annual Review of Phytopathology* **37**: 427–446.
- 789 **Kavimandan SK. 1985.** Root nodule bacteria to improve yield of wheat (*Triticum aestivum* L.). *Plant and Soil* **86**:  
790 141–144.
- 791 **Kazamia E, Aldridge DC, Smith AG. 2012.** Synthetic ecology – A way forward for sustainable algal biofuel  
792 production? *Journal of Biotechnology* **162**: 163–169.
- 793 **Kazamia E, Helliwell KE, Purton S, Smith AG. 2016.** How mutualisms arise in phytoplankton communities:  
794 building eco-evolutionary principles for aquatic microbes (G Fussmann, Ed.). *Ecology Letters* **19**: 810–822.

- 795 **Kelty MO, Cook CB. 1976.** Survival during starvation of symbiotic, aposymbiotic, and non-symbiotic *Hydra* In:  
796 *Coelenterate Ecology and Behavior*. Boston, MA: Springer, 409–414.
- 797 **Kessler RW, Weiss A, Kuegler S, Hermes C, Wichard T. 2018.** Macroalgal-bacterial interactions: Role of  
798 dimethylsulfoniopropionate in microbial gardening by *Ulva* (Chlorophyta). *Molecular Ecology* **27**: 1808–1819.
- 799 **Kinlan BP, Gaines SD. 2003.** Propagule dispersal in marine and terrestrial environments: a community perspective.  
800 *Ecology* **84**: 2007–2020.
- 801 **Kleiner M, Dong X, Hinzke T, et al. 2018.** Metaproteomics method to determine carbon sources and assimilation  
802 pathways of species in microbial communities. *Proceedings of the National Academy of Sciences of the United*  
803 *States of America* **115**: E5576–E5584.
- 804 **Klinger D, Naylor R. 2012.** Searching for solutions in aquaculture: charting a sustainable course. *Annual Review of*  
805 *Environment and Resources* **37**: 247–276.
- 806 **Kopf A, Bicak M, Kottmann R, et al. 2015.** The ocean sampling day consortium. *GigaScience* **4**: 27.
- 807 **Kubo I, Hosoda K, Suzuki S, et al. 2013.** Construction of bacteria–eukaryote synthetic mutualism. *Biosystems* **113**:  
808 66–71.
- 809 **Lebeis SL, Paredes SH, Lundberg DS, et al. 2015.** Salicylic acid modulates colonization of the root microbiome  
810 by specific bacterial taxa. *Science* **349**: 860–864.
- 811 **Lehnert EM, Burriesci MS, Pringle JR. 2012.** Developing the anemone *Aiptasia* as a tractable model for  
812 cnidarian-dinoflagellate symbiosis: the transcriptome of aposymbiotic *A. pallida*. *BMC Genomics* **13**: 271.
- 813 **Lenneman EM, Wang P, Barney BM. 2014.** Potential application of algicidal bacteria for improved lipid recovery  
814 with specific algae. *FEMS Microbiology Letters* **354**: 102–110.
- 815 **Leys SP, Kahn AS, Fang JKH, Kutti T, Bannister RJ. 2018.** Phagocytosis of microbial symbionts balances the  
816 carbon and nitrogen budget for the deep-water boreal sponge *Geodia barretti*. *Limnology and Oceanography* **63**:  
817 187–202.
- 818 **Llewellyn MS, Boutin S, Hoseinifar SH, Derome N. 2014.** Teleost microbiomes: the state of the art in their  
819 characterization, manipulation and importance in aquaculture and fisheries. *Frontiers in Microbiology* **5**: 207.
- 820 **Loh J, Pierson EA, Pierson LS, Stacey G, Chatterjee A. 2002.** Quorum sensing in plant-associated bacteria.  
821 *Current Opinion in Plant Biology* **5**: 285–90.
- 822 **de Lorgeril J, Lucasson A, Petton B, et al. 2018.** Immune-suppression by OsHV-1 viral infection causes fatal  
823 bacteraemia in Pacific oysters. *Nature Communications* **9**: 4215.
- 824 **Madsen EL. 2011.** Microorganisms and their roles in fundamental biogeochemical cycles. *Current Opinion in*  
825 *Biotechnology* **22**: 456–464.
- 826 **Marchesi JR, Ravel J. 2015.** The vocabulary of microbiome research: a proposal. *Microbiome* **3**: 31.
- 827 **Margulis L, Fester R. 1991.** *Symbiosis as a Source of Evolutionary Innovation: Speciation and Morphogenesis*.  
828 MIT Press.
- 829 **Martin-Platero AM, Cleary B, Kauffman K, et al. 2018.** High resolution time series reveals cohesive but short-  
830 lived communities in coastal plankton. *Nature Communications* **9**: 266.
- 831 **Martin W, Baross J, Kelley D, Russell MJ. 2008.** Hydrothermal vents and the origin of life. *Nature reviews*.  
832 *Microbiology* **6**: 805–14.
- 833 **Martínez-Pérez C, Mohr W, Löscher CR, et al. 2016.** The small unicellular diazotrophic symbiont, UCYN-A, is a  
834 key player in the marine nitrogen cycle. *Nature Microbiology* **1**: 16163.
- 835 **Marzinelli EM, Campbell AH, Zozaya Valdes E, et al. 2015.** Continental-scale variation in seaweed host-  
836 associated bacterial communities is a function of host condition, not geography. *Environmental Microbiology* **17**:  
837 4078–4088.
- 838 **Marzinelli EM, Qiu Z, Dafforn KA, Johnston EL, Steinberg PD, Mayer-Pinto M. 2018.** Coastal urbanisation  
839 affects microbial communities on a dominant marine holobiont. *Biofilms and Microbiomes* **4**: 1.
- 840 **McFall-Ngai MJ. 2014.** The importance of microbes in animal development: lessons from the squid-*Vibrio*  
841 symbiosis. *Annual Review of Microbiology* **68**: 177–194.
- 842 **McFall-Ngai M, Hadfield MG, Bosch TCG, et al. 2013.** Animals in a bacterial world, a new imperative for the life  
843 sciences. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 3229–3236.
- 844 **Meng A, Marchet C, Corre E, et al. 2018.** A de novo approach to disentangle partner identity and function in  
845 holobiont systems. *Microbiome* **6**: 105.
- 846 **Mereschkowski C. 1905.** Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Biologisches*  
847 *Centralblatt* **25**: 593–604.
- 848 **Middelboe M, Brussaard CPD. 2017.** Marine viruses: key players in marine ecosystems. *Viruses* **9**: 302.
- 849 **Millennium Ecosystem Assessment Panel T. 2005.** *Ecosystems and human well-being: synthesis / Millennium*  
850 *Ecosystem Assessment*. Washington, USA: Island press.

- 851 **Mitra A, Flynn KJ, Burkholder JM, et al. 2013.** The role of mixotrophic protists in the biological carbon pump.  
852 *Biogeosciences Discussions* **10**: 13535–13562.
- 853 **Moeller AH, Caro-Quintero A, Mjungu D, et al. 2016.** Cospeciation of gut microbiota with hominids. *Science*  
854 **353**: 380–2.
- 855 **Moitinho-Silva L, Steinert G, Nielsen S, et al. 2017.** Predicting the HMA-LMA status in marine sponges by  
856 machine learning. *Frontiers in Microbiology* **8**: 752.
- 857 **Moran NA, Sloan DB. 2015.** The hologenome concept: helpful or hollow? *PLOS Biology* **13**: e1002311.
- 858 **Musat N, Musat F, Weber PK, Pett-Ridge J. 2016.** Tracking microbial interactions with NanoSIMS. *Current*  
859 *Opinion in Biotechnology* **41**: 114–121.
- 860 **Nardon P, Charles H. 2001.** Morphological Aspects of Symbiosis In: *Symbiosis*. Dordrecht: Kluwer Academic  
861 Publishers, 13–44.
- 862 **Nemergut DR, Schmidt SK, Fukami T, et al. 2013.** Patterns and processes of microbial community assembly.  
863 *Microbiology and Molecular Biology Reviews* **77**: 342–56.
- 864 **Neufeld KM, Kang N, Bienenstock J, Foster JA. 2011.** Reduced anxiety-like behavior and central neurochemical  
865 change in germ-free mice. *Neurogastroenterology and Motility* **23**: 255–64, e119.
- 866 **Nichols D, Cahoon N, Trakhtenberg EM, et al. 2010.** Use of ichip for high-throughput in situ cultivation of  
867 “uncultivable” microbial species. *Applied and Environmental Microbiology* **76**: 2445–50.
- 868 **Not F, Probert I, Gerikas Ribeiro C, et al. 2016.** Photosymbiosis in marine pelagic environments In: *The Marine*  
869 *Microbiome*. Cham: Springer International Publishing, 305–332.
- 870 **O’Malley MA. 2017.** From endosymbiosis to holobionts: Evaluating a conceptual legacy. *Journal of Theoretical*  
871 *Biology* **434**: 34–41.
- 872 **Ochsenkühn MA, Schmitt-Kopplin P, Harir M, Amin SA. 2018.** Coral metabolite gradients affect microbial  
873 community structures and act as a disease cue. *Communications Biology* **1**: 184.
- 874 **Ohdera AH, Abrams MJ, Ames CL, et al. 2018.** Upside-down but headed in the right direction: review of the  
875 highly versatile *Cassiopea xamachana* system. *Frontiers in Ecology and Evolution* **6**: 35.
- 876 **van Oppen MJH, Gates RD, Blackall LL, et al. 2017.** Shifting paradigms in restoration of the world’s coral reefs.  
877 *Global Change Biology* **23**: 3437–3448.
- 878 **van Oppen MJH, Oliver JK, Putnam HM, Gates RD. 2015.** Building coral reef resilience through assisted  
879 evolution. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 2307–13.
- 880 **Overstreet RM, Lotz JM. 2016.** Host–symbiont relationships: understanding the change from guest to pest In:  
881 Hurst CJ, ed. *The Rasputin Effect: When Commensals and Symbionts Become Parasitic*. Springer, Cham, 27–64.
- 882 **Pan J, Stephenson AL, Kazamia E, et al. 2011.** Quantitative tracking of the growth of individual algal cells in  
883 microdroplet compartments. *Integrative Biology* **3**: 1043.
- 884 **Peixoto RS, Rosado PM, Leite DC de A, Rosado AS, Bourne DG. 2017.** Beneficial microorganisms for corals  
885 (BMC): proposed mechanisms for coral health and resilience. *Frontiers in Microbiology* **8**: 341.
- 886 **Pesant S, Not F, Picheral M, et al. 2015.** Open science resources for the discovery and analysis of Tara Oceans  
887 data. *Scientific Data* **2**: 150023.
- 888 **Petersen JM, Kemper A, Gruber-Vodicka H, et al. 2016.** Chemosynthetic symbionts of marine invertebrate  
889 animals are capable of nitrogen fixation. *Nature Microbiology* **2**: 16195.
- 890 **Pita L, Rix L, Slaby BM, Franke A, Hentschel U. 2018.** The sponge holobiont in a changing ocean: from  
891 microbes to ecosystems. *Microbiome* **6**: 46.
- 892 **Pollock FJ, McMinds R, Smith S, et al. 2018.** Coral-associated bacteria demonstrate phyllosymbiosis and  
893 cophylogeny. *Nature Communications* **9**: 4921.
- 894 **Ponnudurai R, Kleiner M, Sayavedra L, et al. 2017.** Metabolic and physiological interdependencies in the  
895 *Bathymodiulus azoricus* symbiosis. *ISME Journal* **11**: 463–477.
- 896 **Porrás-Alfaro A, Bayman P. 2011.** Hidden fungi, emergent properties: endophytes and microbiomes. *Annual*  
897 *Review of Phytopathology* **49**: 291–315.
- 898 **Quinn RA, Vermeij MJA, Hartmann AC, et al. 2016.** Metabolomics of reef benthic interactions reveals a  
899 bioactive lipid involved in coral defence. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20160469.
- 900 **Raina J-B, Clode PL, Cheong S, et al. 2017.** Subcellular tracking reveals the location of  
901 dimethylsulfoniopropionate in microalgae and visualises its uptake by marine bacteria. *eLife* **6**: e23008.
- 902 **Raina J-B, Tapiolas D, Willis BL, Bourne DG. 2009.** Coral-associated bacteria and their role in the  
903 biogeochemical cycling of sulfur. *Applied and Environmental Microbiology* **75**: 3492–501.
- 904 **Rix L, de Goeij JM, van Oevelen D, et al. 2017.** Differential recycling of coral and algal dissolved organic matter  
905 via the sponge loop. *Functional Ecology* **31**: 778–789.
- 906 **Rohwer F, Seguritan V, Azam F, Knowlton N. 2002.** Diversity and distribution of coral-associated bacteria.

- 907 *Marine Ecology Progress Series* **243**: 1–10.
- 908 **Rolland J-L, Stien D, Sanchez-Ferandin S, Lami R. 2016.** Quorum sensing and quorum quenching in the  
909 phycosphere of phytoplankton: a case of chemical interactions in ecology. *Journal of Chemical Ecology* **42**: 1201–  
910 1211.
- 911 **Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. 2007a.** The role of microorganisms in coral  
912 health, disease and evolution. *Nature Reviews Microbiology* **5**: 355–362.
- 913 **Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I. 2007b.** The hologenome theory disregards the  
914 coral holobiont: reply from Rosenberg et al. *Nature Reviews Microbiology* **5**: 826–826.
- 915 **Rosenberg E, Sharon G, Atad I, Zilber-Rosenberg I. 2010.** The evolution of animals and plants via symbiosis  
916 with microorganisms. *Environmental Microbiology Reports* **2**: 500–506.
- 917 **Rosenberg E, Zilber-Rosenberg I. 2018.** The hologenome concept of evolution after 10 years. *Microbiome* **6**: 78.
- 918 **Roughgarden J. 2019.** Holobiont evolution: model with vertical vs. horizontal microbiome transmission. *bioRxiv*:  
919 465310.
- 920 **Rubin-Blum M, Antony CP, Sayavedra L, et al. 2019.** Fueled by methane: deep-sea sponges from asphalt seeps  
921 gain their nutrition from methane-oxidizing symbionts. *ISME Journal* **13**: 1209–1225.
- 922 **Saha M, Berdalet E, Carotenuto Y, et al. 2019.** Babylonian towers in a blue world—using chemical language to  
923 shape future marine health. *Frontiers in Ecology and the Environment* **in press**.
- 924 **Saha M, Weinberger F. 2019.** Microbial “gardening” by a seaweed holobiont: Surface metabolites attract  
925 protective and deter pathogenic epibacterial settlement. *Journal of Ecology* **in press**: 1365-2745.13193.
- 926 **De Schryver P, Vadstein O. 2014.** Ecological theory as a foundation to control pathogenic invasion in aquaculture.  
927 *ISME Journal* **8**: 2360–2368.
- 928 **Segev E, Wyche TP, Kim KH, et al. 2016.** Dynamic metabolic exchange governs a marine algal-bacterial  
929 interaction. *eLife* **5**.
- 930 **Selosse M-A, Bessis A, Pozo MJ. 2014.** Microbial priming of plant and animal immunity: symbionts as  
931 developmental signals. *Trends in Microbiology* **22**: 607–613.
- 932 **Seyedsayamdost MR, Case RJ, Kolter R, Clardy J. 2011.** The Jekyll-and-Hyde chemistry of *Phaeobacter*  
933 *gallaeciensis*. *Nature Chemistry* **3**: 331–335.
- 934 **Seymour JR, Amin SA, Raina J-B, Stocker R. 2017.** Zooming in on the phycosphere: the ecological interface for  
935 phytoplankton–bacteria relationships. *Nature Microbiology* **2**: 17065.
- 936 **Shapira M. 2016.** Gut microbiotas and host evolution: scaling up symbiosis. *Trends in Ecology & Evolution* **31**:  
937 539–549.
- 938 **Shreiner AB, Kao JY, Young VB. 2015.** The gut microbiome in health and in disease. *Current Opinion in*  
939 *Gastroenterology* **31**: 69–75.
- 940 **Singh Y, Ahmad J, Musarrat J, Ehtesham NZ, Hasnain SE. 2013.** Emerging importance of holobionts in  
941 evolution and in probiotics. *Gut Pathogens* **5**: 12.
- 942 **Skillings D. 2016.** Holobionts and the ecology of organisms: multi-species communities or integrated individuals?  
943 *Biology & Philosophy* **31**: 875–892.
- 944 **Smriga S, Sandin SA, Azam F. 2010.** Abundance, diversity, and activity of microbial assemblages associated with  
945 coral reef fish guts and feces. *FEMS Microbiology Ecology* **73**: 31–42.
- 946 **Spoerner M, Wichard T, Bachhuber T, Stratmann J, Oertel W. 2012.** Growth and thallus morphogenesis of  
947 *Ulva mutabilis* (Chlorophyta) depends on a combination of two bacterial species excreting regulatory factors.  
948 *Journal of Phycology* **48**: 1433–1447.
- 949 **Theis KR. 2018.** Hologenomics: systems-level host biology. *mSystems* **3**: e00164-17.
- 950 **Thompson AW, Foster RA, Krupke A, et al. 2012.** Unicellular cyanobacterium symbiotic with a single-celled  
951 eukaryotic alga. *Science* **337**: 1546–1550.
- 952 **Thompson LR, Sanders JG, McDonald D, et al. 2017.** A communal catalogue reveals Earth’s multiscale  
953 microbial diversity. *Nature* **551**: 457.
- 954 **Tolstoy LN. 1878.** *Анна Каренина*. Moscow: Русский вестник.
- 955 **Troussellier M, Escalas A, Bouvier T, Mouillot D. 2017.** Sustaining rare marine microorganisms:  
956 Macroorganisms as repositories and dispersal agents of microbial diversity. *Frontiers in Microbiology* **8**: 947.
- 957 **Vallet M, Strittmatter M, Murúa P, et al. 2018.** Chemically-mediated interactions between macroalgae, their  
958 fungal endophytes, and protistan pathogens. *Frontiers in Microbiology* **9**: 3161.
- 959 **de Vargas C, Audic S, Henry N, et al. 2015.** Eukaryotic plankton diversity in the sunlit ocean. *Science* **348**:  
960 1261605.
- 961 **Vellend M. 2010.** Conceptual synthesis in community ecology. *The Quarterly Review of Biology* **85**: 183–206.
- 962 **Venn AA, Loram JE, Douglas AE. 2008.** Photosynthetic symbioses in animals. *Journal of Experimental Botany*



- 963 59: 1069–80.
- 964 **Wallin IE. 1925.** On the nature of mitochondria. IX. Demonstration of the bacterial nature of mitochondria.
- 965 *American Journal of Anatomy* **36**: 131–149.
- 966 **Webster NS, Taylor MW, Behnam F, et al. 2010.** Deep sequencing reveals exceptional diversity and modes of
- 967 transmission for bacterial sponge symbionts. *Environmental Microbiology* **12**: 2070–82.
- 968 **Wichard T. 2015.** Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales
- 969 (Chlorophyta). *Frontiers in Plant Science* **6**: 86.
- 970 **Wichard T, Beemelmans C. 2018.** Role of chemical mediators in aquatic interactions across the prokaryote–
- 971 eukaryote boundary. *Journal of Chemical Ecology* **44**: 1008–1021.
- 972 **Williams SCP. 2014.** Gnotobiotics. *Proceedings of the National Academy of Sciences of the United States of*
- 973 *America* **111**: 1661.
- 974 **Wiltshire KH, Kraberg A, Bartsch I, et al. 2010.** Helgoland Roads, North Sea: 45 years of change. *Estuaries and*
- 975 *Coasts* **33**: 295–310.
- 976 **Wolfowicz I, Baumgarten S, Voss PA, et al. 2016.** *Aiptasia* sp. larvae as a model to reveal mechanisms of
- 977 symbiont selection in cnidarians. *Scientific Reports* **6**: 32366.
- 978 **Zaneveld JR, McMinds R, Vega Thurber R. 2017.** Stress and stability: applying the Anna Karenina principle to
- 979 animal microbiomes. *Nature Microbiology* **2**: 17121.
- 980 **Zengler K, Palsson BO. 2012.** A road map for the development of community systems (CoSy) biology. *Nature*
- 981 *Reviews Microbiology* **10**: 366–372.
- 982 **Zilber-Rosenberg I, Rosenberg E. 2008.** Role of microorganisms in the evolution of animals and plants: the
- 983 hologenome theory of evolution. *FEMS Microbiology Reviews* **32**: 723–35.
- 984
- 985