

Dear Drs. Arnaud-Haond and Vacher.

Thank you for the suggestions and corrections to our manuscript. Please find uploaded to the PCI server a version of the manuscript with tracked changes, as well as below a detailed response to each of your comments (your comments in black, our response in blue). As you will see we have incorporated most of your suggestions, but we also disagree on some points notably concerning the objectives of this paper, and we explain our position below. We hope that, in light of these explanations, you will accept our arguments and recommend this revised version of the manuscript. Regardless of your decision, we are grateful for the significant contributions you have made.

Thank you very much in advance,
Simon Dittami, Catherine Leblanc, and Fabrice Not (on behalf of the authors)

Dear Dr. Dittami,

We thank you for this revised version. Please receive our sincere apologies for the long delay in revising it.

We feel the article is substantially improved with this new revision.

We believe there is room for a more significant contribution, would the consortium take side on the realistic and operational scale of boundaries to apply to the holobionts concept (lines 246->253) the way they discuss it in the rest of the paper. Would the consortium advocate its extension to extremely large scale of ecosystem, would they consider it pragmatic and useful considering the research and application axis they forecast ?

We understand the value of this suggestion, yet we do not feel comfortable going beyond what we initially stated here. This paper is an opinion paper based on the results of a workshop with a wide range of participants from different fields. While these participants could all agree on the importance of the holobiont concept and potential uses as well as advocating its consideration in future work, at this point it is difficult to generalize and suggest a common operation scale. This very much depends on the system and the question asked, as well as on methodological approach (and future developments). This is why we concluded that the concept should be used with a degree of malleability (Conclusion section).

We also feel there is a need to clarify the fundamental questions raised considering the holobionts concept (lines 255->268).

We have now expanded the introduction to this paragraph including other relevant questions as outlined below in our response to comment C12, but in this paragraph, we would nevertheless like to focus on one (arguably the most fundamental) question, i.e. that of the evolution of holobionts. Other (arguably more practical) questions are then discussed in more detail in the "Challenges and opportunities" section (transmission, chemical interactions, community assembly).

We attach a version including comments and suggestions for editing. We still feel more detailed recommendations would be needed in the last section on new methods, to enhance the interest and the future use.

We understand the wish for clear and concrete recommendations and we believe that we do already make several relevant suggestions along these lines. For instance, we argue for a need to maintain culturing efforts and develop a wider range of model systems to cover marine diversity. We also underline the need of integrating studies on such model systems with large-scale studies and the incorporation of the holobiont concept in mathematical models (from distribution of species potentially up the geochemical cycles). Finally, we highlight the relevance of the holobiont concept for applied research (management, aquaculture), but call for caution when it comes to manipulating holobionts for these purposes including global change resistance.

Making even more concrete recommendations would, in our opinion, also require focusing on concrete problems or models, and this would best be accomplished in targeted reviews. As stated above, this paper is the outcome of a foresight workshop bringing together scientists from different disciplines (from philosophy to molecular biology). It aims to strike a balance between all of these different perspectives, models, and approaches, and extract common issues related to holobionts in the marine realm. This was possible only at the expense of some level of detail. We nevertheless believe that, even if it remains general, this paper has its place and conveys an important message.

We however appreciate the long time now since first submission, and are willing to recommend your manuscript on receipt of the next version.

All the best,
Sophie Arnaud-Haond and Corinne Vacher

Response to comments from the annotated pdf:

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

This part of the sentence was removed as requested

[SAH2]: Here we propose that one of the first significant challenge (some very important ones are also required)

This sentence was revised as requested

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

I suggest rephrasing focusing on what such focus 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 and 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 holobiont, and to account for the holobiont concept in applied area such as the conservation or exploitation of marine ecosystems and resources"?

This sentence was now revised as follows: "This first step is crucial to decipher the main drivers of the dynamics and evolution of holobionts and to account for the holobiont concept in applied areas, such as the conservation, management, and exploitation of marine ecosystems and resources". Management was added in response to comment C34.

[C4]: A reference should be included here

Holt and Miller 2010 was added as reference and "quantitative" was added to the definition.

[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.

The definition was revised as follows: "Community assembly process – the processes that shape community composition in a given habitat, according to Nemergut et al. (2013) the four main forces relevant for community assembly are evolutionary diversification, dispersal, selection, and ecological drift)."

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

This was changed as requested, see C5.

[SAH7]: The word organism is restrictive when it comes to the definition of a term as large as holism (i.e. beyond ecology). The definition in the early part of the introduction fits better. In order to avoid repetition, maybe the Oxford dictionary 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."

The definition was changed as requested, except that we did not include the last sentence, which is not relevant to this paper.

[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.

This definition was now expanded: "Holobiont – an ecological unit of different species living together in symbiosis. The term is frequently used for the unit of a host and its associated microbiota but can be extended to larger scales. Whether or to what extent holobionts are also a unit of evolution is still a matter of debate (Douglas and Werren 2016)."

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

This citation was added, but we also kept the original reference, as the proposed reference is focused on diseases.

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

This was added as requested.

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

Yes, community was added as requested.

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

We have now expanded this sentence but would still like to highlight the question of evolution as fundamental. This paragraph is essentially about the latter question as it derives directly from the holobiont concept. Other more practical questions are then discussed in more detail in the challenges section. We now state here: "Such a conceptual perspective raises fundamental questions not only regarding the interaction between the different compartments of holobionts and processes governing their dynamics, but also that of the relevant units of selection and the role of co-evolution".

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

This is a statement, but it raises the question how widespread this phenomenon is. We now added to this: "plant and animal evolution involves new functions co-constructed by members of the holobiont or elimination of functions redundant among them (Selosse et al. 2014), and it is likely that these processes are also relevant in marine holobionts."

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

This was added as requested.

[SAH15]: See references on green algae as well fitting the description here such as articles by Hollants et al.

We added one sentence on *Bryopsis* and one reference, but please note that it is not possible to include all models in this opinion paper, which does not aim to extensively cover bibliography resources as a review paper. We merely wish to illustrate our point of view by a few examples.

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

[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

This was changed as requested (we did not come up with anything better than your suggestion).

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

This comment was now incorporated: "During microbiota transmission (whether vertical or horizontal), "selection" by the host and/or by other components of the microbiome, is a key process in establishing or maintaining a holobiont microbial community."

[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.

We now removed "as opposed to drift", see C18.

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

This was now rephrased: "In addition to selection, ecological drift, dispersal and evolutionary diversification have been proposed as key processes in community assembly, but are difficult to estimate in microbial communities (Nemergut *et al.* 2013)".

[C21]: Several frameworks were developed 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

Thank you for pointing out these interesting references. We now cite them as a perspective to be used also in the marine environment: "Considering the high connectivity of aquatic environments, differences in marine microbial communities are frequently attributed to a combination of selection and drift, rather than limited dispersal (e.g. Burke, Steinberg, *et al.* 2011), a conclusion which, in the future, could be refined by conceptual models developed for instance for soil microbial communities (Stegen *et al.* 2013; Dini-Andreote *et al.* 2015)."

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

This was added as requested.

[SAH23]: Except for primates?

The primate example was now entirely removed, see also comment SAH24 (just below).

[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 co-speciation 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 above for the reserves to interpret congruent phylogenetic patterns as co-speciation indices

This sentence was now revised and the suggested literature added: “However, co-speciation is challenging to prove (de Vienne *et al.* 2013; Moran and Sloan 2015) and only few studies have examined this process in marine holobionts to date, each focused on a restricted number of actors (*e.g.* Peek *et al.* 1998; Lanterbecq *et al.* 2010).”

[SAH25]: why here ?

The idea here was not to say that it is more challenging in the context of global change, but rather that global change is a challenge that will require us to employ the holobiont concept to be able to predict and mitigate it. The sentence was revised to clarify this: “Increasing our knowledge on the contribution of these processes to holobiont community assembly in marine systems is a key challenge, which is of particular urgency today especially in the context of ongoing global change.”

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

This sentence was now rephrased to be more precise: “Yet, the contribution of the microbiome is still missing in most quantitative models predicting the distribution of marine macro-organisms, or additional information on biological interactions would be required to make the former more accurate (Bell *et al.* 2018)”

[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*

This reference was now added: “At the same time, and despite the recent advances in community modeling (Ovaskainen *et al.* 2017), hypotheses drawn from large scale-studies remain correlative and require experimental validation of the mechanisms driving the observed processes.”

[SAH28]: invasion

This example was added as requested

[SAH29]: lineage ?

“species” was replaced by “lineages”

[SAH30]: contribution ?

“role” was replaced by “contribution”

[SAH31]: compartments ?

“components of the holobiont” was replaced by “compartments of the holobiont”

[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

This part of the sentence was deleted to avoid ambiguities.

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

This was added as requested: “We argue that maintaining or even extending cultivation efforts, possibly via the implementation of “culturomics” approaches as successfully carried out for the human gut microbiome (Lagier *et al.* 2012), remains essential to capture the maximum holobiont biodiversity possible and will facilitate the experimental testing of hypotheses and the investigation of physiological mechanisms.”

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

We now specifically added management to the last sentence of the abstract, but we do not want to put the main focus on management by adding the entire sentence.

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

Yes, we agree, but believe this was already mentioned: “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 bacteria detrimental to the host and to prevent disease outbreaks in aquaculture settings”.

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

In this example we refer to bacteria detrimental to the host – this was now rephrased see C35.

[SAH37]: Side consequences ?

Ecological consequence was replaced by “side effects”

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

This was changed as requested

[SAH39]: Amount of

We added “amounts of”.

[SAH40]: How so ? it may be worth detailing in one sentence the idea developed in this article?

This was now done – thank you: “A holistic microbial management approach, *e.g.* by reducing the use of sterilization procedures and favoring the selection of healthy and stable microbiota consisting of slow-growing K-strategists, may provide an efficient solution to these latter problems, reducing the sensitivity of host to opportunistic pathogens (De Schryver and Vadstein 2014).”

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

“(often hidden in their hosts)” was added in parentheses after “introduction from exotic regions”

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

This question was added in the conclusion section as requested.

[SAH43]: And dispersal

This was added as requested.

[SAH44]: Undersanding of biochemical cycles?

This was added as requested.

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

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

The figure legend was updated to be more precise: “Impact of emerging methodologies (light green) on the main challenges in marine holobiont research identified in this paper (blue). Turquoise and purple correspond to the two main clusters of activity identified in Figure 3.”