

The manuscript by Rådecker and Meibom reports on a long-term starvation experiment of the sea anemone *Aiptasia*, an emerging model system for the study of cnidarian-Symbiodiniaceae symbioses. *Aiptasia* is a promising symbiosis model system. *Aiptasia* are small mixotrophic anemones which feed on free-living prey (and maybe particulate or dissolved organic matter??) and on photosynthate transferred from their algal endosymbionts. It can be reared and manipulated in the laboratory and can be used to improve our understanding of Cnidarian-Symbiodiniaceae symbioses. Among other reasons, studying this model is relevant because ultimately it may be used to tackle important basic biology questions about economically, ecologically and evolutionarily relevant ecosystems, such as coral reefs which are based on similar symbiotic interaction with Symbiodiniaceae. With their long-term experiment, the authors aimed at studying the relative contributions to nutrition from the symbionts and from external sources.

The main conclusions of the manuscript are:

1. The contribution of symbiont derived nutrients to the host metabolism remained unaffected by long-term starvation due to an increase in algal photosynthesis and more efficient carbon translocation.
2. Feeding on external prey “is not essential to fulfilling the energy requirements of the holobiont on a one-year timescale”
3. Feeding on external prey “is a critical source of nitrogen required for holobiont growth under oligotrophic conditions”.

Overall, I found the manuscript relatively clear, the model and the research questions interesting, however, I think the authors may be overinterpreting their data, some essential controls are not provided, and the number of replicates is very low. The conclusions are based on a very small number of replicates, only three anemones were analyzed in each batch. Although I understand that NanoSIMS is not high throughput, for dry weight, symbiont cell count and protein content, however more than three individuals could have been analyzed easily.

A small terminology comment: It would be okay to talk about the autotrophic versus the heterotrophic nutrition for the symbiosis but throughout the manuscript, the authors refer to symbiont derived photosynthate as *autotrophic nutrients* for the host and to external food sources (i.e. *Artemia*) as *heterotrophic nutrients*. In my opinion, that this is inadequate and confusing. How do you define an autotrophic or heterotrophic nutrient? In both cases the nutrients are organic compounds and the *Aiptasia* host feeds on them heterotrophically. Some external prey in the environment may be autotrophs, heterotrophs, particulate organic matter, or dissolved compounds therefore *heterotrophic/autotrophic nutrient* is, I think, not a good way to go.

A concern I have is the technique used to quantify the symbiont density. The authors homogenized the host, then centrifuged the homogenate and considered the supernatant as the “host fraction” and the pellet as the “symbiont fraction”. Is this an established and validated method in *Aiptasia*? Without any reference for this method or any form of control provided by the author, it is hard to believe that this is a reliable way to separate host and symbiont. Was the supernatant (considered “host fraction”) also analyzed by the CellDrop cell count? This would be an easy and essential control to check whether the host fraction was really symbiont free. Similarly, was the pellet observed under light microscopy to confirm that it is composed of symbiont cells only?

The reason I'm pointing this out is that these data are essential to conclusion #1 and the starvation may be inducing some changes in the symbiont cells as shown in some papers ([Bedgood et al 2020](#); [Ladriere et al 2008](#)). The 80% symbiont density could be in part due to a technical bias. If the symbionts in starved host have smaller size and/or density they may not pellet as much. Another easy way to better support the drastic reduction of symbiont density would be to add micrographs of histological staining (Toluidin blue) of semi-thin section from both treatments. A 80% reduction in symbiont density would be quite obvious.

Regarding the NanoSIMS ROI analysis, why is the number of ROI so different between host (Fed: n=25, Starved n=22) and symbiont (Fed n=144, starved n=140)? This can affect the outcome of the statistical tests. Figure 2B has a different scale (10 folds higher in starved host) than 2A, I suppose this is an error because the box plot data do not show a higher enrichment in the starved host. Please, correct or comment on this.

Line 150 the authors mention a unlabeled control but no data are provided, could the author add these data?

Line 222: "Indeed, patterns of host ^{13}C enrichment (Fig. 2A-C) were not affected by heterotrophic starvation indicating that photosynthate availability for the host was not impaired". The current NanoSIMS data provided in the manuscript do not show a clear ^{13}C enrichment in the host above that of the natural abundance of ^{13}C . To support this, the authors need to provide the ROI data of the unlabeled control, and ideally an aposymbiotic host control incubated with ^{13}C -bicarbonate as well (to control for host incorporation of inorganic C via anaplerosis, see recent papers from Harald R. Gruber-Vodicka [here](#) and [here](#)). At the very least, the unlabeled control should be added to the Fig.2.

Line 184-186: " ^{13}C enrichment from ^{13}C -bicarbonate assimilation/translocation was highest in the algal symbionts with host ^{13}C enrichment primarily observed in lipid bodies". What makes you think that these are lipid bodies? Lipids are not preserved by glutaraldehyde or paraformaldehyde fixation (OsO₄ fix lipids) and therefore they are washed away during samples dehydration and result in "empty vacuoles" on resin section. In absence of TEM correlation, how can you make confirm that these enriched area are not tangential sections of enriched symbiont cells?

The authors have kept 2 batches of *Aiptasia anemone* in the lab for one year. One batch was regularly fed with *Artemia* while a second batch relied solely on the nutritional contribution of their photosynthetic symbionts for nutrition. The study clearly shows that at least some *Aiptasia* survived and maintained their association with their nutritional symbionts even after one year of diet restriction. This seems to be relevant information on its own and would benefit from being discussed against similar starvation experiments from the literature. However, the authors should provide the total number of *Aiptasia* individuals at the beginning and at the end of the experiment in the two batches. How many anemones survived after 1 year starvation, how many died? How does that compare to the batch fed with *Artemia*? This essential to know in order to draw the conclusion #2.