

Response to Martina Prazeres

We would like to thank Dr. Martina Prazeres for her contribution on the discussion from the viewpoint of benthic foraminiferal symbiosis. A point-by-point response to the comments is included below.

Haruka Takagi

(on behalf of all co-authors)

Ln 56-57: Is kleptoplasty a possibility?

Reply 3-1: In planktonic foraminifera, kleptoplasty has never been reported despite its years of study. In this study, we investigated the functionality of chlorophyll but did not identify the algal symbionts. At this point, the possibility of functional kleptoplasty is left. However, we are suspicious about it because most of the species whose symbionts has not been identified to species level has once been observed under TEM and the plausible symbionts reported was at least algae (see Table 1), not chloroplasts.

Ln 249-250: Is this based on the chlorophyll functionality or have the symbionts been identified? Please, clarify.

Reply 3-2: It is solely based on the chlorophyll functionality. We change the sentence as follows; “... five species were newly confirmed as symbiotic based on the functionality of chlorophyll; *S. dehiscens*, ..., ...,”.

Ln 257-258: Could it be the other way around? They require more pigment because photosynthesis is not that efficient?

Reply 3-3: Probably, we should delete the word “effective” which makes the confusion. In this sentence, we intended to mention that the more chlorophyll they have, the more photosynthesis they can perform. The effectiveness of photosynthesis is another story (in fact, the F_v/F_m value is the parameter of the effectiveness, and the value of these species are high).

Ln 270-272: Unless physiological studies have been conducted confirming the nature of the algal-host relationship, they might in fact all be ‘facultative’.

Reply 3-4: We totally agree. That is why we do not want to use the word “facultative” or “oblige”. Please also check the comment from Reviewer #1 and our reply to it (Reply 1-12).

Ln 276-278: If the host can acquire food, then increasing the algal biomass might not be necessary.

Reply 3-5: That is true. But it is also the case for species with positive correlation in test size-Chl *a* relationship. Planktonic foraminifera actively acquire food even if they have symbionts. They cannot rely solely on their symbionts. The extent of their dependence on symbionts is still unknown, which is an interesting subject in the future. In any case, this sentence will be deleted in the revised manuscript as suggested by Reviewer #2.

Ln 280-281: Yes, but it can also mean that what authors are calling 'obligated' symbiont-bearing species are actually mixotrophics (as in most cases), which are obligate symbionts but also acquire energy through feeding.

Reply 3-6: As explained in Reply 3-5, all planktonic foraminifera species are basically heterotrophic, and for symbiotic species, they can be called mixotrophic. We think this point should be clarified in the earlier part of the text. We will change the first sentence of Introduction to "Planktonic foraminifera are unicellular heterotrophic marine zooplankton with calcite tests.".

Ln 281-284: Please, clarify how this can be true. In benthic forams, there is no sure thing as 'certain algae', as host species are very conserved when it comes to choosing an algal partner (please see Prazeres & Renema 2019, Biological Reviews). Also, symbiosis is a very fine tuned relationship.

Reply 3-7: In this part, we mentioned the possibility of "retention of photosynthesis-capable algae". This is a hypothesis to explain the absence of test size-Chl *a* correlation for species in Cluster 3. We will state more clearly that it is a hypothesis. In addition, we will tone down this part as suggested by Reviewer #2.

Ln 288: I am not sure that's how symbiosis work, at least not in benthic forams. Please, clarify this assertion.

Reply 3-8: This sentence will be deleted as it is a second guess derived from the above hypothesis.

Ln 289-290: This is actually not a good reference, given that it not symbiosis at all, just kleptoplasty, which actually contradicts what authors are saying.

Reply 3-9: We are sorry for making confused. We cited them for the reference of a way of maintaining symbiosis (incorporation of algal cells, non-permanent retention, and replenishment). Since a similar thing is reported for

kleptoplasty, we here cited them. We will replace the reference right after the word “..kleptoplasts” in the sentence, which will make it more straightforward to read.

Ln 293: The types of symbioses mentioned here need to be defined early on. Whats the difference between: ‘facultative’ and ‘transient’? Are they being used interchangeably?

Reply 3-10: The terms “obligate” and “facultative” are defined in Introduction (Lines 76-84) with a short historical review of these terms used in previous studies. In contrast, the terms we newly used from this section, “persistent” and “transient”, are chosen to illustrate the mode of symbiosis indicated from our results. We think “obligate symbiosis” and “facultative symbiosis” are technical terms including the meaning of the dependency of the symbiotic relationship. Therefore, “facultative” and “transient” are not interchangeable in our text.

Ln 304-305: I would be very careful stating that planktonic forams are phototrophics, as they are more likely to be mixotrophic to some degree.

Reply 3-11: Thank you for the comment. It is true that they are mixotrophic. The word ‘acquired phototrophy’ here is interchangeable to ‘mixotrophy’. Since the former was used in the paper we referred to show the concept of photosymbiosis (Stoecker et al., 2009), we preferred to use ‘acquired phototrophy’. In addition, since the photosymbiosis in planktonic foraminifera should be established at every new generation, ‘acquired’ seems to be suitable to describe its nature (please see Reply 1-12 as well). In the sentence, we will change in the parenthesis as “... (higher extent of acquired phototrophy/mixotrophy) ...”.

Ln 338-340: This can also indicate mixotrophy, or a less dependency on the algal symbionts. It seems to me that the authors are assuming that all energy is coming from the symbiosis with algae, which might not be the case. Nowhere in the text that authors mention mixotrophy (except when talking about benthic forams). If this is not the case, the authors need to add citations with compelling evidence that planktonic forams that host dinoflagellates are only photoautotrophs.

Reply 3-12: Thank you for pointing it out. We never assume that planktonic foraminifera solely relies on phototrophy. We admit that the references for mixotrophy in Introduction (Line 76) were insufficient. We will add Stoecker (1998) and Caron (2000) since they discuss and review the mixotrophy in marine plankton including planktonic foraminifera.

Ln 351-352: Please, re-write. A sentence should never finish in a preposition. It is fine in spoken English but not in written English.

Reply 3-13: We will change the last part of this sentence as “...regardless of the phylogenetic position of the host”.

Ln 360-362: In the case of planktonic forams, the symbiont selects the host? Please, clarify.

Reply 3-14: In contrast to benthic species, host-symbiont association so-far reported for planktonics is one-to-one relationship alone. It seems that they have strong symbiont specificity. However, the selection process of symbionts is still unknown. We do not know whether the host attracts symbionts or the symbionts attract host, or alternatively, the acquisition of symbionts is controlled by a chance; i.e., individuals that could not acquire its partner in early life stage will die.

In this part, we tried to interpret the host's habitat depth relating to the light preference of the symbionts. Since the host must acquire specific symbionts, we assume that the light requirement of the symbiont may regulate the host's depth. We think the term “control” may be confusing, hence we change this sentence as “... the symbiont acclimation potential may be one of the factors restricting the habitat depth of the host species”.

Ln 381-382: Since the authors mentioned kleptoplasty in benthic foraminifera, and later on suggested for planktonic forams, just having active chlorophyll is not convincing, it is indicative.

Reply 3-15: Sorry for making confused. We did not suggest kleptoplasty in planktonic foraminifera, but suggest the algal retaining behavior we presume is similar to what is reported for kleptoplasty in benthic species. We will rewrite the earlier part (Line 289-290) as explained in Reply 3-9 to avoid a misinterpretation.

Figure 11: Typo. Please amend from 'Aquired' to "Acquired".

Reply 3-16: We will correct it.

References:

Caron, D. A.: Symbiosis and mixotrophy among pelagic microorganisms, in: *Microbial Ecology of the Oceans*, edited by: Kirchman, D. L., Wiley-Liss, Inc., New York, 495–523, 2000.

Stoecker, D. K.: Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications, *European Journal of Protistology*, 34, 281–290, 1998.