Comments to the Author

Manuscript ID: bg-2019-382

The manuscript of Richirt and coauthors on “Foraminiferal community response to seasonal anoxia in Lake Grevelingen (the Netherlands)” represents the assemblage fluctuation of benthic community in response to hypoxic/anoxic environments. These analyses are important to understand the foraminiferal tolerance to hypoxia/anoxia and hydrogen sulfide, and to understand life histories under the extreme environments. However, the structure of manuscript is very poor and experimental design and data validations are problematic. Therefore, I strongly suggest reconstructing throughout the manuscript, and also data validation is needed.

The biggest problem is that authors only used specimens of 125µm or more. Juvenile specimens have the size smaller than 125µm. If you are looking at population dynamics, you must deal with juvenile specimens.

In the methods section, the authors should explain more detailed procedures. I also found several methods sentences in the result and also in the discussion. Also, in the section 3.4, the authors described methods, although this paragraph is in the results section. These explanations should be move to appropriate section.

In the first section of the discussion (4.1), the author described both advantages and disadvantages of both CTG methods and Rose Bengal staining respectively. However, the CTG method has already described in Bernhard et al. (2006), and therefore it is not necessary to explain in detail.

In the Section 4.2, I strongly suggest that the author should describe environmental setting of the sampling points. However, these descriptions must be explained in the beginning of this article. The authors also should explain vertical profile of oxygen in the water column and in the sediment in the "environmental settings of Den Osse Basin" section. This information can help readers to understand the habitat where foraminifera live in.

In the section 4.3, I cannot understand what you want to discuss about. The authors referred (quoted) about previous studies in the first two paragraphs. The authors should move these paragraphs to the introduction, Ah...you would like to discuss relationship between sulfidic condition and foraminiferal assemblages? The discussion starts from line 321...
I strongly recommend to make clear and re-structure throughout the manuscript.

Other comments.

Line 34, “Elphidium selseyense and Elphidium magellanicum are much less affected by anoxia and free H2S than Ammonia sp. T6”

Is the light reaching the lake bottom? Is it not necessary to consider the photosynthesis of kleptoplasts? Ammonia T6 has a nitrate pool in the cell. Nomaki et al. (2014, Limnol. Oceanogr., 59, 1879–1888) points out that this species potentially use an anaerobic respiration.

Line 47-, “Benthic faunas are strongly impacted by these events (Diaz and Rosenberg, 1995) although the meiofauna, especially foraminifera, appears to be less sensitive to low Dissolved Oxygen (DO) concentrations than the macrofauna”

Virgulinella, Bulimina, etc. may be sensitive to anoxic environments. Cannariato et al (1999, Geology, 27, 63-66) has analyzed community changes over the last 60,000 years at Santa Barbara Basin. As a result, low-oxygen torelant species are clearly replaced. Bolivina tumida, Buliminella tenuata and Globobulimina auriculata are low oxygen torelant species (dysoxic species). Interestingly, the response to hypoxia varies from species to species. Buliminella tenuata increase at the beginning of dysoxic. On the other hand, Bolivina tumida increases toward to the end of the dysoxic period.
Bolivina tumida has symbiotic microbes in its cells. Bolivina pacifica, Uvigerina peregrina, and Loxostomum pseudoberyichi retain microbes outside (in the pore) (Bernhard et al. 2018, Mar. Micropal. 138, 33-45). Based on these phenomena, it is expected that the response pattern to anoxia will differ depending on the symbiotic mode. The authors should explain/discuss this phenomenon in introduction and discussions.

Line53-, “Neutral molecular H2S can diffuse through cellular membranes and inhibits the functioning of cytochrome c oxydase (a mitochondrial enzyme involved in ATP production), finally inhibiting an aerobic respiration (Nicholls and Kim, 1982; Khan et al., 1990; Dorman et al., 2002).”

What do you think about an anaerobic respiration? The authors should explain about an anaerobic repiration.

Line 89, “In order to avoid this problem, we used CellTracker™ Green (CTG) to recognise living foraminifera. CTG is a fluorescent probe which marks only living individuals with cytoplasmic (i.e. enzymatic) metabolic activity (Bernhard et al., 2006)”

This method is not new. The authors should only mention that CTG staining was used to distinguish live benthic foraminifera populations.

Line 115-, “Measurements of oxygen concentrations in the bottom water (1 m above the sediment-water interface using a CTD) for 2011 are from Donders et al. (2012), whereas the 2012 data are from Hagens et al. (2015) and Seitaj et al (2017). Oxygen Penetration Depth (OPD) and depth of free H2 S detection were determined using O2 and H2S microsensors by Seitaj et al., (2015) for station 1, and the data for station 2 were acquired similarly (Supplementary Table 1).”

The authors should explain about environmental settings both station 1 and 2 in the beginning. This information can help reader to understand faunal assemblage changes (and population dynamics). This information is in the end of this manuscript.

Line 121-, “The uppermost centimetre of each core was labelled with CellTracker™ Green CMFDA (CTG, 5-chloromethylfluorescein diacetate, final concentration of 1µM following Bernhard et al., 2006) and fixed in 5 % sodium borate buffered formalin after 24 h of incubation.”

Where did you done this experiment? What kind of tools did you use? Did you sliced top 1cm and then put in the petri dish or some other container for CTG incubation? or jut put CTG directly onto the top of core? Need detailed experimental procedures.

Line 129, “125µm”

As the authors mention about juvenile specimens, it is important point. Juvenile specimens have smaller than 125µm in size in many cases. If you are looking at population dynamics, you should deal with juvenile specimens. For this reason, it is difficult to see when the juvenile specimens have been reproduced.

Line 145, “Supplementary Figure 1 shows…”

I found there are two types in these specimens. Specimens #145 and 152 have a larger proloculus than specimens #147 and #155. In my opinion, these differences in morphology correspond to different generations, megarospheric and microspheric. It is important points to find these generations to understand population dynamics. I strongly recommend to check which generations are abundant in each month.

Line 145, “the penultimate chamber”

Are there any differences in the pore size for each month (season)?

Line 165, “population dynamics”

Need juvenile specimens for analyze.

Line 169, “Fig. 1”

I think the authors should explain much more detail in this paragraph. Detailed procedures were written in figire1 caption!

Line 175, “>125 µm, our analysis mainly concerns adult specimens, anddoes not include juveniles. This limitation should be kept in mind when interpreting the results”

If the authors discuss about population dynamics, it is necessary to check juveniles.

Section3.1
Any statistical analyses?

Line 185, “very low in Janually”

I strongly suggest that the author should describe environmental setting of the sampling points. However, these descriptions must be explained in the beginning of this article. The authors also should explain vertical profile of oxygen in the water column and in the sediment in the "environmental settings of Den Osse Basin" section. This information can help readers to understand the habitat where foraminifera live in.

Line 193, section 3.2

It is better to explain one by one. The authors should explain about station 1 and then explain about station 2.

Line 221-, “then progressively decreased until the end of 2012 (= 48.1 ± 26) in November 2012). Trochammina inflata showed a similar pattern as Ammonia sp. T6”

It is necessary to indicate statistical analyses. Statistically significant?

Line 237, “of larger individuals (>400 μm)”

Are there any ecological meanings?

Line 239-, “The low number of Ammonia sp. T6 individuals at station 1 does not allow us to draw any firm conclusion concerning the size distribution at this station”

In the result section, the authors should describe "results" in detail. For example, there are several large sized individuals in May, simultaneously 200~250 μm-sized individuals are there.
How about propagules? Alve and Goldstein (2002, Journal of Micropaleontology, 21, 95-96; 2003, Limnology and Oceanography, 28, 2163-2170) discussed about propagules in their literatures.

Line 243, “but started to diminish in December”

Are there any data? Please provide.

Line 244, “decrease of the median to 339 μm”

Ammonia has two generations, asexual and sexual phases. These two generations are commonly found in spring and autumn. The authors have to think about the life cycle of foraminifera.

line 245-, “Our tentative to distinguish cohorts by using a deconvolution method to separate the total size distributions into a sum of Gaussian curves was not conclusive”

Please indicate in the methods section.

Line 246-251,

It is not a result.
If the goal is to evaluate foraminiferal behavior in an anaerobic environment, an experimental desing that analyzes small individuals should be considered. Objective 2 cannot be achieved.

Line 255, “thin (Fig. 8c– e) and rather coarse”

Are there any data?
To explain how it differs from the normal case, the authors should show the data.

Line 257-, “Because the crust stayed cohesive after exposition to 0.1 M of EDTA (EthyleneDiamineTetraacetic Acid) diluted in 0.1 M cacodylate buffer (acting as a carbonate chelator)”

This sentence should be moved to the methods section.

Line 259-,
This sentence should be move to the discussion section.

Line 269-281,
It is not necessary to explain detailed about disadvantages of Rose Bengal staining method and advantages of CellTracker Green.
Yes, the CellTracker Green labeling is suitable and reliable method to identify live specimens. However, incubation is required for the CTG method. I think this method includes some artifacts. During the staining, samples were transferred to petri dishes or bottles for 24 hours. The specimens were exposed different environmental condition from their habitat.
This paragraph can be more shorten. Because this method was already described in Bernhard et al (2006), so the authors do not need a detailed description of this method.

Line 291, Fig. 10
You can omit this figure. Because, this is not your data. You can mark the timing of blooming on your figures 11 and 12.

Line 328-,
In the case of symbiontic bacteria-bearing foraminifera, oxic condition is not suitable. Because symbiotic bacteria cannot consume hydrogen sulfide, methane or nitrate in an oxic

condition, and the host foraminifera cannot use organic matter and/or anaerobic respiration from microbes.

Line 368-
There is little data in 2011. This sentence is overstatement.
At both stations 1 and 2, low oxygen was observed from May to August. This situation is totally different from 2012. This characteristic situation will affect next year's (2012) assemblages.

Line 381-, “leading ultimately (in November) to almost complete disappearance of the foraminiferal fauna.”

I’m worried about incubation time (duration) for CTG staining. For example, oxygen penetration depth is about 4mm in October at station 1, but sulfide layers still existed in the deep layer below 4mm. When the authors used top 1cm of the sediment for incubation, sulfidic conditions will be constructed in the experimental bottle (or other gear). For this reason, when living specimens still exist in top 4mm in October, sulfidic conditions may affect living ones. However, the authors did not explain detailed procedures of CTG staining methods. Long time exposure of sulfidic condition may affect living specimens. How did you evaluate for this effect in your experiment?

Line 384-, “inhibited reproduction, and eventually, increased mortality”

Need juvenile data.

Line 390, Section 4.4
It is not appropriate section title. Need improvement.

This section includes many topics related to environmental characteristics and food availability for foraminiferal responses. The authors should rearrange and clarify what authors want to discuss. This paragraph also includes the results. Need reconstruction.

Line 391-, 1st paragraph

Is this a topic sentence in this section? I think this information should be move to the Materials & Methods section. This section is also long and confusing. The authors have to reconstruct.

Line 413, “take place throughout the year”

Are there any evidences that reproduction took place throughout the year? The authors should describe detailed results in the Result section. There exist relatively small-sized specimens that increased in May and September-October-November. In my opinion, it looks reproduction occurred twice in 2012. However, it is difficult conclude that there are no three or four chambered juveniles.