Reviewer: 1

Comments to the Author

My main concern with this ms is that there really is only a limited connection between its data and M. rubrum. The paper is about cryptophytes. While that is the focus of the title, the ms itself spends a lot of time speculating about control of M rubrum blooms, about which there is no data here. My first question is whether there is any way to confirm that this was "post-bloom" and not just "no bloom this year"? A recent paper by Dierssen et al in PNAS used ocean color to quantify M rubrum. If the data from the present ms could be put into the context of some real data about an actual prior bloom in this estuary, it would make the connection between the ciliate and the cryptophyte abundance more concrete.

We added data of bulk orange fluorescence, a proxy for biomass of phycoerythrin-containing cells such as cryptophytes and *M. rubrum,* collected at the same sampling site. Phycoerythrin biomass was high the week before the start of the survey and decreased below the detection limit during the survey (**Fig. S2**). Together with high chlorophyll *a* fluorescence (**Fig. 1**), high orange fluorescence observed before the start of the survey indicates the presence of *M. rubrum* blooms. The sharp decline and subsequent low chlorophyll *a* and phycoerythrin fluorescence indicates that the survey took place during the decline of *M. rubrum* blooms. We added the figures and discuss the results in the revised manuscript (lines 215-217, 322-326).

At 0.1-0.3 x 10^6 per L Mesodinium was still pretty high during the study. What about physical mechanisms leading to bloom decline? The authors seem too quick to make the connection between DIN, cryptophytes and bloom decline without direct data. In this regard, the Conclusion section is a bit of an overreach. The data do not "show" nutrient-limited division rates, much less that this leads to bloom decline of the ciliate. These words should be tempered.

We agree with the reviewer that our results only suggest that nutrient limitation may contribute to the bloom decline and that physical processes may also play an important role in the process. We now discuss the role of physical processes as a potential mechanism responsible for the decline in *M. rubrum* abundance (lines 328-332).

Some specifics:

Confused wording about the sampling (lines 96-100). There is either a redundancy here or I did not follow.

We deleted the redundant sentence (line 99).

Put more information on the figures, for example add the symbols on the axes. Nowadays, the reader expects to be able to understand the figure without reading the caption unless there is confusion.

We added symbols on the axes or inside the panel for Fig 1, 3 and 5.

Maria – can you add symbols next to the axis labels for Fig 1 (a vertical black line before “salinity” and “red fluo”, a black circle for “DIN”; a vertical grey line for “temperature” and “oxygen”, a grey circle for “DIP”). I took care of Fig. 3 and 5.

It is bit disappointing not to have SSU to confirm that the cells were indeed Teleaulax. Matt Johnson has published some interesting data on different clades of cryptophytes co-occurring with M rubrum, but perhaps that is beyond the scope of this ms.

Cryptophyte cells were sorted by flow cytometry to be examined under a light microscope and could not be used for sequencing due to fixative. However, a DNA sample was collected each week of the survey to confirm the presence of *T. amphioxeia* by qPCR using *T. amphioxeia* specific primer [TxD2 1F (TGAAAAAGGGCCTGAAATTG) /TxD2 USE 2R (ATCATTCACTCGCATGCCCC)] (P. Zuber, unpublished data).

What is the tidal excursion - the spatial extent of advection at the fixed site? This would help evaluate the scale of "patchiness" for the cryptophytes.

Tidal excursion in the Columbia River estuary in September is in the order to 3 days (see Karma & Baptista 2016 Water age in the Columbia River. Estuarine, Costal and Shelf Science 183: 249-259). (line 293-294)

Point out to the reader that figure 3 is a log scale.

We pointed to log scale of Fig 3 in the caption (line 505).

lines 259-261 confusion. "Population growth rates" were 0.2-1.5, whereas "doubling rates" or "division rates" were 0.3-2.1 per day. As written, the authors use "division rates" in two different senses.

The reviewer mistakenly assumed that doubling rate and division rate are synonymous. Division rate (also called specific growth rate) represents the rate of cell division, while doubling rate represents the number of cell division in one day, which is calculated by dividing the division rate by the natural log of 2 (doubling rate = division rate / ln(2)). In our study, division rates ranged from 0.2-1.5 d-1, equivalent to a doubling rate of 0.3-2.1 d-1. We have now replaced “number of cell division per day” by “doubling rate” to make it less confusing.

We choose deliberately not to use the term “population growth rate” to avoid confusion, as it can refer to “specific growth rate” (= division rate) or “net growth rate” (= the rate of change in cell abundance and depends on both the rate of cell division and the rate of net population cell loss),

Reviewer: 2

Comments to the Author

minor issues:

Intro

line 43: Regarding the Qiu reference… this is fine as it is written here but you may want to add after this reference (but see Johnson et al. 2017). I’ll send you a copy of my recent PNAS letter if you like, a response to Qiui et al., titled “Mesodinium rubrum: the symbiosis that isn’t”. The bottom line is that I think they were wrong and that M. rubrum does not harbor intact endosymbionts that divide. More accurately it farms organelles (as previously described).

In light of the reviewer’s recent PNAS letter, we have removed the reference to Qiu et al. from the introduction, but very briefly touch on the idea of “Mesodinium-farming-Teleaulax” and it’s controversy in the discussion section of the manuscript. (line 354-357). We agree that the results of Qiu et al. may be considered artifacts of the methodology (in particular, with regards to the TEM images), but wish to still include the reference in order to reflect the most recent literature on the specifics of the cryptophyte-*M. rubrum* relationship.

Methods:

Line 135 and 153: 0.025% and 0.01% gluteraldehyde seem low. Are you sure these are correct?

We thank the reviewer for pointing that mistake. Final concentrations in the text were changed to 0.25% and 0.1% Glutaraldehyde.

Discussion:

Line 322: again, I don’t think that this report by Qiu et al is correct, but you can leave this in if you like.

Discussed in the above response

major issues

The title and theme: How confident are you of your assessment that these are all Teleaulax cryptophytes? I have trouble believing that the populations are all Teleaulax since there is no rigorous proof to back this up. Checking for cell morphology and size of populations that are sorted is not enough to say for sure in cryptophytes (they are notoriously difficult to identify). T. amphioxeia does have a distinct morphotype but there are no statistics showing what % of observed cells matched this (certainly not 100%). I think its OK to say either Teleaualx-like cryptophytes or Teleaulax dominated cryptophyte populations. Also, since you were not measure grazing, I don’t think you should use “prey” in the title. Certainly cryptophytes are more than just food. I think you should change the title to “Dynamics of cryptophytes during the decline of a red water bloom in the Columbia River Estuary”.

We agree with the reviewer that we don’t know whether cells belonging to the cryptophyte population identified by flow cytometry are all Teleaulax, so cells should be named Teleaulax-like cryptophytes. We modified “Teleaulax cryptophyte prey” in the title by “Teleaulax-like cryptophyte”.

In Fig. 3 you show a pattern for Teleaulax abundance that change but do not appear to be explained by spring/neap tide cycles. One possibility that you don’t bring up could be vertical movement. While your patterns don’t appear to be explained by diurnal cycles of migrations, a study in the Baltic showed that Teleaulax cryptophytes tended to be deeper during the day and more shallow at night (counter to ones expectations for a phototroph and different from other cryptophytes they observed). Why this would be the case is not clear (escaping predators?), but Teleaulax is pretty fast for a cryptophyte so migration could be a possibility for your variation.

Baltic paper:

Susanna Hajdu, Helena Höglander, Ulf Larsson, Phytoplankton vertical distributions and composition in Baltic Sea cyanobacterial blooms, Harmful Algae, Volume 6, Issue 2, February 2007, Pages 189-205, ISSN 1568-9883, http://dx.doi.org/10.1016/j.hal.2006.07.006.

(//www.sciencedirect.com/science/article/pii/S1568988306000990)

The reviewer brings up an interesting point, but in an estuary dominated by lateral circulation, the evidence of cryptophyte vertical migration would likely be obscured by the effects of advective transport. We have added estimates of tidal excursion in our discussion of cryptophyte abundance and patchiness (line 295-296).

Table S1. There is a reference to “see Materials and Methods” for Table S1 (i.e. LSU D2 region data) in the figure legend, but no such methods exist. Also there is no reference to see Table S1 within the manuscript text. Therefore I assume this was included by mistake and should be removed? Also, these data don't support your assertion that Teleaulax dominated the cryptophyte populations very well.

We apologize for the confusion; the table has now removed from the manuscript. Just for clarification, the data reported in the table represented the percent of amplicons from *Teleaulax amphioxeia* to the total number of amplicons from all cryptophytes, not just the cryptophyte population identified by flow cytometry, hence the low percent of *Teleaulax amphioxeia*.