



## ***Interactive comment on “Upper Arctic Ocean water masses harbor distinct communities of heterotrophic flagellates” by A. Monier et al.***

**Anonymous Referee #1**

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**Overview:** This study makes a nice contribution to our understanding of structure and composition of heterotrophic flagellates in upper water column Arctic, and its most interesting contribution is data suggesting vertical structuring of these communities consistent with distinct water layers.

**General Comments:** 1. Within methods somewhere authors should discuss how they sorted pyreores "unambiguously classified as HFL" out of the total pool of reads assigned a taxonomy. Was everything retained that was obviously not a phototroph? Certainly HFL is not a taxonomic group per se, and many groups of protists can include autotrophic taxa as well as heterotrophic taxa, and so some understanding of what was excluded would be useful to the reader. This is particularly important since a small fraction of the 59,409 reads was retained for this analysis. The rest of the data

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were undoubtedly used for a separate paper, but this criterion needs to be clarified. Also, the explanation of percentages of HFL in different samples on page 3404 lines 5-9 is not clear. 2. Results 3404: Vertical HFL distributions: The p values are hard to interpret without knowing the criteria for designating HFL vs non-HFL above. While this is likely not a problem, it would be good to state the percentage of reads that were "unclassified" 3. Results page 3406 lines 16-27: I don't agree that there are "clear trends" between water masses and MAST diversity from OTU distributions. Looking at Figure 4 there are not terribly significant differences for many groups of MAST between water depths. Perhaps tone this down or include p values to support these statements. Same problem with statement on line 4 of page 3407 which states "clear water mass influenced OTU distribution." While this may be true, with these data it is hard to tell for many MAST groups. In contrast, the Rhizarian groups appear much more influenced by water mass. 4. Figure 5 right cladogram might be more readable if you worked with sequences representing OTUs, and simply noted the number of reads behind each OTU next to each identifier in your cladogram. As is, is difficult to read. 5. Discussion page 3410 lines 1-3, I suggest toning down the statements that analysis of MAST communities shows strong vertical specificity at all phylogenetic levels. Not all phylogenetic levels are presented, and certainly looking at Figure 4, there is not a large difference in representation of MAST clades 1a for ex. at station 760 or 540, or clade 1b at station 540, or 8 at 760, or 2 at 430, etc.

**Specific Comments:** 1. Would not say "rapidly becoming" on line 28, since Next Gen methods are already commonly used for comparing eukaryotic and prokaryotic communities in marine environments. 2. Methods section please specify total water volume filtered for each sample analyzed. 3. Methods: Since the data for the 0.2-3 micron fraction of each water sample are not presented in this paper, omit some of this text or explain around line 13 why those data are omitted here. Most likely the authors want a separate publication on those data, but since many of the taxonomic groups they discuss in this paper are also represented in the >3 micron fraction, there has to be some rationale stated for why they are not presented here. 4. Methods: Update "X" on lines

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19-20. 5. Methods: line 16 specify amplicons from each of X libraries were mixed. . . . to clarify whether this is amplicons from samples from 4 depths from each of 4 stations, or what. 6. Methods: line 17 state 1/4 not 2/8 7. Methods: page 3402 line 4-5 please clarify why SCM and SCMa samples from each station were pooled. You explain this somewhat later in results, but should be explained here instead. 8. Methods page 3402 lines 28-29 specify the number of sites included in alignment, and on page 3403 lines 5-7 specify if only the common sites were used for the phylogenetic analysis, and regardless, how many sites this was. 9. Results: page 3403 lines 10-top of next page: most of this is methods and should be moved. 10. Results page 3407: move some of lines 5-8 to methods or delete. 11. Discussion line 19 page 3407. I would not open with the statement that this is the first comprehensive survey of HFL. . . . using high-throughput. . . . . Consider Framvaren Fjord literature, and Comeau et al. 2011, etc. Maybe just rephrase to one of the first or something like that. 12. Page 3408 line 5: extra "cryothecomonas" in sentence 13. Search MASTS and be consistent with MASTs 14. Consider adding a statement in the Future directions section indicating that an investigation of seasonality may reveal interesting patterns in abundances of particular groups in different water layers.

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