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

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Reply to referees' comments

Dear Prof. Herndl,

We are deeply grateful for these thoughtful reviews. We have incorporated the majority of suggestions and our specific responses to all of the comments are detailed below; Both referees raised questions regarding our classification of HFL sequences; we apologize for lack of clarity, we have modified text elaborating the basis of our approach and have added relevant references. We now provide more details on HFL taxonomic classification of pyroreads, which taxonomic groups were filtered out and the reasoning behind these decisions. Overall we feel the reviews have greatly strengthened our MS

and we hope that you find it acceptable for publication in Biogeosciences.

Best wishes, Adam Monier & Connie Lovejoy

-Referee #1

General comments: 1- Referee: Within methods somewhere authors should discuss how they sorted pyroreads "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 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.

Reply: This is indeed an important point since HFL are polyphyletic and many belong to lineages that may also be mixotrophic. Since the main point of our study was to discover whether non-photosynthetic flagellate communities were structured within the euphotic zone we chose a conservative taxonomic filtering approach. Pyroreads were matched to the lowest taxonomic level possible and we retained only those classified as belonging to taxa that had confirmed heterotrophic life styles. For phyla with mixotrophic representatives, we retained only clades with no representatives that could be considered obligate phototrophs, defined as requiring energy from photons for basic metabolic maintenance. HFL pyroreads were flagged after taxonomic classification at the OTU level (using the mothur Bayesian classifier with an in-house, manually curated, reference sequence database, as in Comeau et al., PLoS One (2011). For taxonomically valid HFL taxa, initial screening was based on Ald et al. (J Eukaryotic Microbiology 2012), as well as recent literature for newer phyla and clades known only from environmental 18S rRNA gene surveys. In sum, we retained the following lineages for our

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HFL classification:

- Marine Stramenopiles (MASTs), (Massana et al., Environ Microbiol 2006) - Bilphytes (now Picozoa); single-cell genomics and culture isolate studies recently showed that this phylum is not phototrophic. (Seenivasan et al., PLoS One 2013). -Choanoflagellates (Adl et al., J Eukaryotic Microbiology 2012). -Rhizarians (Adl et al., J Eukaryotic Microbiology 2012). -Telonemia (Shalchian-Tabrizi et al., Proc Biol Sci 2006). -Centroheliozoa (2 sequences) (Adl et al., J Eukaryotic Microbiology 2012). -Katablepharidophyta (1 sequence) (Lee and Kugrens, Microbiological Reviews 1992).

We also searched for the following HFL reported in Adl et al. (J Eukaryotic Microbiology 2012) but none were present in our datasets: -Apusozoa -Amoebozoa -Heterolobosea -Jakobida -Malawimonadidae

This list of HFL clades is now given in the results section and we have added the above detail to the methods section. We now also state which taxonomic groups were excluded. Along with the obligate phototroph species, we excluded ciliates and dinoflagellates, in the Alveolata supergroup, since these are not usually classed as HFL (Sherr and Sherr, Microbial Ecol 1994). In sum we excluded: - Alveolata -Chlorophyta - Cryptophyta - Euglenozoa - Glaucocystophyceae - Haptophyceae - Rhodophyta - non-MAST stramenopiles including chrysophytes and pelagophytes

In addition, we removed all sequences classified as belonging to multicellular organisms (Fungi/Metazoa and Streptophyta).

We agree that the explanation of percentages of HFL in different samples was confusing (information at both the OTU and sequence levels). We kept only information at the sequence level, it now reads:

"[. . .] HFL sequences represented 14.6% of all sequences (8,697 out of a total of 59,409 sequences; Table S3). HFL sequences (Figure 1B) ranged from 7.5% of all sequences (station 460 SCM) to 28.7% (station 760 SCMb)."

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2- Referee: 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"

Reply: We have now added the percentage of reads that were filtered out of our study (i.e., non-HFL) in the method section. We also added the HFL percentage per sample, at the read and OTU levels, to the table S2.

3- Referee: 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 inïňĆuenced 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.

Reply: We agree that the paragraph was confusing. The MAST communities showed a strong water-mass influence, but this was detected only at the phylogenetic level using UniFrac distances, as shown in the cluster in figure S4. In contrast, the Rhizaria showed a strong water-mass influence at the OTU level.

We therefore analyzed the MAST-1 clade at a finer phylogenetic level (phylogenetic mapping) as a case study to determine trends missed by naïve OTU distribution. We re-wrote this result section; we hope it now reads more clearly and that our goal here is better stated. We now begin the section with:

"Overall water-mass influence on MAST diversity was at the clade and subclade level (Figure 4)... We next investigated MAST taxonomic distribution in the water-masses using higher taxonomic resolution, with the goal of detecting patterns potentially missed by OTU analysis."

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4- Referee: 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.

Reply: Because the figure, which summarizes phylogenetic mapping results, conveys diversity estimates, it was more appropriate to include pyroread counts rather than OTU counts. Adding the read number emphasizes relative abundance to MAST-1 phylogenetic diversity. We increased the font size of the read number to make the figure easier to read.

5- Referee: 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.

Reply: Agreed, we have deleted “all phylogenetic levels”, and the sentence now reads:

“Our analysis of MAST communities showed vertical specificity at the phylogenetic level (Fig. S4), ...”

SpeciïňĄc Comments: 1- Referee: 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.

Reply: We removed “rapidly becoming” from the sentence.

2- Referee: Methods section please specify total water volume ïňĄltered for each sample analyzed.

Reply: We filtered 6 L of water, we have now added more methodological details:

“Six L of water were sequentially filtered through 3 μm pore size 47mm diameter polycarbonate filters then 0.2 μm Sterivex filter units (Millipore) as described earlier (Galand

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et al 2009: Lovejoy and Potvin 2011). The Sterivex units were stored at -80 °C with buffer (1.8 ml of 40 mmol L-1 EDTA; 50 mmol L-1 Tris pH = 8.3; 0.75 mol L-1 sucrose). "

3- Referee: 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.

Reply: Thank you for pointing this out, there was a typographical error and lack of detail in the methods section. DNA was extracted from filters representing the 0.2-3 μm size fraction (see also previous reply). This has been rewritten:

"For this study DNA was extracted from cells collected on the Sterivex units with an aim to enrich the sample with smaller plankton (Terrado et al., 2011)."

4- Referee: Methods: Update "X" on lines19-20.

Reply: All pyrosequences were deposited into the NCBI SRA database under the accession SRA063446. We updated the text to reflect this change.

5- Referee: 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.

Reply: We rewrote this section to make it clearer:

"Primers for individual samples included a Roche multiplex identifier sequence, which enabled pooling of amplicons from all samples in equal quantities. The pooled samples were run on $\frac{1}{4}$ plate, on the Roche 454 GS-FLX Titanium platform at the IBIS/Université Laval Plateforme d'Analyses Génomiques."

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6- Referee: Methods: line 17 state 1/4 not 2/8

Reply: We changed to 1/4 plate (previous reply).

7- Referee: 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.

Reply: We moved to methods the paragraph explaining why we pooled the SCM and SCMa together:

"After preliminary taxonomic analyses, we merged the pyroreads from both SCMa and SCM samples because the communities were globally similar in terms of composition for those two depths at all stations; in addition, a detrended correspondence analysis using untransformed values of temperature, salinity, nitrate concentration, fluorescence and PAR also grouped the SCMa and SCM together."

8- Referee: 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.

Reply: We added the alignment length information. After curation, the alignment was composed of 650 positions. For the phylogenetic placement of MAST-1 pyroreads, we tested only common positions between the reference and pyroread sequences; we added to the phylogenetic placement methodology:

"[. . .] only common alignment positions between MAST-1 reference and pyroread sequences were used for phylogenetic placements."

9- Referee: Results: page 3403 lines 10-top of next page: most of this is methods and should be moved.

Reply: We agree this was redundant with information in the methods section. We shortened it to:

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"After sequence quality filtering (Table S2) and clustering (≥98% identity), OTUs were taxonomically classified; we then targeted and kept only sequences assigned to known HFL taxonomic groups."

10- Referee: Results page 3407: move some of lines 5-8 to methods or delete.

Reply: We removed these lines from the results.

11- Referee: 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.

Reply: We removed "first". It now reads:

"Here we provided a comprehensive survey of HFL communities using high-throughput sequencing [...]"

12- Referee: Page 3408 line 5: extra "cryothecomonas" in sentence

Reply: Thank you for catching that. We removed the extra reference to Cryothecomonas.

13- Referee: Search MASTS and be consistent with MASTs

Reply: All changes were made throughout the manuscript.

14- Referee: 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.

Reply: We agree that this is important to emphasize and have added the following concuding sentence:

"The dramatic seasonality in the Arctic means that microbial communities may rapidly shift due to both succession (Terrado et al., 2008) and water mass movements (Terrado

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et al., 2011) and future studies will likely unveil addition factors favoring the prevalence of HFL taxa".

Referee #2

Referee: [. . .] The main weakness of the study is in the identification of sequences belonging to the functional group of HF. This is done by excluding sequences not known to belong to organisms from this group. Since this excludes an unknown part of the community, at the same time as it seems to me that one easily could include photosynthetic members with similar V8 regions (?) it seems difficult to know whether there is an over- or under-estimation of diversity.

Reply: Our aim was not to estimate diversity but to identify potential niche differentiation among a particular functional category in the upper water column; we have now emphasized this goal in the introduction:

"To test whether the HFL communities can indeed be considered a single guild in the upper water column or if they reflect a potential functional partitioning."

We chose to focus on smaller flagellates that require bacteria or colloidal particles for energy acquisition, to limit confounding effects of light capturing ability found in mixotrophs. We now state our selection criteria more clearly in the methods section:

"Sequences classified as known HFL based primarily on Adl et al 2012 were selected and all other sequences were discarded from this study"

Overall a very small proportion (1.1%) of the sequences were not identified to a trophically known taxonomic level and were also discarded from our HFL study.

Referee: I also could not find a discussion of the fundamental conceptual problem of dividing the flagellate community in two separate parts, as either hetero- or auto-trophs. There is an increasing literature on mixotrophy in the Arctic, with the underlying intriguing question of whether mixotrophy is important in systems with a long dark season. Since two reasons for mixotrophy have been proposed, either the need for energy /car-

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bon or the advantage of taking up limiting nutrients in “pelleted” form, this aspect seems relevant to the differences reported in this manuscript between communities above, in and below the deep chlorophyll max (usually associated both with the nutricline and the transition to the aphotic zone). The discussion on these technical and conceptual problems should be more visible than in the present manuscript.

Reply: We focused on HFL as a means of tracking a single functional group that would not be influenced by light level per se. The aim of our study was to indentify differences in that one functional category; this is now more clearly stated. The diversity of mixotrophs is a very interesting question, and deserves to be dealt with separately given their need to trade of light and nutrients. We agree that an overall description of how different functional groups might interact is interesting, but we strongly believe that the present study, verifying the independent occurrence of taxa placed into the functional category HFL, which occur within different upper water masses, was required prior exploring interactions. We have now added the following as a means of pointing the reader to the need for additional studies.

“The HFL taxa also likely compete with mixotrophic grazers and overall the complexity of microbial eukaryotic communities is underappreciated. Additional studies aimed at revealing predator-prey interactions and detection of significant co-occurrences using a network analysis approach linking eukaryotic, bacterial and archaeal diversities are needed”.

Interactive comment on Biogeosciences Discuss., 10, 3397, 2013.