Review of the manuscript submitted to *Biogeosciences* by Amelie Stieg, Boris K. Biskaborn, Ulrike Herzschuh, Andreas Marent, Jens Strauss, Dorothee Wilhelms–Dick, Luidmila A. Pestryakova, and Hanno Meyer
Diatom shifts and limnological changes in a Siberian boreal lake: impacts of climate warming and anthropogenic pollution

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RC2: 'Comment on egusphere-2024-2470', John Smol, 29 Oct 2024

The authors provide a multi-proxy paleolimnological study from an understudied region in eastern Siberia, providing important limnological data on how lakes from this part of the world are responding to climate and other environmental change. The methodology and study design are sound and their arguments are generally well supported with literature (but see below my comment with respect to how chrysophyte scales were interpreted incorrectly as well as N deposition). The paper is nicely organized and generally well-written. Some grammatical fixes are needed and I've tried to help where I can. The manuscript should be acceptable for publication with minor revisions.

ANSWER: Thank you for this positive assessment. In line with the comments from Reviewer #1, we have revised substantial parts of the manuscript. The minor comments are addressed below.

The 2 issues I note above are detailed below: (but result in only minor changes in writing)

- *The authors mis-read one of my early paper on chrysophyte scale fossils and acidification. The authors cite my 1984 Nature paper – this showed how chrysophyte scales (well 2 species of chrysophytes) could be used for indicating lake acidification and led to many other papers. BUT almost all chrysophytes thrive in circumneutral and alkaline waters – it was mainly only 2 taxa that replaced the many circumneutral and alkaline chrysophytes) that indicated acidification. There are many papers from my lab and elsewhere documenting this. So, the first issue: there is no evidence for acidification from chrysophytes or any other indicator.*

ANSWER: Thank you for the clarification. Our previous interpretation of recent lake acidification at Lake Khamra has also been addressed by Reviewer #1 and is discussed in detail there and in the comments below. In summary, we revised the interpretation of the *Mallomonas* index and omitted the interpretation of acidification from our text. Instead, we agree with the correction and discuss the increase in *Mallomonas* in the context of potentially enhanced thermal stratification of Lake Khamra due to rising temperatures, which is consistent with the observed shift in diatom species in recent decades.

- *Second issue from the chrysophyte interpretation is the incorrect statement and interpretation that chrysophytes indicate higher nutrients. The authors cite a 40 or so year old paper by Munch in support. I have not re-read that old paper but if Munch wrote this over 40 years ago, she was incorrect. Chrysophytes thrive especially in oligotrophic waters – and thrive with declines (not increases) with N and P*

additions. Opposite to what was said in paper. Chrysophytes have diverse nutritional strategies and are flagellated… allowing them to thrive in well-stratified and very nutrient-poor waters. In fact, back when I was a student, I even suggested an index of diatom frustules to chrysophyte cysts to indicate eutrophication (i.e. higher chrysophytes indicating more nutrient-poor waters, not eutrophication). See Smol, J.P. 1985. The ratio of diatom frustules to chrysophycean statospores: a useful paleolimnological index. Hydrobiologia 123: 199- But there are many other papers and reviews documenting this. So, the chrysophyte indicate lower nutrient, not higher.

ANSWER: Thank you for this comment. We have removed the statement regarding chrysophytes indicating higher nutrients. Instead, we provide additional background information on chrysophytes in the discussion (Chapter 4.2.1) and now relate the increase to rising air temperatures and the increased potential for a likely longer ice-free period and enhanced thermal stratification at Lake Khamra. Please find further details in the comments below.

- *The above are actually minor fixes in the paper. And in fact the chrysophytes STRONLY support the diatom and other proxies in that there was NO nutrient nor pH additions from deposition, and the changes you are seeing are solidly linked to declining ice cover and increased thermal stratification. We (and many others) have ben using the rise of chrysophyte scales in sediments to indicate warming and especially increased thermal stratification. Scaled chrysophytes thrive in oligotrophic, and well stratified waters. They are flagellated and are especially competitive in stratified waters since they can control their position in the photic zone etc. So again, the chrysophytes strengthen your argument that this is a climate (ice cover and thermal stratification story) and argues against any aerial deposition story.*

We have many papers on the above, showing chrysophyte scales increase with stratification – but here is one very recent one just published:

Favot, E.J., Rühland, K.M., Paterson, A.M., and Smol, J.P. 2024. Sediment records from Lake Nipissing (ON, Canada) register a lake-wide multi-trophic response to climate change and its possible role for increased cyanobacterial blooms. International Journal of Great Lakes Research 50: 102268.

Or go back earlier and see:

Ginn, B.K., Rate, M., Cumming, B.F., and Smol, J.P. 2010. Ecological distribution of scaled-chrysophyte assemblages from the sediments of 54 lakes in Nova Scotia and southern New Brunswick, Canada. J. Paleolimnology 43: 293-308.

ANSWER: We agree with your interpretation and thank you for suggesting these two important papers. We have incorporated them into the text, particularly in chapter 4.2.1.

In summary, then, it seems that the profiles you have match perfectly with your climate interpretations. What we have been seeing and publishing in many lakes is an increase in scaled chrysophytes, like Mallomonas, with increased thermal stratification and other climate-related

variables. Being planktonic, they can thrive in well-stratified waters, controlling their position in the photic zone. Similar to the Discostella change, we often see a rise in chrysophyte scales with warming. It seems this matches your interpretations perfectly? So, warming seems to be the driver, but not acidification nor eutrophication.

ANSWER: Thank you for this summary comment. We agree with the suggested interpretation and have revised our manuscript accordingly. Please find our answers in detail below.

I address some of this further below with some minor suggestions, especially with respect to the discussion.

Minor comments

Line 19 remove comma after Siberia

ANSWER: done.

Line 23 and elsewhere in text. I think you should put approximate signs (~) whenever you say ~xx year ago… and ca. before all dates. All our dates are approximate.

ANSWER: Thank you for your suggestion. We have incorporated approximate signs (~) and "ca." before all dates where appropriate to reflect that all our dates are approximate.

Line 25. Change less-examined to understudied

ANSWER: Abstract has been revised. This part of the sentence has been deleted.

Line 29 spell out Discostella as first use.

ANSWER: done.

Line 38 same for Asterionella (but see my comments on Asterionella Formosa and climate and not nutrients – Sivarajah et al – as one example)

ANSWER: We revised the abstract and adjusted the argumentation on *A.formosa* accordingly (please find the corresponding answer in the comments below). The sentence is no longer included.

Lines 38 to 39 – see my comments that an increase in Mallomonas does NOT indicate acidification and nutrients, but oligotrophy and warming

ANSWER: Thank you. We revised the abstract and adjusted the argumentation on *Mallomonas*, as suggested.

Line 58. Change experiences to has experienced

ANSWER: done.

Line 62. Unclear what is meant by anthropogenic alteration on the ecosystems. Can you provide examples?

ANSWER: Thank you for your comment. We have reworded the sentence to provide more clarity:

"*Furthermore, climate warming is associated with an increase in wildfire activity (AMAP, 2021), also observed in Yakutia, which is partly due to anthropogenic alterations to the ecosystems <u>including traditional agro-industrial burning practices and the expansion of industrial areas</u> (Kirillina et al., 2020).*"

Line 64. Change There are evidences to There is evidence

ANSWER: We have slightly modified the paragraph, incorporating feedback from Reviewer #1. The sentence now reads as follows:

"*Evidence suggests that recent warming and human-induced pollution are impacting limnological conditions in Siberia.*"

Line 95. Change form to from

ANSWER: done.

Line 99. Suggest, In this study, we examine subfossil diatom assemblages in Lake Khamra and explore whether any changes are consistent with recent climate warming as has been documented in many temperate lakes throughout North America and Europe.

ANSWER: Thank you for your suggestion. We rephrased the sentence as follows:

"*In this study, we analyse subfossil diatom assemblages in Lake Khamra to determine whether taxonomic changes are consistent with recent climate warming, as documented in many temperate lakes in North America and Europe*."

Line 105. Suggest adding the timeframe of the study to objective 1. (I) identify historical lake ecosystem changes within a continuous diatom assemblage record spanning the past ~220 years,

ANSWER: done.

Fig. 1 caption. Change "drilling position of sediment core" to "coring location of"

ANSWER: done.

Methods. The core sectioning details are unclear to me. When it is stated that "The rim material (<0.5 cm) was removed to avoid possible contamination due to mixing", does this mean the core was split and sectioned horizontally? Was there no loss of the surface-most sediments? Was anything done to preserve the sediment-water interface (gel seal? etc) when transporting the core back the lab?

ANSWER: The short core was opened and completely subsampled from top to bottom, as the multiproxy approach had a high demand of sample material. A water-absorbent floral foam (*Mosy*) was used to keep the core as intact as possible during transport, and no gel seal was applied. We have rephrased the relevant sentences and added additional information for clarity:

"*The sediment core with a total length of 42 cm was sealed with water-absorbent floral foam and transported in a PVC tube to the Alfred Wegener Institute (AWI) in Potsdam, where it was stored dark and cool at 4°C until further analysis. The sediment core was subsampled downcore in horizontal 1 cm continuous increments (n=39) in October 2021. To avoid potential contamination between depths due to mixing, rim material (<0.5 cm) was carefully removed from each sample layer. All subsamples were freeze–dried for at least 48 h before further processing."*

Line 213. Change The slide preparation followed the common procedure to Preparation of slides for siliceous microfossils followed common procedures

ANSWER: In line with Reviewer #1's comments, we have revised the sentence as follows:

„*Diatom slides were prepared for each of the 39 samples of the short core EN18232-1 to analyse the species assemblages. Preparation of slides for siliceous microfossils followed Battarbee et al. (2001).”*

Lines 228 to 229 – good to have this index BUT it does not indicate acidification and increased nutrients, but increased thermal stratification etc… see comments above.

ANSWER: Thank you for the clarification. We agree and have revised the sentence and included suggested studies as follows:

“*In addition, silicified chrysophyte Mallomonas scales were counted without further specification to calculate the Mallomonas index, which measures Mallomonas in relation to diatom cells (M/D), to evaluate the degree of thermal stratification and the trophic status (Smol, 1985; Ginn et al., 2010).”*

Line 309, you write “cyclotelloid genus Aulacoseira” -- Aulacoseira is not a cyclotelloid diatom -- I think you meant centric or colonial or centric colonial diatom

ANSWER: Thank you. We changed it to “centric”.

Lines 358 to 359 – yes, clear indication of warming and stratification in your Mallomonas index.

ANSWER: Thank you for your valuable expertise!

Line 435. Thrives instead of thrive ---- but true you can have A Formosa in higher nutrient waters, but also thrives in oligotrophic. See for example:

Sivarajah, B., Rühland, K.R., Labaj, A.L., Paterson, A.M., and Smol, J.P. 2016. Why is the relative abundance of Asterionella formosa increasing in Boreal Shield lakes as nutrient levels decline? J. Paleolimnology 55: 357-367.

This taxon has been increasing strikingly in an area of known declining N deposition and in ultra-oligotrophic waters. We had long-term N deposition in this area showing striking declines as well as in-lake N water chemistry data– and that is when A Formosa thrived and increased.

ANSWER: Thank you for this comment. Regarding the comments of Reviewer #1 we revised and shortened this section of the paper and now primarily focus on the two dominant diatom taxa, *Aulacoseira subarctica* and *Aulacoseira ambigua*. This sentence has been removed. However, we incorporated the suggested reference in the discussion on nitrogen deposition (Chapter 4.4), as noted in a response in a later comment.

Line 458. Hill's N2

ANSWER: The paragraph has been revised. However, we specified the Hill number as follows:

„*After the dry and cold period at the onset of the record, both Aulacoseira species show a clear increase in abundance until the 1940s, reaching their highest abundance of the entire record (Fig. 3). The dominance is accompanied by a decline in effective species richness, as indicated by decreasing Hill's N2 (Fig. 3).*"

Lines 443, 509. Basionym rather than synonym

ANSWER: done.

Line 513. From the northern hemisphere

ANSWER: done.

Line 543. Italicize Fragilaria

ANSWER: done.

Line 568. Replace broader with "most ecologically significant"

ANSWER: In response to Reviewer #1's comment regarding the use of the word 'significant', we have revised it as follows: „*most ecologically important shift*"

Lines 656, 698. Besides, not beside

ANSWER: done.

Lines 703-704 – incorrect interpretation of Mallomonas and acidification as noted above.

ANSWER: As mentioned above and in agreement with Reviewer #1's comments, we omitted the interpretation of *Mallomonas* in relation to acidification and nutrient enrichment. Thank you for the clarification! This previous interpretation has been removed from the text. Instead, we discuss the increase in *Mallomonas* in the context of possibly enhanced stratification of Lake Khamra due to rising air temperatures, in line with the observed diatom species shift:

„*Additionally, we observe a rapid increase in the silicified chrysophyte Mallomonas scales since the 1990s, inferred from the Mallomonas index (M/D) (Fig. 3). Chrysophytes are common in oligotrophic environments (Smol, 1985). Their motility, enabled by flagella, allows them to thrive in stratified lakes by maintaining their position in the photic zone. This gives them an advantage over non-motile, colonial diatoms such as Aulacoseira (Ginn et al., 2010; Mushet et al., 2017). The observed increase in chrysophytes at Lake Khamra suggests changes in the lake's mixing regime. Further it provides evidence for a likely longer ice-free period and enhanced thermal stratification during summer months in recent decades. Similar increases in scaled chrysophytes have been reported in other lake systems, associated with climate warming and increased thermal stability (Paterson et al., 2004; Ginn et al., 2010; Favot et al., 2024).”*

Lines 709-714 – same error (see previous comments)

ANSWER: The interpretation of *Mallomonas* has been revised, please see comment above.

Line 720 – see Sivarajah et al paper on A Formosa and N that I discussed earlier

Line 720. As noted at start of review, an argument is made that atmospheric N enrichment may have caused diatom assemblage shifts and limnological changes. However, the authors do not discuss their d15N profile, which is the primary proxy to either support or refute this hypothesis. The d15N profile in Fig 2 shows no trend of depletion that is outside natural variability over the past 220 years. This is important because it allows the authors to conclude that the increase in D. stelligera and A. formosa in recent years is not related to atmospherically-derived nutrients, as is often questioned with these types of diatom shifts. It is fine to cite these other studies, but your data clearly show no influence of atmospherically-derived N deposition. Also worth reiterating here is that long-range transport of contaminants, eg Hg, is noted to occur at this lake.

ANSWER: Thank you for this valuable comment and clarification. We revised the corresponding section to include information of $\delta^{15}N$ and *A. formosa* and agree that Lake Khamra is likely not influenced by nitrogen atmospheric deposition, based on our data. We now address this in the discussion as follows:

“*In contrast to the recent marked increase in mercury levels and $\delta^{13}C$ depletion, $\delta^{15}N$ in Lake Khamra sediments has shown only minimal variation over the last ~220 years, fluctuating by ±0.5‰, with a slight decrease of ~0.3‰ since the 1970s (Fig. 5). Human activities, such as fossil fuel combustion and fertilizer production, are relevant sources of reactive nitrogen (Nr) that contribute to the deposition of $\delta^{15}N$-depleted nitrogen in lake sediments, typically in a range from 1–3‰ (Gruber and Galloway, 2008; Holtgrieve et al., 2011; Wolfe et al., 2013). Despite the possible influence of atmospheric pollution, no substantial $\delta^{15}N$ depletion is observed in Lake Khamra. However, not all lakes display $\delta^{15}N$ depletion, as nitrogen cycling in lakes is complex, and factors such as nitrogen inputs, water residence time, and aquatic activity play crucial roles (Meyers and Teranes, 2001; Galloway et al., 2003; Anderson et al., 2018). For example, increased abundance of A. formosa in oligotrophic alpine lakes in North America has been linked to atmospheric nitrogen deposition (Saros et al., 2005; Saros et al., 2010). At Lake Khamra, we observe only a slight increase in planktonic A. formosa in the most recent diatom zone 5 (Fig. 2). We suggest that this slight increase in abundance is more likely a response to*

climate warming and related changes in like water mixing and thermal stability rather than nitrogen enrichment by atmospheric deposition (Sivarajah et al., 2016). This further supports the argument that Lake Khamra is primarily influenced by recent climate warming, which is altering the lake's properties, rather than by atmospheric nitrogen deposition from human sources."

As noted above, the author also incorrectly used the increase in Mallomonas scales as indicating aerial transport, but the chrysophyte results indicate exactly opposite interpretations to this conclusion. The important change in chrysophytes near the surface indicates low nutrients and do not indicate acidification. In fact, we and many others, see changes like this is scaled chrysophytes indicating warming – these taxa thrive in thermally stratified waters (their flagella etc give them important advantages in oligotrophic and stratified waters).

The authors have a much simpler (but important) story here – a clear indication of the lake changing markedly with less ice and stronger and longer thermal stratification.

Nice contribution.

John Smol