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Re: Review of Agnieszka Szczerba's PhD thesis – "Tracking climate signals in lakes of northeastern Poland: modern sedimentation studies using chrysophyte cysts and diatoms.

There is an urgent need to better assess the effects of climate change and other environmental stressors on Earth's ecosystems, and this is especially true for our freshwater resources. The problems are complex, as we are dealing with multiple stressors operating at different spatial and temporal scales. The lack of long-term monitoring data only makes these challenges more difficult to address.

Given the lack of long-term monitoring data, scientists have developed indirect proxy methods to reconstruct past environmental changes over critical time periods – most often over the last 200 or so years (the period of accelerated anthropogenic impacts). Fortunately, for lake systems, sediments provide an archive of past environmental changes that skilled scientists can use to reconstruct the missing monitoring data -- namely the field of paleolimnology. Paleolimnology has made tremendous progress over the last few decades and has become a major tool for ecosystem assessment. However, in order for paleolimnologists and other scientist to effectively use biomarkers (such as diatoms and chrysophytes, which are used in this thesis) in

lake sediments, we need an understanding of the present-day ecology of indicators, including their seasonality, to fine-tune these reconstructions.

For reasons that I outline below, Ms Szczerba's PhD thesis is a major contribution to the field of phycology and limnology, and then also paleolimnology, addressing many key issues, using state-of-the-art approaches.

Before I address the scientific contributions in this thesis, I feel I should make some comments on the flawless English used in the thesis. I am frankly amazed how well written this thesis is, given the first language of the candidate is not English. It certainly is better than the writing of many PhD student I know whose native language is English. Very impressive.

Before I go into individual papers, in a general sense, the thesis is highly impressive from a number of fronts. First, many PhD candidates work on only one proxy – as becoming expert in, for example, diatoms (which are also used in these papers) is a very difficult task, with thousands of species. Learning just diatom taxonomy is very time consuming. Not only does Ms Szczerba master diatoms, but she also works on chrysophyte cysts. My lab did some of the earliest work on chrysophyte cysts, about 20-30 years ago, and even we have slowed that work as it is very difficult. The taxonomy is complex and difficult requiring very high-resolution microscopy as well as other challenges noted in this thesis. Yet, we were certain, even 30 years ago, that they are highly sensitive biomarkers. Ms Szczerba's PhD thesis clearly demonstrates that our early hunches were correct, as she has effectively shown how they respond to several key environmental stressors. In addition, her thesis effectively covers a thorough analysis of limnology and statistical treatments and other approaches. Such integrative approaches are required to assess the complexities linked to limnological changes induced by climate warming and other stressors. This is an impressive piece of work.

The thesis opens with the Introduction, which includes the rationale for the study and a literature review. There is no doubt that this study is of key importance, given the many changes our freshwater resources are subject to. Key factors linked to warming, such as declining ice covers and changes in thermal stratification, are correctly identified as being especially important and are well reviewed with the key literature cited. The State of Research section is equally excellent, documenting an important review of the state-of-the-science of using both diatoms (which are reasonably well studied) and chrysophyte cysts (which is a very novel aspect of the thesis). This section provides a thorough background and documents a clear understanding of the literature. Chapter 1 ends with clear statements of the hypotheses and objectives of the remaining 3 chapters.

In order to use proxies such as diatoms and chrysophyte cysts to reconstruct the missing data sets from the sedimentary record, we have to understand what environmental drivers are key in determining the composition of these assemblages. This thesis directly addresses these pressing questions.

To achieve the goals of this thesis, and as summarized in the Materials and Methods section, the thesis represents three and a half years of high-resolution monitoring records from three strategically selected Polish lakes, where the physical and chemical limnological variables were measured in the water column, diatom and chrysophyte cyst assemblages were collected and analyzed from sediment traps, and meteorological data were used as a potential factor explaining changes in assemblage composition. These data and insights can then be used to help interpret paleolimnological changes in these proxies. The results of this thesis work are summarized in 2 published papers, and one paper listed as submitted.

The first paper, published in 2021 in *Ecological Indicators*, uses sediment trap studies to explore the relationship between chrysophyte cyst assemblages and meteorological conditions. As noted earlier, chrysophyte cysts are poorly studied, but have great potential as bio-indicators – and more-so after the results of this study. This study is very novel – first for the taxonomy and use of chrysophyte cysts (a very under-studied but important group), and second for using sediment traps. The bi-weekly samples of assemblages and limnological variables, coupled with the meteorological data, reveal an indirect relationship of climate change on cyst assemblages, namely changes in lake mixing regimes (not a trivial connection as these changes affect all aspects of lake ecology). This provides a clear foundation for using chrysophyte cysts in other limnological and especially paleolimnological applications, tracking changes in lake ecology linked to warming and other stressors. Information on seasonality is key. We now have data on these relationships based on this thesis work.

The companion paper, also published in *Ecological Indicators*, but in 2023, now examines diatom assemblages to changes in lake conditions and especially those linked to meteorological changes. Three years of high-resolution data were gathered from two lakes. Air temperature and wind speed – two variables closely linked to recent climate warming – were identified as key drivers, which would alter limnological conditions and hence diatom assemblages. As with the previous paper, this is a very well executed study requiring the mastering of a completely new set of difficult taxonomic identifications, limnological analyses, statistical treatments, and interpretations. In contrast to chrysophyte cysts, diatoms are the most commonly used proxy indicators used in paleolimnological studies – nonetheless so new insights, such as those provided here, will have a very broad audience.

The third paper, submitted to *Freshwater Biology*, blends both chrysophyte cysts and diatom dynamics, as well as detailed limnological changes, with meteorological observations in a

eutrophic Polish lake. The results again point to a direct influence of meteorological conditions on the physico-chemical changes and taxonomic changes in diatoms and chrysophyte cyst morphotypes as well as fluxes. Temperature and wind speeds are again identified as key variable influencing mixing regimes. Interestingly (and very importantly), the period of study (luckily for scientists) included one warm winter when no ice formed – the authors seized on this opportunity to more fully explore the role of ice cover on assemblages. Once again, this study shows (in addition to a lot of hard work!), insights on the complex relationships of key algal assemblages and multiple stressors, not least of which are changes in climate. I am sure this third paper will soon be published.

On page 37 of the thesis, Ms Szczerba provides important guidance for future research based on her and her colleagues' findings.

This is an excellent and well-executed thesis, demonstrating expertise in taxonomy, ecology, limnology, chemistry, physics, and statistics – and of course field work. In my view, the thesis clearly passes any international threshold required for the granting of a PhD. Given the many new insights, the complexities of the issues addressed, the high quality of the analyses and writing, *I would recommend that this thesis be passed with distinction*.

Please let me know if you require any additional information.

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