

# Responses to the Reviewer's Questions

**PhD Dissertation of Bassam Hussein Ibrahim Abubaker**

**“REVEALING DIATOM DIVERSITY, RARE TAXA AND THEIR ENVIRONMENTAL DRIVERS THROUGH COMPLEMENTARY eDNA METABARCODING AND MICROSCOPIC APPROACHES”**

Reviewer: Dr. Danijela Vidaković

**1. Given the current limitations of DNA metabarcoding, including incomplete reference libraries, PCR bias, and difficulties in abundance estimation, what level of confidence can we realistically place in metabarcoding-based ecological status assessments compared to traditional microscopy-based approaches?**

**Response:**

DNA metabarcoding can currently be regarded as a highly informative and reliable approach for detecting community-level ecological patterns and responses to environmental gradients, but it should not yet be considered a fully interchangeable quantitative replacement for microscopy in all biomonitoring contexts. The main sources of uncertainty include incomplete reference libraries, PCR amplification bias, and the imperfect relationship between sequence-read abundance and the actual abundance or biomass of organisms. These limitations are particularly important when species-level taxonomic assignment or direct abundance-based interpretation is required.

At the same time, the results of this dissertation show that these limitations do not prevent DNA metabarcoding from recovering strong and ecologically meaningful signals. In Chapter III, the community–environment relationship was significant for all three biological datasets, but the strongest co-structure with environmental variables was obtained for diatom metabarcoding ( $RV = 0.36$ ), followed by diatom microscopy ( $RV = 0.27$ ). Metabarcoding also produced the highest median per-sample richness, with 60 taxa compared with 33 taxa for diatom microscopy and 21 taxa for phytobenthos microscopy. These results indicate that molecular data can be highly sensitive to environmentally structured changes in diatom communities.

For routine regulatory biomonitoring, the most appropriate current strategy is a complementary one in which DNA metabarcoding is increasingly integrated with microscopy while reference libraries, standardized protocols, cross-laboratory quality assurance, and quantitative approaches continue to improve.

**2. One of the most interesting findings is that many rare species occupy core positions in the ecological network. How do you explain the apparent contradiction between low abundance and high ecological importance?**

**Response:**

Low abundance and high ecological importance are not necessarily contradictory because they describe different properties of a species within a community. Abundance describes how many individuals of a species are present, whereas network position describes how that species is associated with the structure of the community. A taxon may therefore occur at low abundance but still occupy a central position if its occurrence is strongly connected with many other taxa or if it contributes to the organization and turnover of the community.

The interpretation of the results in Chapter II. supported the community-variability analyses. Rare-species subcommunities contributed disproportionately to total community variability: the contribution was about 50% higher for small-population and geographically restricted species, about 40% higher for absolutely rare taxa, and about 25% higher for habitat specialists. Rare taxa also made a strong contribution to beta-diversity and species replacement among sites. Together, these findings support the idea that rare species can act as a hidden structural component of the community and may contribute to ecological stability, resilience, and the maintenance of diversity.

**3. Given the difficulties associated with sampling effort, changing taxonomy, species detectability, and the definition of rarity itself, how confident can we be in our current understanding of rare diatom species, and what should be the priorities for future research in this field?**

**Response:**

Our current understanding of rare diatom species is sufficiently strong to conclude that rarity is an ecologically important component of benthic diatom communities, but greater caution is required when classifying an individual species as universally rare. Rarity is context-dependent and can change with spatial scale, sampling intensity, environmental conditions, taxonomic revisions, and the probability of detecting a species. For this reason, a species that appears rare in one river network or sampling period may not necessarily be rare in another region or under different ecological conditions.

To reduce the limitations of a single abundance threshold, Chapter II used a multidimensional framework based on population size, habitat specificity, and geographic range. This approach provides a more ecologically meaningful interpretation of rarity than defining rare taxa only by low abundance or low frequency of occurrence.

Future research should therefore focus on improving both the reliability of rarity classification and our understanding of its ecological consequences. Longer-term and geographically broader sampling would help distinguish persistent rarity from apparent rarity caused by limited sampling. Continued taxonomic revision, expert morphological verification, and the expansion of high-quality genetic reference libraries would improve species detection and taxonomic

consistency. Combining microscopy and DNA metabarcoding would also help reduce method-specific detection bias.

**4. In your dissertation, you state that “rather than asking which method is universally better, we should ask under what conditions each method performs best, what kinds of diversity each captures, and how their outputs can be integrated without losing ecological information.” After completing this research, how do you envision the future role of these methods in biomonitoring? What would an ideal monitoring framework look like, and which tasks would be best addressed by each method?**

**Response:**

The main conclusion of this dissertation is that the future of diatom biomonitoring should be based on complementarity rather than on replacing one method with another. Microscopy and DNA metabarcoding capture partly different dimensions of biodiversity, and each method has specific strengths that are valuable for ecological assessment.

Microscopy remains essential for morphological interpretation, taxonomic validation, and continuity with long-term historical monitoring datasets and established diatom indices. It is especially valuable when diagnostic morphological characters must be examined, when newly described or poorly barcoded taxa are present, or when direct cell-based relative abundance is required. Its main limitations are the time required for identification, dependence on highly trained taxonomic expertise, and the possibility of overlooking cryptic or very low-abundance taxa.

DNA metabarcoding is particularly well suited to high-throughput biodiversity assessment, the detection of rare and cryptic diversity, comparisons across large numbers of samples, and the analysis of fine-scale spatial and temporal community patterns. The results of Chapter III support this role: metabarcoding produced the highest per-sample richness and the strongest relationship between community structure and measured environmental gradients. Retaining ASV-level information can also preserve ecological variation that may be lost when sequences are forced into incomplete taxonomic reference frameworks.

Based on the results of this dissertation, an ideal monitoring framework would combine both approaches in a coordinated way. A practical model would use standardized DNA metabarcoding as a broad and scalable routine screening tool, while microscopy would be retained for taxonomic verification, calibration, quality control, difficult or unrepresented taxa, and maintenance of continuity with established ecological-status systems.

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