From molecules to monitoring: integrating genetic tools into freshwater quality assessments

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SUMMARY

Freshwater is an important resource, not only for the ecosystem services it provides to humankind, but also as a habitat for many species. Freshwater ecosystems are, however, at great risk of species decline due to habitat loss and modification, pollution and over-exploitation, and invasive alien species. European and national regulation dictate the monitoring of freshwater quality in the Water Framework Directive (WFD). Quality assessment of freshwater makes use of different criteria that define its health and impact status. The biological elements of these assessments focus on the organisms living in freshwater systems, such as fish, macroinvertebrates, and plants. Traditional monitoring of quality relies on labor-intensive and expensive collection and morphological identification of specimens. Recent developments in molecular techniques allow for easier identification through (meta)barcoding and species detection using environmental DNA (eDNA).

Comparative studies assessing both traditional methodology and DNA-based analyses are important in the transition from the former to the latter for ecological quality ratio (EQR) assessments. Since traditional methods have been set up with limitations of these methods in mind, DNA-based techniques do not necessarily line up with requirements set forth in the WFD. One important point of contention is the use of abundance data in EQR scoring. DNA metabarcoding methods are prone to technical biases that obfuscate the original biomass or specimen counts. In Chapter 2 we investigated the influence of abundance data on the EQR scoring according to the Dutch EQR calculation system. By comparing EQR scores on historical data with abundances removed to the original EQR scores of those samples, we found that abundance data was of limited influence. The strong correlation between the scores with and without abundance would allow for DNA-based species lists to be used for WFD assessments, opening the way for the introduction of barcoding-based methods into routine quality monitoring.

Studies comparing traditional morphology-based assessments with DNA and eDNA metabarcoding also highlight differences caused by underlying issues such as the difficulty of identification with morphological keys for certain taxa, or the inability to distinguish other taxa using DNA barcodes. Chapter 3 illustrates these issues, where we obtained a similar number of taxa using DNA metabarcoding as were observed using morphological assessments. There were, however, large

differences between the taxa lists of both methods, with less than 60% overlap between the two. Simple taxonomic sorting alleviated some of the before-mentioned technical biases, and our results clearly show the effects of preferential amplification in complex bulk samples. Impact of the differences between the species lists on EQR scoring were considerable, but DNA metabarcoding allowed for much more detailed information in morphologically hard to identify taxonomic groups, such as chironomids. Integration of DNA-based identifications for such groups would allow for more accurate EQR status assessments.

In addition to DNA-based identifications, environmental DNA is a game-changer for freshwater assessments, as it allows for simpler, cheaper, and more easily standardized sampling. There are, however, many unanswered questions regarding the behavior—or "ecology"—of eDNA within the aquatic environment. In Chapter 4 we explored the impact of replicates in various steps of the analyses on richness estimations and community patterns. While the effect of PCR replicates was limited, the effect of sampling replicates was considerable. Dissimilarities between replicates were high, revealing the heterogenous distribution of eDNA within a waterbody. Furthermore, the weekly sampling of the same two study sites showed that temporal replicates were even more dissimilar than the spatial replicates. This suggests that turnover effects might be more important for the dynamics of eDNA than its spatial heterogeneity. Many studies fail to incorporate these dissimilarities into their study design, meaning that between-site comparisons done over longer time periods are probably affected by inflated dissimilarities.

One of the main issues with using environmental DNA is that it produces many DNA profiles (Molecular Operational Taxonomic Units, or MOTUs) that cannot be directly linked to a known taxon, due to incomplete databases, but also due to undescribed diversity that has not been morphologically observed. However, eDNA still lends itself for comparative studies that look at patterns between, for example, impacted and non-impacted sites. In Chapter 5 we performed such an impact assessment using eDNA, to investigate the effects of the neonicotinoid insecticide thiacloprid and fertilizer, two of the main agricultural stressors on freshwater systems. Using eDNA, we assessed three different taxonomic groups that represented three trophic levels in the ecosystem: bacteria (composers), phytoplankton (primary producers) and chironomids (consumers and key indicator species). This experiment was performed in a unique "Living Lab", allowing for a controlled experiment in a semi-natural environment. Using a full-factorial setup with many replications also allowed for the disentanglement of single stressor effects. By conducting the experiment at the same time as a morphology-based assessment on the same impacted

sites we were also able to compare results directly to traditional methods. For all three groups assessed, similar patterns of stressor impact were observed over time for both stressors, suggesting that agricultural stressors affect the entire food web, either directly or through cascade reactions. The patterns were also consistent with morphological assessments, with a lower number of technical replicates. This shows that the use of multi-marker environmental DNA provides a more comprehensive assessment of stressor impacts on an ecosystem as a whole, with a higher taxonomic resolution than traditional surveys. We also found over a thousand MOTUs that were indicative of stressor absence or presence, some of which can be putative new bio-indicators for both agricultural stress of freshwater.

There are numerous questions that still need answers, as discussed in Chapter 6. Not only on how DNA data is translated into traditional taxa, but also on the optimization of sampling strategies and the ecology of eDNA. The research presented in this thesis, however, along with the increasing number of publications on similar topics, show that DNA-based methods have great potential for freshwater quality monitoring and impact assessment. The incorporation of these techniques will contribute to a better ecosystem understanding and allow for more effective monitoring and management of freshwater systems, safeguarding the ecosystem services provided to humankind. For successful integration into ecosystem assessments, it is also important in this perspective to involve monitoring agencies and policy makers, by demonstrating the possibilities of DNA-based methods and including them in the development of molecular tools.