A *tufA* metabarcoding approach for *Ulva* and related seaweeds

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Received: 04 Mar 2021 | Published: 04 Mar 2021

Citation: Weinberger F, Steinhagen S, Karez R, Bonthond G (2021) A *tufA* metabarcoding approach for *Ulva* and related seaweeds. ARPHA Conference Abstracts 4: e65495. [https://doi.org/10.3897/aca.4.e65495](https://doi.org/10.3897/aca.4.e65495)

**Abstract**

*Ulva*-like green algae are notoriously difficult to distinguish due to their morphological variability and/or similarity. DNA barcoding approaches are therefore currently essential for their reliable identification. However, such approaches often fail when rare or inconspicuous species are to be detected in large mixed populations of Ulva species, for example, at early stages following the introduction of species into new habitats. We therefore developed a detection method based on next-generation DNA sequencing. The approach is suitable for the analysis of DNA traces in preserved water samples or in particles enriched by filtration from such samples. A new pair of primers was designed to amplify a 475 bp segment within the *tufA* marker gene. The primers were relatively group specific. 68.5% of all reads obtained after quality filtering represented the genus *Ulva*, 11.1% other Ulvophyceae, and only 20% other Chlorophyta, despite their relatively higher abundance in phytoplankton. The relatively short target amplicon still allows good differentiation of Ulvales and Ulothrichales at the species level. Using a database containing *tufA* sequences of 879 species - 281 of which were Ulvophyceae and 35 *Ulva* - we were able to detect mostly Ulvophyceae that had been previously detected in our study area in northern Germany using Sanger sequencing. However, the number of species detected at individual sites was generally higher than in previous studies, which could be due to drifting DNA: Analysis of samples collected at different distances from shore suggests that a sample collected at a given site may be influenced by Ulvophyceae within a radius of up to about 1 km in winter. In summer, this radius is reduced to less than 100 m,
possibly due to the less frequent occurrence of strong wind events. Nonetheless, rare species may be detected with this new approach: At one site, an undescribed *Blidingia* species that was not previously known from our study area was repeatedly detected. Based on these findings, the species was searched for and found, and its identity confirmed by traditional tufA barcoding.

**Keywords**

Metabarcoding, *Ulva*

**Presenting author**

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**Presented at**

1st DNAQUA International Conference (March 9-11, 2021)