Assessing the conservation effectiveness of Natura 2000 areas for freshwater communities using environmental DNA

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Although covering less than 1% of the Earth's surface, freshwater habitats host a high proportion of the global animal biodiversity and provide essential ecosystem services. Such habitats, however, are experiencing some of the fastest rates of biodiversity decline and are among the most threatened ecosystems worldwide. To evaluate the effectiveness of Natura 2000 protected areas in conserving freshwater related biodiversity, we combined extensive eDNA-based monitoring with the assessment of water physicochemical and geomorphological parameters to compare streams located within protected areas and adjacent unprotected streams of the Northern Apennines hydro-ecoregion (Central Italy). We specifically compared community richness, taxonomic and phylogenetic diversity, species composition, environmental quality, and the occurrence of species of conservation concern within and outside the Natura 2000 sites. Using a multi-marker metabarcoding approach, we detected a broad diversity of freshwater and terrestrial vertebrates and invertebrates, recording a total of more than 1000 taxa. We found that protected and unprotected sites exhibited comparable environmental conditions and did not differ significantly in overall species richness. The streams within protected areas, however, hosted freshwater communities characterised by a significantly higher taxonomic diversity and a large number of aquatic species of conservation interest. Our results support the effectiveness of riverine eDNA monitoring for comprehensive biodiversity assessments and suggest that, although Natura 2000 areas effectively preserve freshwater related community diversity, their design could be further optimised to enhance freshwater conservation outcomes.