Year-round monitoring and large-scale screening of native and invasive crayfishes in lotic systems

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Abstract

Freshwater crayfish are among the most threatened animal taxa in Central Europe. Effective conservation measures for endangered native and management of invasive alien crayfishes requires knowledge about distribution, monitoring of existing and early detection of newly established populations. eDNA has recently emerged as a promising, highly sensitive, and non-invasive detection tool in this regard.

To evaluate eDNA as detection tool for freshwater crayfish, we developed a novel set of specific eDNA-assays for all native (Austropotamobius torrentium, Austropotamobius pallipes, Astacus astacus) and the most relevant invasive crayfish species (Pacifastacus leniusculus, Faxonius limosus, Faxonius immunis) in Central Europe. To ensure specificity each primer pair was tested in silico, in vitro, and in situ, including a total of 13 lotic and lentic waterbodies (Fig. 1). Moreover, we assessed the influence of spatio-temporal variables (distance to upstream population, season, and stream size) on eDNA detection in seven streams using two different detection methods (qualitative endpoint PCR and quantitative droplet digital PCR, ddPCR).
The newly developed eDNA assays successfully detected all crayfish species across different lotic and lentic habitats. Multiple linear mixed-effects analysis revealed a significant effect of distance and season on eDNA detection rate (endpoint PCR) and eDNA-concentration (ddPCR). Year-round detection was successful up to 7 km downstream of the source population, although detectability was lowest in winter. eDNA detection rate further decreased with increasing stream size. Finally, eDNA-concentration correlated positively with estimated upstream population size.

Overall, our study provides easily applicable eDNA assays for six crayfish species, enabling year-round detection, which represents a clear benefit over conventional methods. Due to its high sensitivity, eDNA detection is also suitable for the targeted search of as-yet unrecorded or newly emerging populations. Using quantitative ddPCR might further allow for a rough estimation of population size, provided that the identified spatio-temporal factors are accounted for. We therefore recommend implementing eDNA-detection as a complementary survey tool, particularly for a large-scale screening of data-deficient catchments or a year-round monitoring (Chucholl et al. 2021).

**Keywords**

environmental DNA, species detection, crayfish, freshwater systems, monitoring
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