



Conference Abstract

Environmental DNA monitoring of noble crayfish *Astacus astacus*: Comparison and refining of methodology

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Received: 24 Feb 2021 | Published: 04 Mar 2021

Citation: Strand DA, Johnsen SI, Fossøy F, Rusch JC, Sandercock BK, Vralstad T (2021) Environmental DNA monitoring of noble crayfish *Astacus astacus*: Comparison and refining of methodology. ARPHA Conference Abstracts 4: e65010. <https://doi.org/10.3897/aca.4.e65010>

Abstract

During the past decade, environmental DNA (eDNA) methodology has become an important non-invasive tool to monitor aquatic micro- and macro-organisms, including freshwater crayfish. In Europe, noble crayfish *Astacus astacus* is the most widespread native freshwater crayfish. However, the species is threatened in its entire distribution range. It is therefore included on the International Union for Conservation Nature (IUCN) red list, and on several national red lists. Reliable monitoring is essential for implementation of conservation measures. For crayfish, traditional population trends have been obtained from catch per unit effort (CPUE) data. In order to successfully apply and use eDNA monitoring for noble crayfish, or any species, it is a prerequisite to know the strengths and weaknesses of the applied methods and how they perform compared to traditional methodology. Sampling strategy and analysis methodology also depends on choice of species to be monitored, and which questions to be answered. Further, refinement of the employed methods may improve the detection probability for eDNA monitoring. Here we report the results from 1) a recently published study on noble crayfish eDNA monitoring (Johnsen et al. 2020) and 2) an ongoing study comparing and optimising the methods used for monitoring noble crayfish.

1) We compared eDNA monitoring (transects with ten 5L samples) with traditional trapping (transects with 50 traps) for noble crayfish in lentic habitats, in order to evaluate detection probability and if eDNA concentration correlates with relative density of crayfish. We also compared two commonly used analytical methods [quantitative real-time PCR (qPCR) and droplet digital PCR (ddPCR)] for eDNA monitoring. We found that qPCR outperformed ddPCR in detection frequency (Fig. 1), most likely due to some inhibition in the ddPCR analysis. eDNA monitoring provided reliable presence/absence data for noble crayfish, even in lakes with very low crayfish densities. Detection frequency increased with increasing CPUE (Fig. 1). However, we did not observe any correlation between relative crayfish densities and eDNA concentrations of crayfish. eDNA concentrations were consistently very low, even in lakes with very high crayfish densities. For lakes with very low crayfish densities, we estimated that ~5 samples (5L samples) are needed for 95 % detection likelihood, while for lakes with high densities 2 samples were needed.

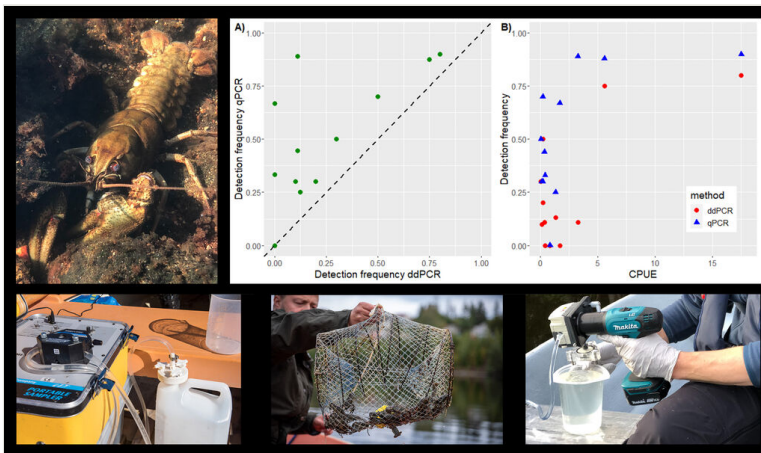


Figure 1. [doi](#)

Top left: Noble crayfish (*Astacus astacus*). Top right: Graph showing A) a comparison of the observed detection frequency (proportion of positive eDNA samples in a lake) between qPCR and ddPCR. The dotted line shows the 1:1 ratio. B) A comparison of the observed detection frequency between CPUE against ddPCR and qPCR. Bottom photos: eDNA sampling and trapping.

2) We compared two eDNA sampling strategies (sampling from bottom or the surface), commonly used for crayfish or fish in Norway to investigate how both strategies perform. The sampled filters were divided and two DNA extraction protocols were evaluated (CTAB based vs Column based). We found that the DNA yield was higher from the column based DNA extraction protocol, and that eDNA concentrations from fish (brown trout *Salmon trutta*, northern pike *Esox lucius* and European perch *Perca fluviatilis*) were significantly higher than for crayfish. For crayfish and brown trout, there was little difference between detection probability for bottom and surface samples, while for northern pike and European perch the detection probability was higher for the bottom samples. Currently, we are analysing eDNA

samples collected with glass fibre filters and NatureMetrix filters for noble crayfish in both lentic and lotic habitats and the preliminary results will be presented.

We conclude that eDNA monitoring cannot substitute CPUE monitoring for freshwater crayfish, but it offers reliable presence-absence data, provided sufficient sampling efforts. Thus, it is suitable for large scale monitoring of threatened crayfish and combined with eDNA analysis of alien crayfish and diseases such as crayfish plague, this is a cost-efficient supplement offering a more holistic approach for aquatic environments and native crayfish conservation. Furthermore, the synergy effect of using collected eDNA samples from different projects to monitor additional species is substantial.

Keywords

eDNA, occupancy modeling, qPCR, ddPCR, CPUE, detection frequency, relative density, method optimisation,

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Presented at

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

References

- Johnsen SI, Strand DA, Rusch JC, Vralstad T (2020) Environmental DNA (eDNA) Monitoring of Noble Crayfish *Astacus astacus* in Lentic Environments Offers Reliable Presence-Absence Surveillance - But Fails to Predict Population Density. *Frontiers in Environmental Science* 8 <https://doi.org/10.3389/fenvs.2020.612253>