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Conference Abstract

# Getting rid of rain and stars: mitigating inhibition effects in ddPCR assays, the case of the invasive crayfish *Pacifastacus leniusculus* in the streams of Luxembourg

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## Abstract

ddPCR is getting more and more popular in the field of eDNA-based aquatic monitoring. Even if emulsion PCR used in ddPCR confers a partial resistance to inhibition due to the high number of reactions for the same sample (between 10K and 20K), it is not impervious to it. Inhibition impacts the fluorescence amplitude of positive droplets, affecting both their dispersion and their position relatively to the negative droplets cloud. This fluctuation could jeopardize the use of a shared threshold among several samples and thus the objective assignation of the positive droplets.

This is even more critical for low concentration samples such as eDNA samples: the positive droplets are scarce and it is thus crucial to objectively discriminate if they can be counted as positive by establishing an appropriate threshold. Another issue is the artifactual generation of high fluorescence droplets that could be counted as positive with a single threshold solution.

Here we propose a double threshold method to take both high fluorescence droplets and PCR inhibition impact into account allowing for an objective sorting of the positive and negative droplets in ddPCR assays.

## Keywords

ddPCR, inhibition, data treatment, crayfish, Pacifastacus leniusculus, streams, eDNA, invasive species

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