



Conference Abstract

# Application of microfluidic high-throughput quantitative PCR systems for the monitoring and surveillance of aquatic species

Luca Mirimin<sup>‡</sup>, Dennis van der Pouw Kraan<sup>‡</sup>

<sup>‡</sup> GMIT, Galway, Ireland

Corresponding author: Luca Mirimin ([luca.mirimin@gmit.ie](mailto:luca.mirimin@gmit.ie))

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## Abstract

Quantitative PCR (qPCR) has been increasingly used for the detection of target organisms in environmental DNA (eDNA) studies, and this is thanks to high sensitivity and ability to quantify DNA targets copy number. However, prior to their implementation, qPCR species-specific assays must be developed and validated and, when implemented, they are limited to relatively low number of targets that can be screened as a multiplex or in parallel. Thanks to recent technological advances, several qPCR-based platforms have become available to increase the throughput capability of qPCR systems as well as lowering time of execution and costs associated to sample processing. The present study describes the use of a microfluidic high-throughput qPCR/dPCR system (Biomark HD, Fluidigm) for the screening of species of ecologic and economic importance in bulk plankton environmental samples from marine coastal areas around the Irish coast.

Data was generated using the configuration enabling the highest throughput (in terms of data points) of the system, including Integrated Fluidic Control (IFC) units capable of producing 96 x 96 sample/assay combinations in each run (9,216 individual qPCR tests in a single run). Thanks to such a capability, it was possible to execute the following three main development and implementation phases in a relatively short period of time (weeks as opposed to months/years): (i) development of a panel of species-specific assays

targeting a range of crustacean and bivalve species; (ii) assessment of Limits of Detection (LOD), Limits of Quantification (LOQ), and enzymatic inhibition control for selected assays; and (iii) screening of environmental time-series samples ( $n = 242$ ) obtained from a citizen-like sampling effort that involved a range of stakeholders and locations throughout the Irish marine coastal territory between 2019 and 2020.

During the assay-development phase, the IFC system configuration (whereby all assays are tested in parallel against all samples) enabled the rapid screening of species-specific assays against a wide range of (genomic DNA of) non-target organisms, hence enabling for rapid specificity testing. LOD/LOQ experiments showed high levels of sensitivity and thanks to the large number of assays that could be accommodated in a single run, it was possible to include up to four distinct Internal Positive Controls (IPCs) at different concentrations in each run (hence controlling for potential inhibition at different target concentration levels). The inclusion of inhibitor-removal reagents in a pre-amplification step as well as the dilution factor of conducting reactions in small volumes (6.7 nL reaction volumes, hence comparable to a “digital PCR” effect) proved to be an effective strategy to reduce the effect of inhibitors in control experiments (humic acid and EDTA), as well as in actual environmental samples from a range of marine environments. Combining such a high-throughput screening platform with a nation-wide citizen science-like sampling programme enabled the acquisition of large datasets that are being used to monitor occurrence and (spawning) activity of important species that are of conservation concern, commercial value, or non-indigenous and invasive to Irish waters. The Biomark HD system provides a remarkable flexibility to modify existing and/or incorporate new assays because IFCs are customizable just prior to usage (i.e. are not pre-loaded or spotted with primers/probes), thus current work is focussing on increasing the number of species targeted in a single run, and (thanks to the quantitative nature of data) discriminating between different fractions of DNA in heterogeneous bulk samples (e.g. gametes and larvae vs intra- and extracellular eDNA).

Thanks to low sample processing cost, assay flexibility and high-throughput capability, microfluidic qPCR platforms behold the potential to significantly advance biomonitoring of aquatic ecosystems.

## **Keywords**

qPCR, high-throughput, microfluidic, Integrated Fluidic Control, biomonitoring

## **Presenting author**

Luca Mirimin

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