IN VITRO OPTIMIZATION OF A QUANTITATIVE MOLECULAR ASSAY FOR DETECTION OF EXTRACELLULAR DNA (eDNA) FROM ATLANTIC SALMON (*Salmo salar L.*)

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1 BACKGROUND

- Atlantic salmon (*Salmo salar L.*) is ecologically, culturally and economic important and knowledge of habitat and distribution of salmon (e.g. spawning and hatching area) is vital for conservation and management purposes (Atikson 2018).
- Use of environmental DNA (extracellular DNA; eDNA) represent a technique that is harmless to fish compared to traditional survey methods (e.g. electro fishing).
- Species-specific assays targeting Atlantic salmon DNA (mitochondrial DNA cytochrome c oxidase gene; COI) is available for PCR tests (Atkinson, S et al. 2018), but optimization to maximize specificity and efficiency is required (Taberlet, P et al. 2018).

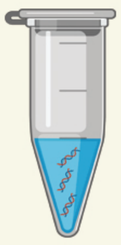
- Optimize quantitative molecular assay and methods for detection of extracellular DNAfrom Atlantic Salmon in environmental samples - Real-time qPCR vs. ddPCR.

2 METHODOLOGY

Muscle tissue
Salmo salar

Salmo trutta | *Oncorhynchus gorbuscha* | *Salvelinus fontinalis* | *Coregonus lavaretus* | *Lepomis gibbosus* | *Perca fluviatilis* | *Esox lucius* | *Ameiurus nebolusus* | *Scardinius erythrophthalmus* | *Phoxinus phoxinus* | *Rutilus rutilus*, *Carassius carassius* | *Gasterosteus aculeatus* | *Oncorhynchus mykiss* | *Leuciscus idus*

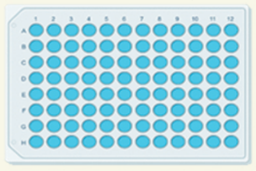
DNA Extraction
DNAeasy Blood and Tissue kit (Qiagen)



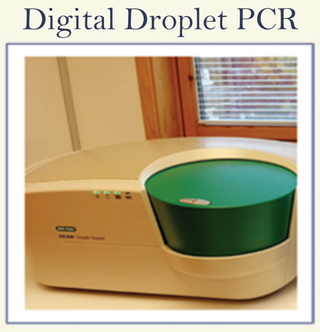
One measurement

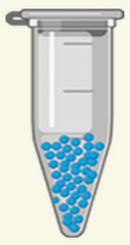
Real-Time PCR

eDNA assay from Atkinson et al (2018)



Digital Droplet PCR





Thousands of measurements

REAL TIME qPCR

- Progress of reaction is monitored after each cycle (Hindson et al 2011).
- Compares sample to a standard to obtain quantitative information (Pinheiro et al. 2012).
- **Possible to be used in field through BioMeme technology.**

DDPCR

- Absolute DNA quantification without external calibration (Hindson et al 2011).
- Target DNA is distributed into droplets (20 000) - replicate reactions.
- Less affected by inhibitory factors from eDNA samples, less chances of false negatives (Mauvisseau et al 2019).
- Droplets contain independent PCR reactions.

4 WHAT COMES NEXT?

- Applying assay and methods for water samples from rivers and lakes to map habitats of Atlantic salmon.
- Transfering the assay to the BioMeme platform (biomeme.com) for *in situ* tests.

3 RESULTS

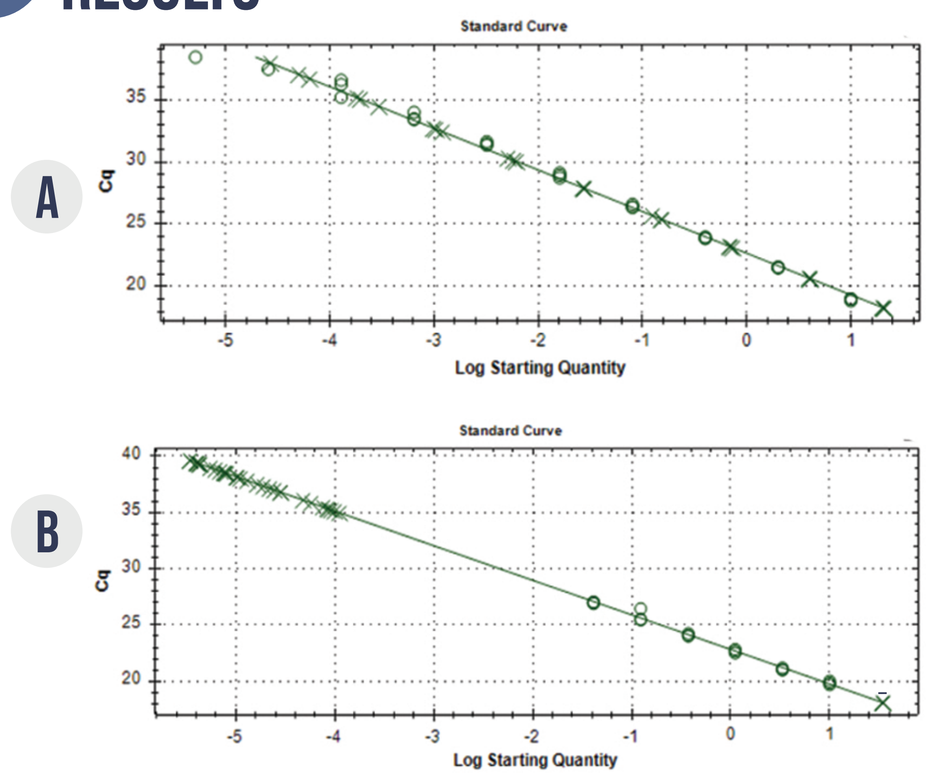


Figure 1 - Detection limit of the Atlantic salmon specific PCR assay in Real-time qPCR runs. The vertical axis represents cycle quantification value (Cq). A) **Efficiency to the assay.** Serial dilution with DNA from Atlantic salmon (o = standard curve, x = Salmon sample) shows high efficiency and precision for Cq values ≤ 34. B) **Specie-specificity of the assay.** Serial dilution with DNA from salmon (o, x̄) and other freshwater fish species (x) as listed in 2 Methodology. Starting amounts of DNA template was equal for all species (10 ng/µl). The figure shows the assay is highly specie-specific for Atlantic salmon at Cq values ≤ 34.

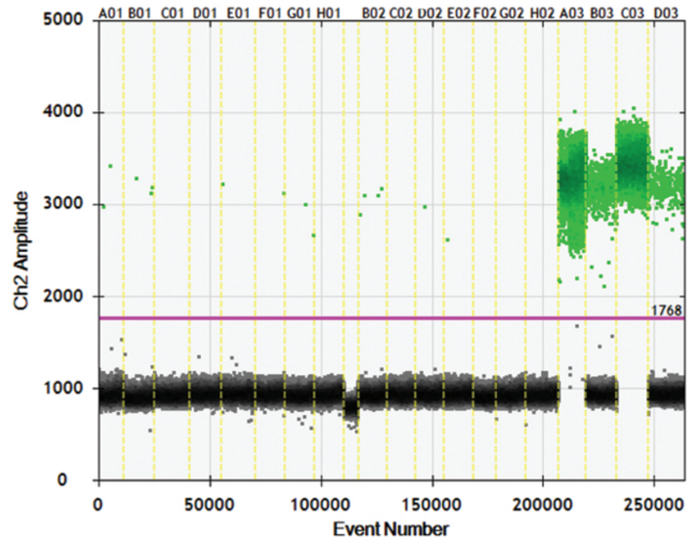


Figure 2 - Specie-specific test using ddPCR. DNA from Atlantic salmon (green) amplified at significant higher level compared to DNA from other fish species (black). Positive results are droplets above the red threshold line. Droplets below this threshold line are negative (no amplification).

CONCLUSIONS

- **The PCR assay is suitable for eDNA detection of Atlantic salmon with use of both qPCR and ddPCR methods.**
- The assay shows a high sensitivity with a limit of detection in qPCR tests (Cq 34) that correspond to a DNA concentration of 32 ng/L.
- The assay also proved to be highly specie-specific to Atlantic salmon in qPCR tests for Cq ≤ 34. That is, Cq values > 34 may represent false positive in lakes and rivers with other fish species than salmon.
- The assay tested with ddPCR show similar sensitivity as qPCR, but ddPCR is more specie-specific with less risk for false positives.

REFERENCES

Atkinson, S., Carlsson, J. E. L., Ball, B., Egan, D., Kelly-Quinn, M., Whelan, K., & Carlsson, J. (2018). A quantitative PCR-based environmental DNA assay for detecting Atlantic salmon (Salmo salar L.). Aquatic Conservation: Marine and Freshwater Ecosystems, 28(5), 1238–1243. https://doi.org/10.1002/aqc.2931

Bio-Rad. (2019). Droplet Digital PCR (ddPCR) Technology: What is Droplet Digital PCR? Retrieved from: https://www.bio-rad.com/en-no/applications-technologies/droplet-digital-pcr-ddpcr-technology?ID=MDV31M4VY

Hindson, B. J., Ness, K. D., Masquelier, D. A., Belgrader, P., Heredia, N. J., Makarewicz, A. J., Colston, B. W. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Analytical Chemistry, 83(22), 8604–8610. https://doi.org/10.1021/ac202028g

Pinheiro, L. B., Coleman, V. A., Hindson, C. M., Herrmann, J., Hindson, B. J., Bhat, S., & Emslie, K. R. (2012). Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. Analytical Chemistry, 84(2), 1003–1011. https://doi.org/10.1021/ac202578x

Mauvisseau, Q., Davy-Bowker, J., Bulling, M., Brys, R., Neyrinck, S., Troth, C., & Sweet, M. (2019). . Combining ddPCR and environmental DNA to improve detection capabilities of a critically endangered freshwater invertebrate. Scientific Reports, 9(1), 1–9. doi.org/10.1038/s41598-019-50571-9

Taberlet, P., Bonin, A., Zinger, L. & Coissac, É. (2018). Environmental DNA: For Biodiversity Research and Monitoring. Oxford University Press. https://doi.org/10.1093/oso/9780198767220.001.0001

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