ParaFishControl

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OUR FUTURE GROWING from WATER

New tools for the diagnosis of the amoebic gill disease (AGD)

ParaFishControl Workshop "North European Fish Parasite Management Strategies in Aquaculture Farms"

Berlin, 9th October 2019

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634429. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

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Challenge and Impact

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- Amoebic gill disease (AGD) is caused by *Neoparamoeba perurans*
- AGD **clinical signs**: multifocal white mucoid spots and patches on the gill surface
- A "gill score" scale, internationally used:
  0 = no lesions
  5 = extensive lesions is·
  The gill score is presumptive

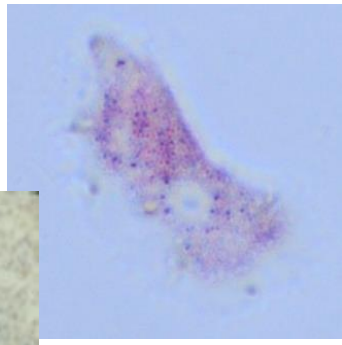

Photo from Journal of Fish Diseases 2011, 34, 411–432

- AGD causes economic losses to salmonid industry worldwide.
- **Cost**s estimated: at A$20m annual (Australia), £30m (UK/EIRE, 2011-2012)
- **Treatments** (e.g. freshwater, H202, peracetic acid..) are **expensive**!!!
- "**Handling**" of the disease: by gill scoring and early treatment
- An **on-site confirmatory test** of the presence of *N. Perurans* is desirable before treatments are conducted for a cost-effective treatment

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Our approach and our team

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The proposed solution:

- To develop a point of care (POC) test for the on-site confirmation of *N. perurans* on non lethal gill-swabs.
- To compare POC DNA extraction methods for field application.

Team involved in the development:

Cefas

Test design

Richard Paley
Irene Cano

Experimental challenges and sample collection

Ava Wayne
Claire Joiner

Amoebae *in vitro* culture

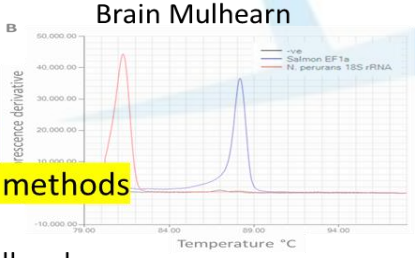
All of them!

POC DNA extraction methods

Robin McCullough
Irene Cano

Sample analysis

Brain Mulhearn



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Our proposed solution (I)

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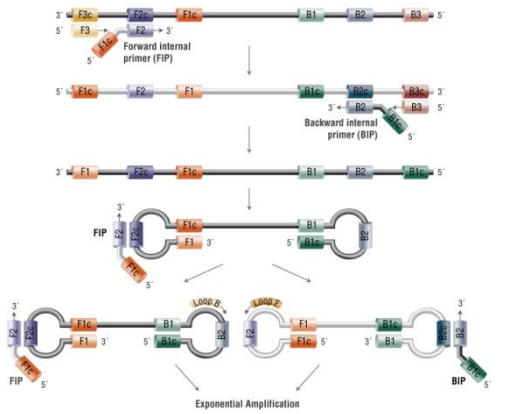
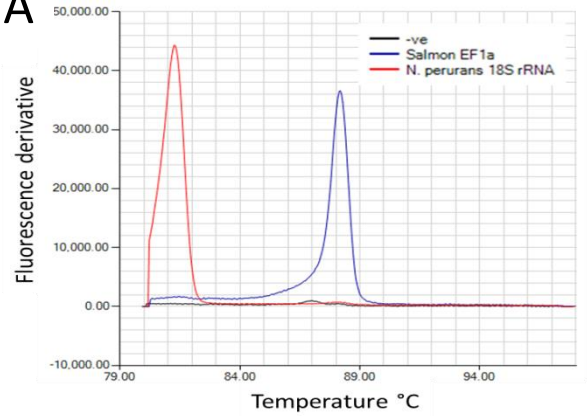
An isothermal amplification (LAMP) assay using portable **weather proof** real-time fluorescence equipment for the **on-site detection** of the 18S rRNA gene of *N. perurans* and the host EF1a gene (internal control) in **non-lethal** Atlantic salmon **gill swabs**.



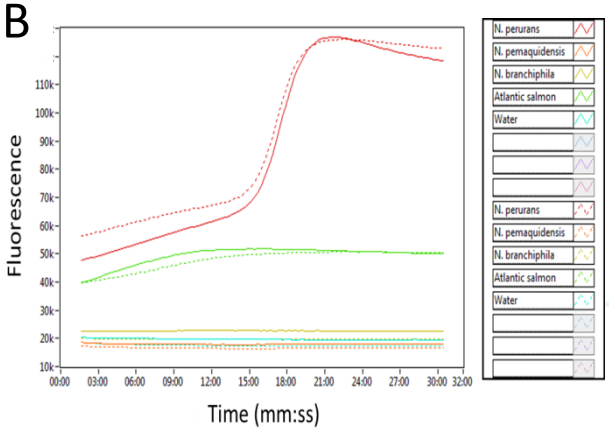
AGD LAMP assay optimization, specificity and analytical sensitivity

A



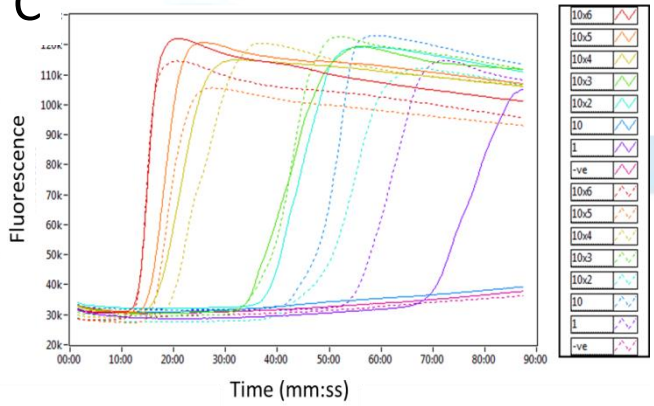
Optimal isothermal amplification at **62.9 ºC**

B



AGD LAMP assay **highly specific!**

C



Detects **10^3 copies** parasite 18S rRNA gene-35 min

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Our proposed solution (II)

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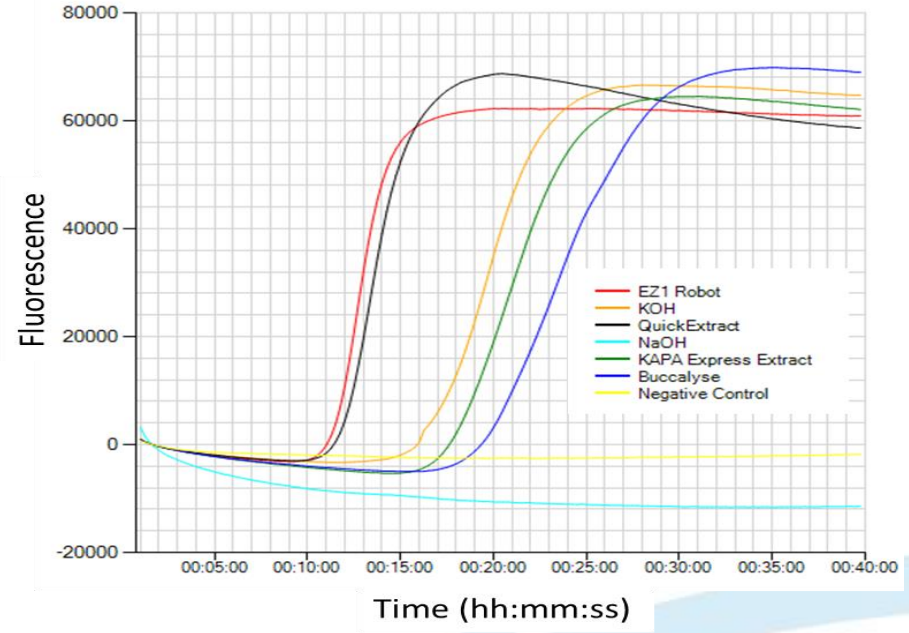
DNA extraction methods for field application

Five "fast and dirty" DNA extraction methods were evaluated:

1. Sodium hydroxide (NaOH)
2. QuickExtract™ DNA extraction solution
3. Potassium hydroxide (KOH)
4. KAPA-express-extract
5. Buccalyse DNA release kit

and compared with the reference laboratory method (EZ1 Robot)



- ✓ QuickExtract faster POC DNA extraction method
- ✓ Compatible with Taqman qPCR chemistry - allows for laboratory validation of field LAMP results

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Our proposed solution (III)

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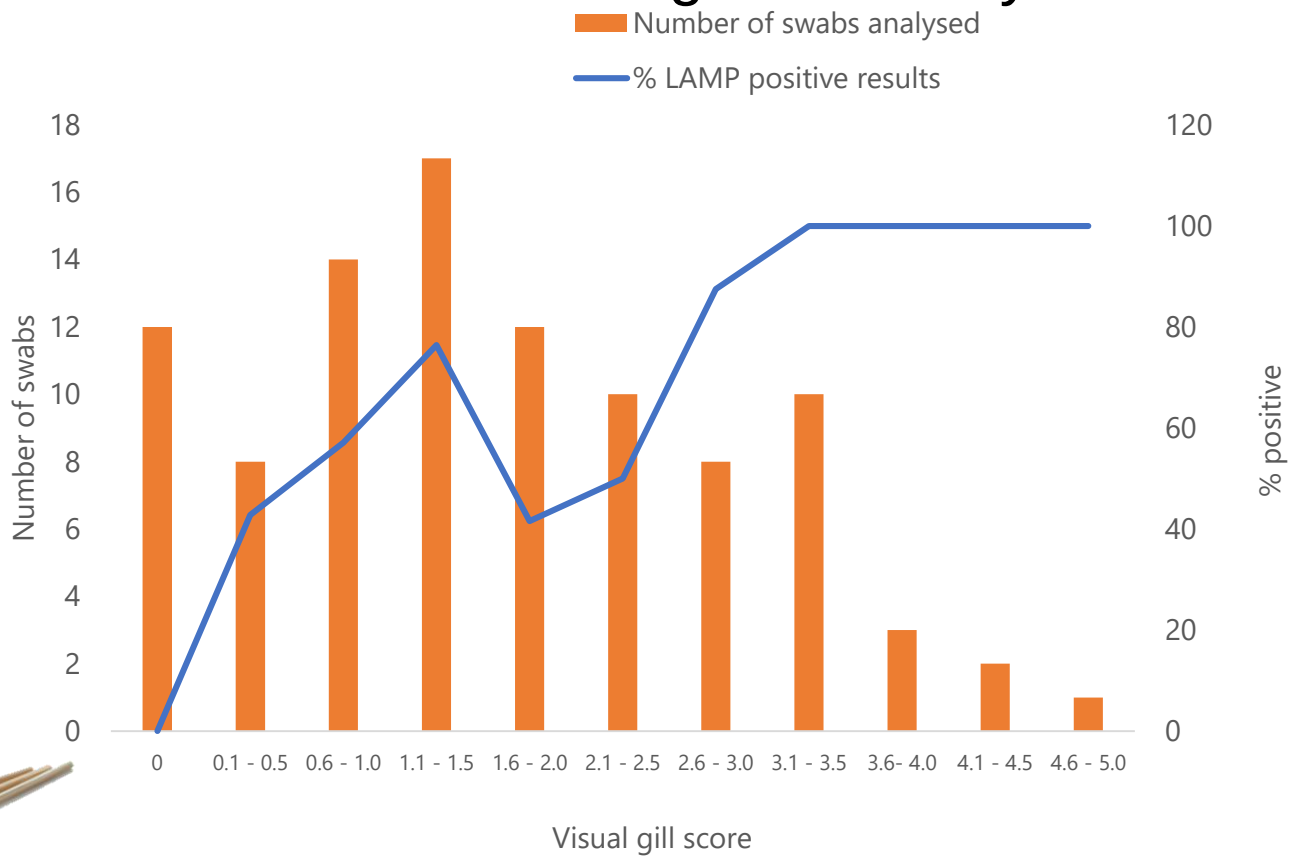
N. perurans detection in non-lethal gill swabs by LAMP



Bars: Atlantic salmon gill swabs analysed

Line: percentage of LAMP-positive tests per gill score

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Expected benefits for the industry

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- **On-site** discrimination of the *N. perurans* from non-lethal gill swabs in clinically infected Atlantic salmon
- In parallel with gill scores can help to cost-effectively plan treatments
- **Easy** assay to perform (low technical requirements)
- **Fast**: Results under 40 min (5 min for DNA extraction and 35 min to detect 10^3 copies of the targeted gene)
- Gross estimated **cost of £2.5 per sample** (including the cost for DNA extraction and the isothermal amplification)

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Current status and next steps

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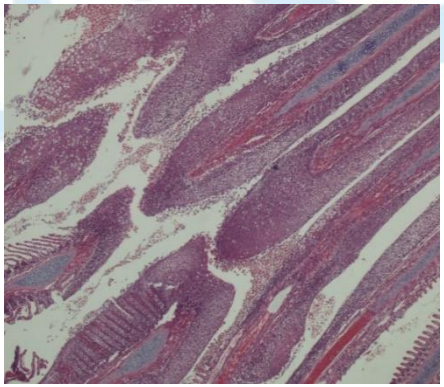
- Test ready for inter laboratory validation
- To develop a commercial kit is possible
- Possible to develop LAMP assays for **other targets** as new sequences of *N. perurans* genes are available, which might help to improve sensitivity and further reduce test time
- Further **refinement** is required for improved detection of lower gill scores



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Conclusions

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- The developed AGD-LAMP assay performs well (100% of positives) for swabs from fish showing gill scores >3.
- Lower gill scores do require a bigger sample size to confirm the presence of *N. perurans* in non-lethal gill swabs.
- Overall, the simplicity, performance and low cost of the present AGD-LAMP assay make this test a good candidate for the on-site confirmation of *N. perurans* in non-lethal gill swabs taken from AGD clinically infected Atlantic salmon.
- Further refinement is required for improved detection of lower gill scores.

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Thank You

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