

Repurposing trypanocidal drugs to tackle amoebic gill disease in Atlantic Salmon

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BACKGROUND

- Amoebic Gill Disease (AGD) is a salmon gill disorder that caused by *Paramoeba perurans*, a parasitic amoeba, and had caused \$ 120 million loss in fish farm each year.
- Almost all *Paramoeba* spp. have a eukaryotic endosymbiont, *Perkinsela*-like organism (PLO) (Figure 1).
- PLO belongs to the kinetoplastida, a diverse group of flagellated parasites including *Leishmania* and *Trypanosoma*. Gene modelling had predicted the interdependence between amoeboid host and kinetoplastid symbiont (Tanifugi *et al.* 2017).
- Developing new market for drugs currently licensed for human and veterinary trypanosomatids may be able to reduce their cost in the developing world.
- Hypothesis: Killing PLO, using current licensed trypanocidal drugs, may kill PLO and may subsequently kill its symbiotic host.

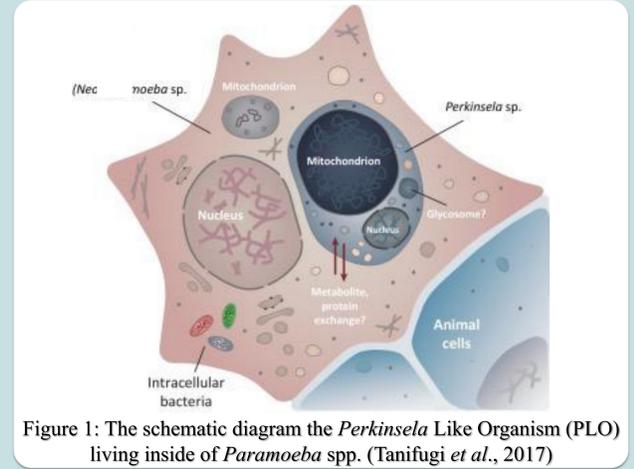
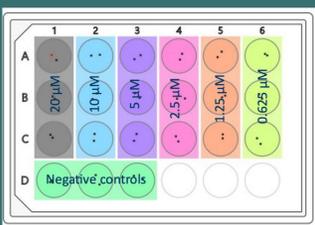


Figure 1: The schematic diagram the *Perkinsela* Like Organism (PLO) living inside of *Paramoeba* spp. (Tanifugi *et al.*, 2017)

In vitro drug assay – Holographic Microscopy



- Seed 1×10^4 cultured *P. perurans* into each well.
- Pipette serially diluted drug into the wells.
- Set Holomonitor to capture an image for each position every minute over the 72 hrs.

Scan QR for the videos

Drug A

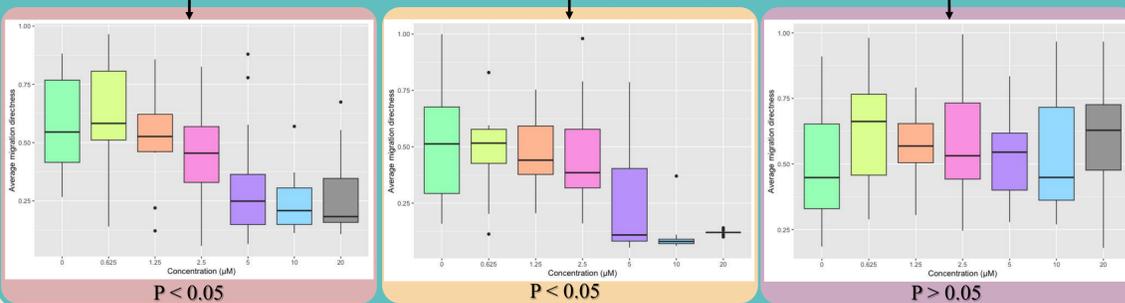
Drug B

Drug C

Single-cell tracking analysis of the initial 6 hours post treatment



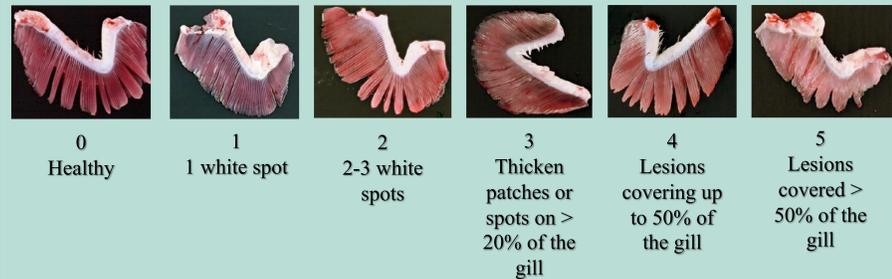
Average migration directness of all cells in different concentration



Objectives

- Develop and deploy an *in vitro* drug assay that can effectively distinguish the dead amoeba from the living ones.
- Undertake an *in vivo* drug trial in Atlantic salmon in a natural exposure setting.

AGD Gill Score



Adapted from Taylor *et al.* (2016)

In vivo drug trial

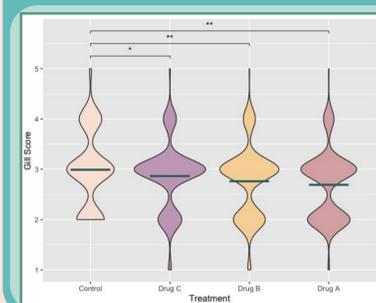
In vivo drug trial setup consisting of 4 treatments (Control, Drug A, Drug B and Drug C) in triplicate at different sentinel pens in 2 large cages.

| | | | |
|-------|---------------------------------------|-------|---------------------------------------|
| Pen 1 | Control (n = 149) Drug C (n = 149) | Pen 4 | Drug C (n = 149) Drug A (n = 149) |
| Pen 2 | Drug B (n = 149) Drug A (n = 149) | Pen 5 | Control (n = 149) Drug A (n = 149) |
| Pen 3 | Control (n = 149) Drug B (n = 149) | Pen 6 | Drug C (n = 149) Drug B (n = 149) |

Study was conducted at Bertraghboy Bay, County Galway, Ireland

* Fin clipped

| Day | Pen 1 | Pen 2 | Pen 3 | Pen 4 | Pen 5 | Pen 6 | |
|-----|-------|-------|-------|-------|-------|-------|--------------------------------|
| 1 | * | * | | | | | 1 st Drug injection |
| 2 | | | * | * | | | |
| 3 | | | | | * | * | |
| 8 | * | * | | | | | 2 nd Drug injection |
| 9 | | | * | * | | | |
| 10 | | | | | * | * | Sampling |
| 15 | * | * | | | | | |
| 16 | | | * | * | | | |
| 17 | | | | | * | * | |



A total of 1,231 fishes were assessed for their gill scores.

The drug-treated fishes have a statistically significant lower gill score when compared to the no-treatment.

Conclusion and Future Direction

- The Holographic Microscope is effective for drug screening of non-axenically *in vitro* grown *P. perurans*.
- Of all the drugs screened previously, three drugs have been selected for a three-week-long *in vivo* drug trial.
- All drugs were shown to have a significantly improved gill score compared to the no-treatment control.
- Future studies will aim to establish the drug mode of action using metabolomic analyses.

References

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Taylor, Richard & Huynh, Christine & Cameron, David & Evans, Brad & Cook, Mathew & Ritchie, Gordon. (2016). Gill Score Guide - Amoebic Gill Disease (AGD) management training document.