



Moredun

Gaston CASPE | GILL HEALTH INITIATIVE MEETING 2023 | 25th September 2023

NeoGiant a natural alternative treatment for Amoebic Gill Disease

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Origen

Polyphenols are plant “non-toxic” components well known for their antioxidant properties.

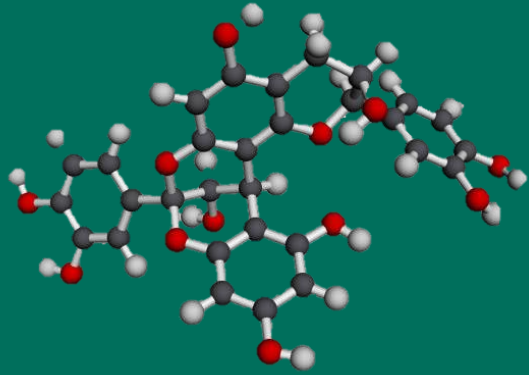
Polyphenols have shown anti-bacterial, anti-parasitic and fungicidal activities.

Amoebic Gill Disease (AGM) is one of the most significant challenges for the salmon industry in Europe.

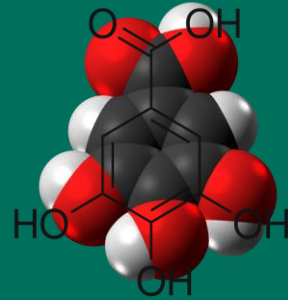
No Vaccine is commercially available.

The aim of this study is to evaluate the amoebicidal activity against *Neoparamoeba perurans* of the Grape extracts based on white grape marc obtained from the NeoGiANT-Horizon 2020 Framework Programme project (101036768).

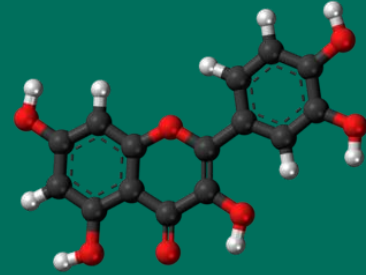
Polyphenols



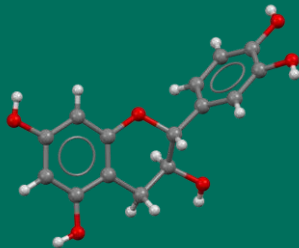
Procyanidines



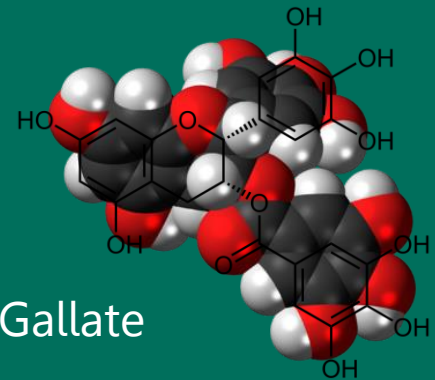
Gallic acid



Quercetin

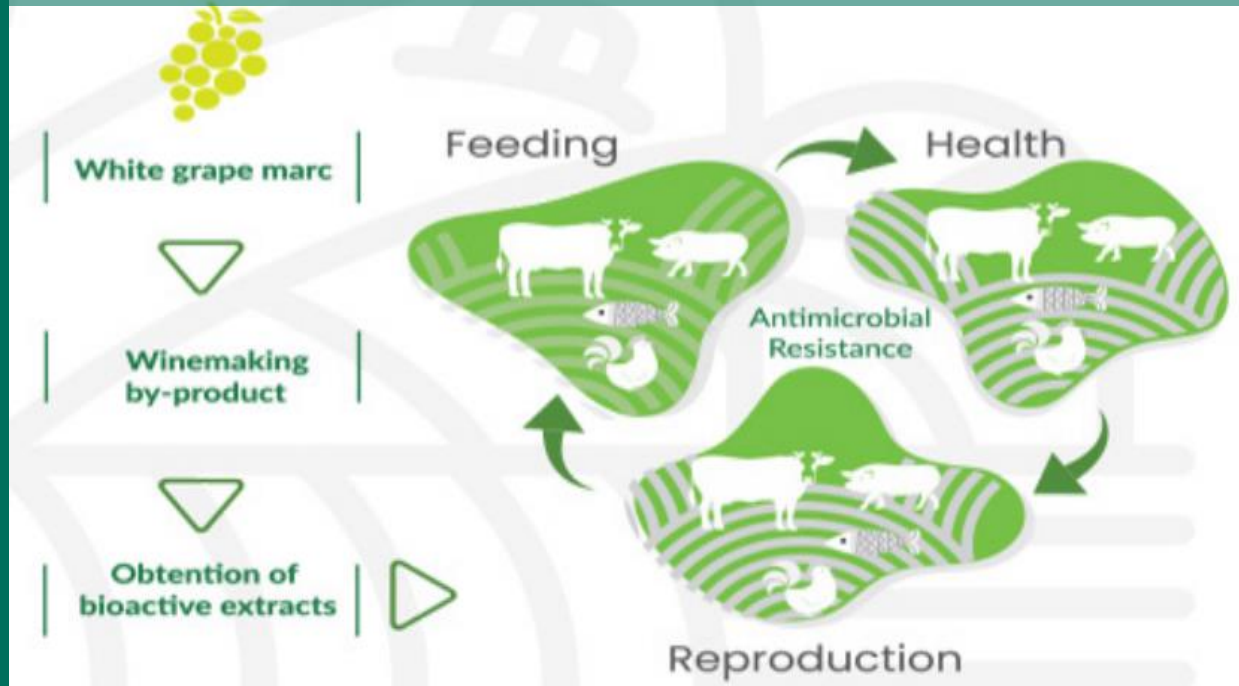


Catechin



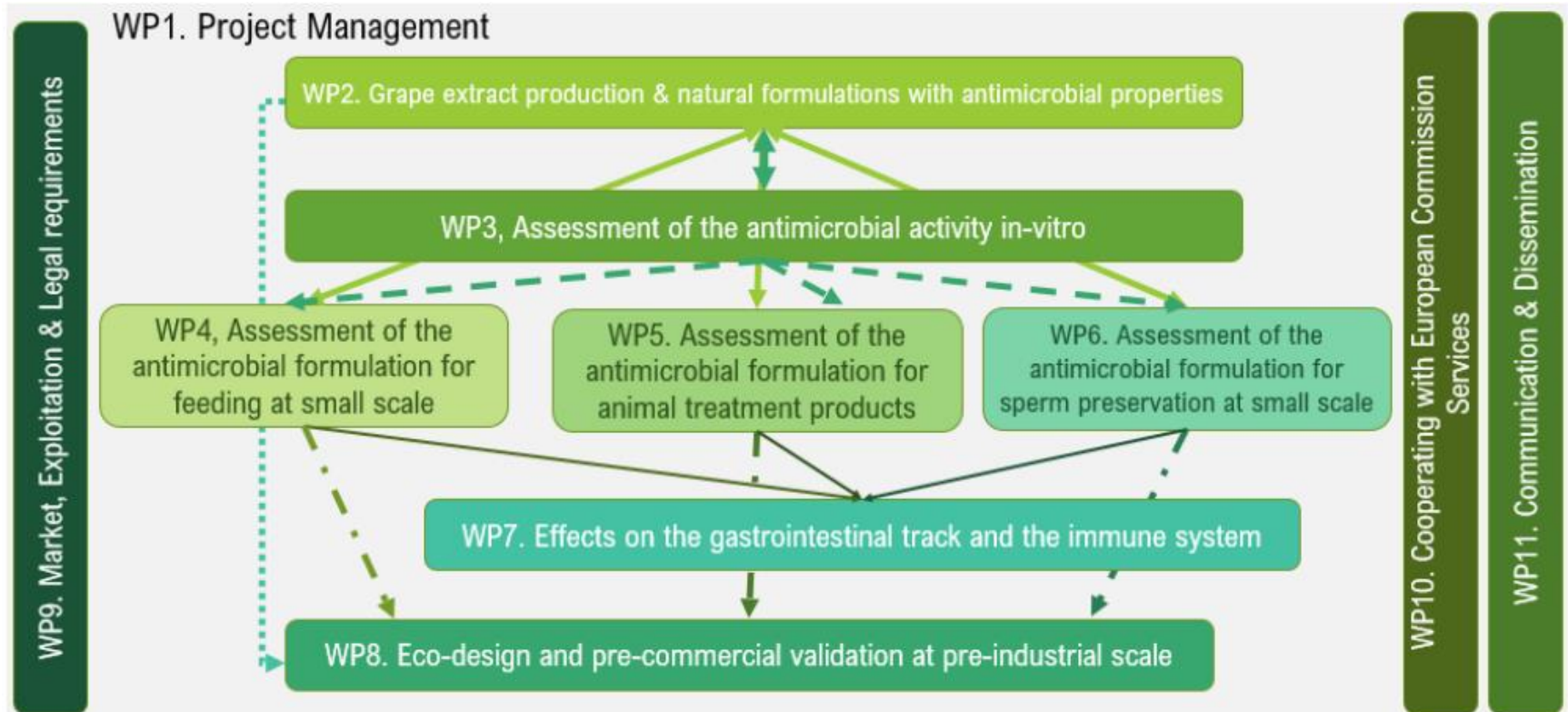
EpicatechinGallate

Origen



NeoGiANT offers an innovative solution based on the known potent natural antimicrobial and antioxidant activities of grape marc extracts, due to their arsenal of phytochemicals

Project Ideas



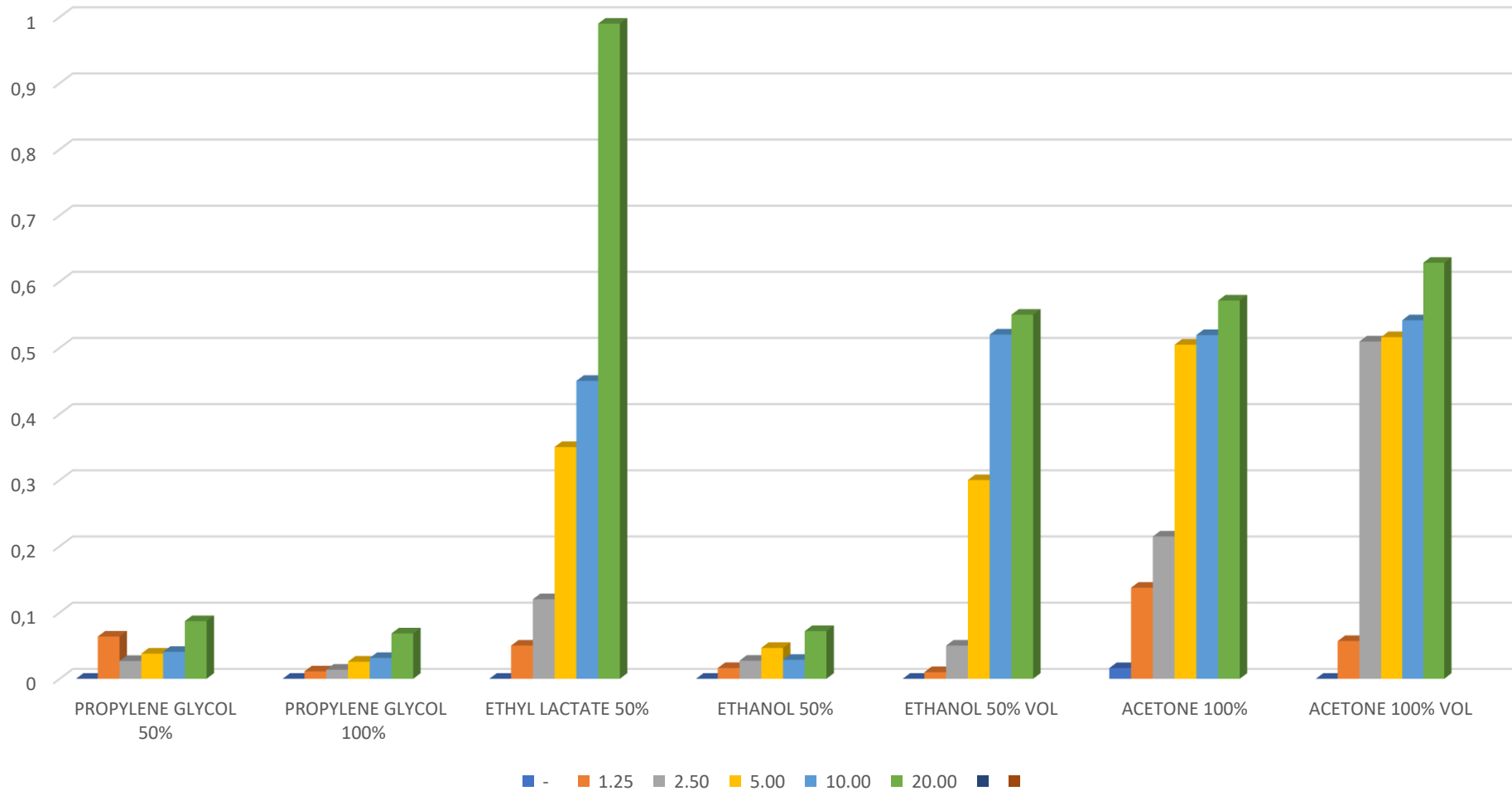
Experimental studies

- *In vitro*: to determine amebicide effect
- *In vivo*: to evaluate both amebicide and Immunological effect

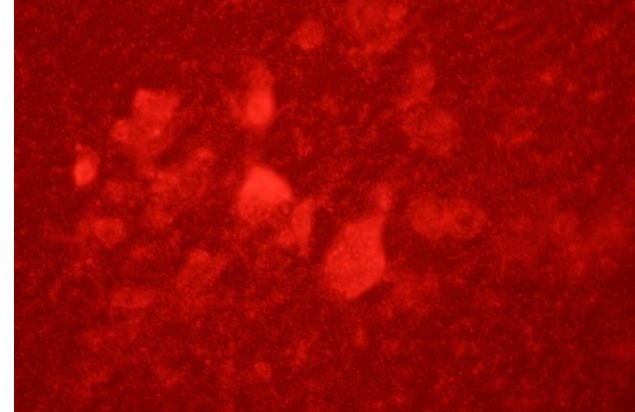
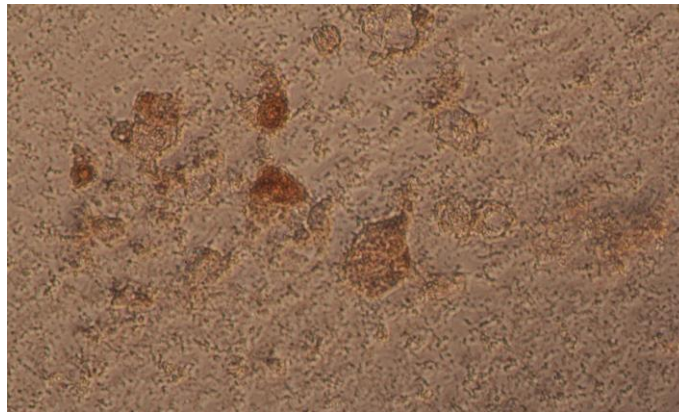
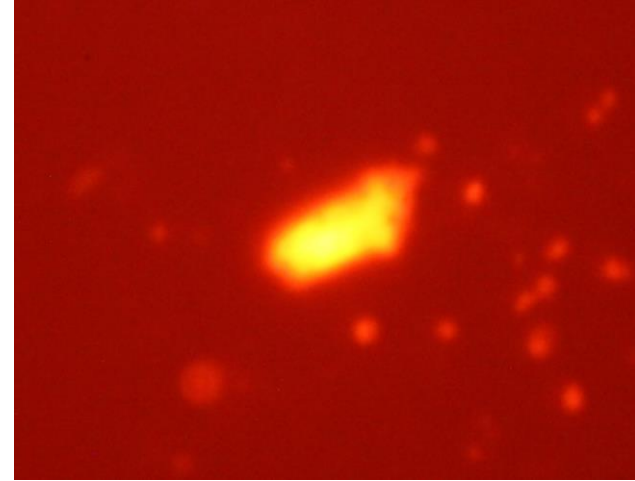
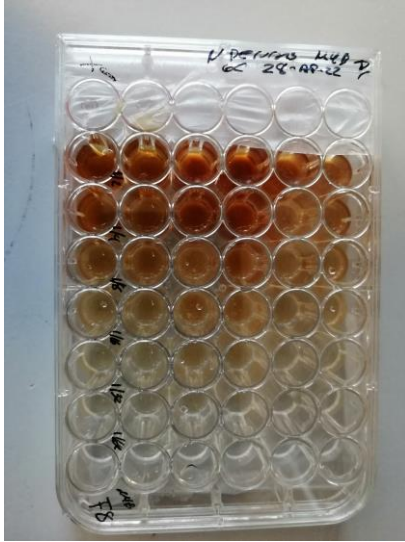
VALUES OF MIC AND IC50

	IC50 (%)		MIC (%)	
	Ext	ve	ext	ve
PROPYLENE GLYCOL 100% (NG01E22PG100RM7C02L01)	≥20	≥20	≥20	≥20
PROPYLENE GLYCOL 50% (NG01E22PG050RM7C02L01)	≥20	≥20	≥20	≥20
ETHANOL 50% VOLATILIZED (NG01E22ET050RM7C01L02V)	15.1461	n/a	≥20	n/a
ETHANOL 50% (NG01E22ET050RM7C01L02)	≥20	≥20	≥20	≥20
ACETONE 100% (NG02E22AC100RM7C02L02)	≥20	n/a	≥20	n/a
ACETONE 100% VOLATILIZED (NG02E22AC100RM7C02L02V)	11.2223	n/a	≥20	n/a
ETHYL LACTATE 50% (NG01E22LE050RM7C01L01)	7.5647	1.2552	12.2356	2.9669

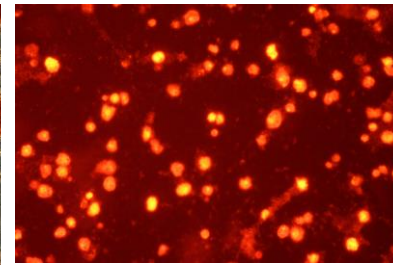
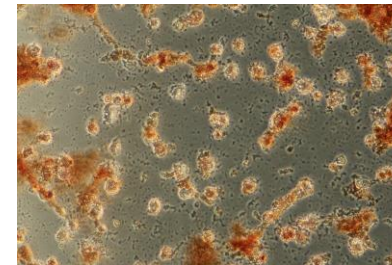
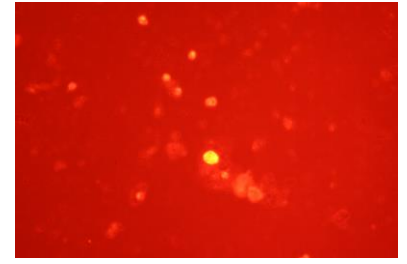
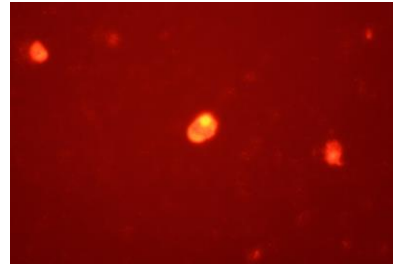
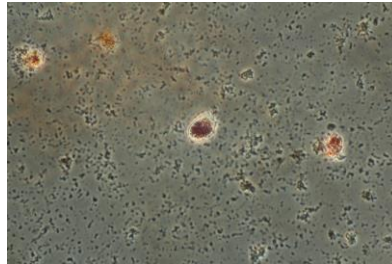
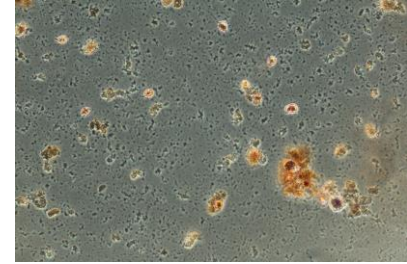
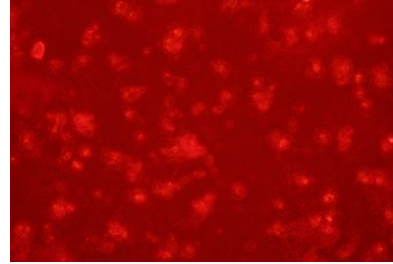
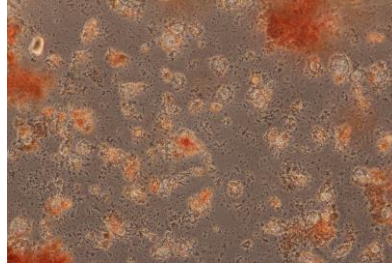
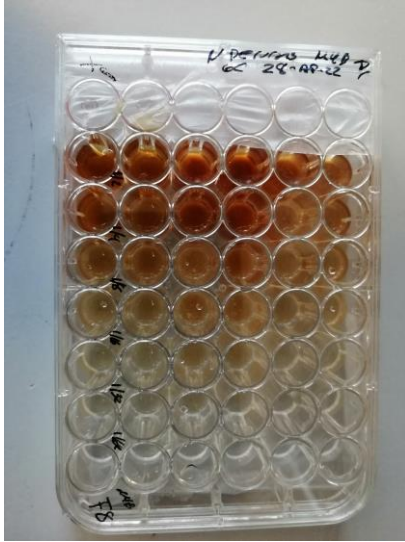
Dead amoebas/total



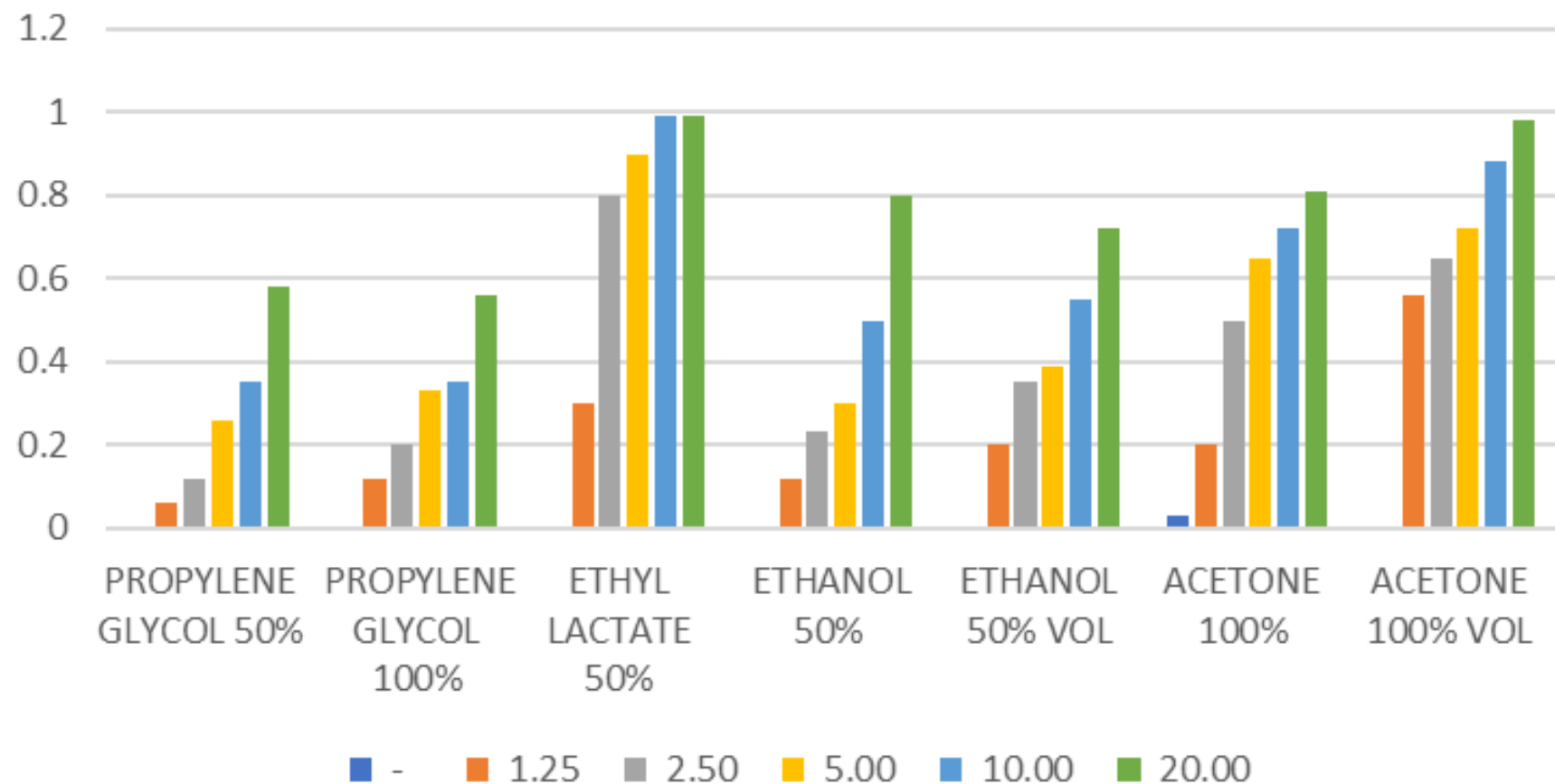
In vitro Trial with *N. perurans*



In vitro Trial with *N. perurans*



"Inactive"/total





In vivo antimicrobial effect of
solid feed

Validation of Amoebic Gill Disease Challenge Model in Atlantic salmon

0

N. perurans challenge
(conc. 200 amoeba/L)

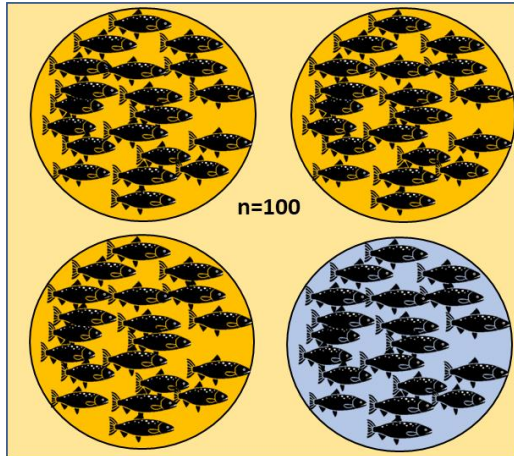
6

AGD Scored and returned to the tank

21

29

Euthanize and
AGD Score

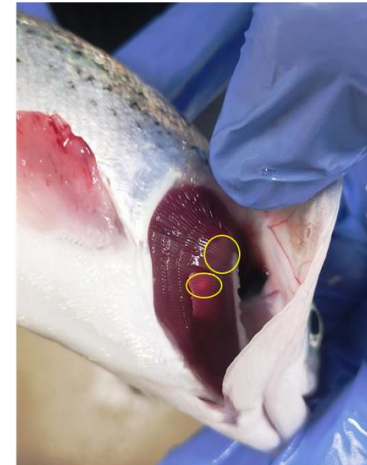


Study objectives:

- Validate the protocol and the virulence of the strain
- Determinate the infective dose to use in the feeding trial
- Define the times to be expected to find clinical signs and rates of morbidity and mortality

n= 100 (25 animals per tank)

- 75 challenged and 25 control



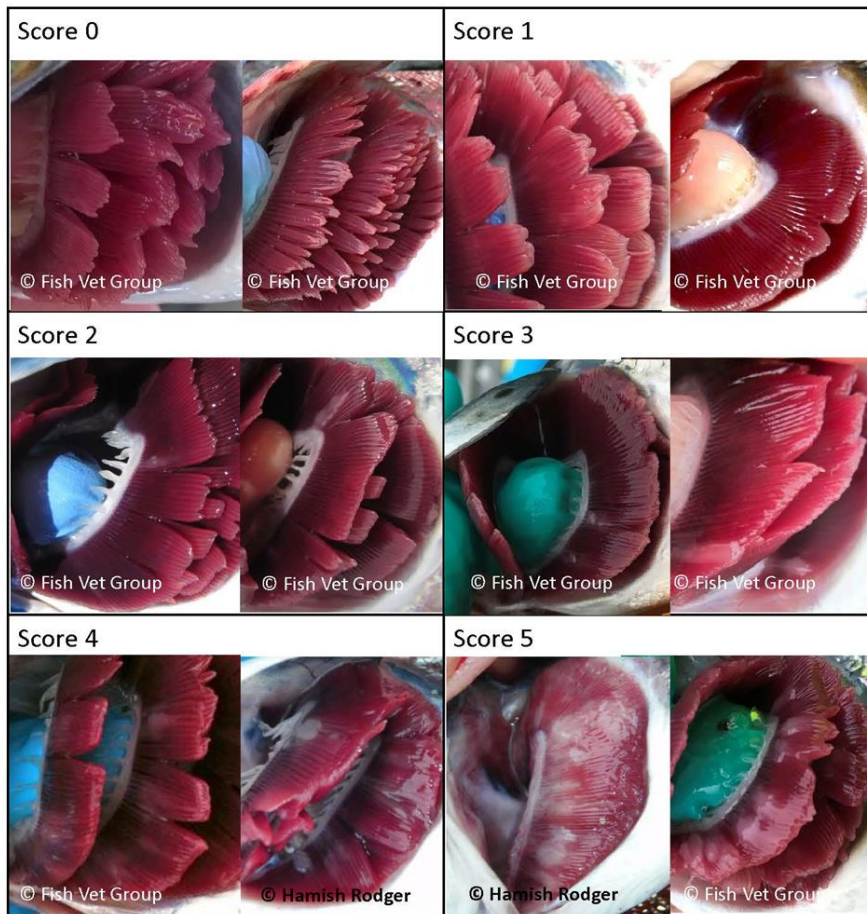
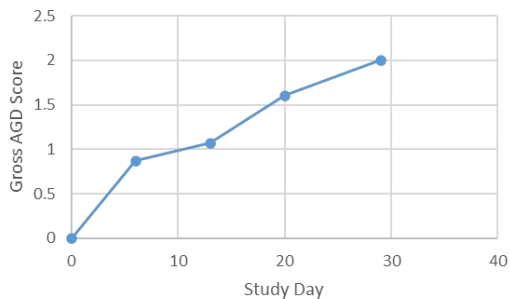
AGD MODEL VALIDATION

AGD scores (average values)

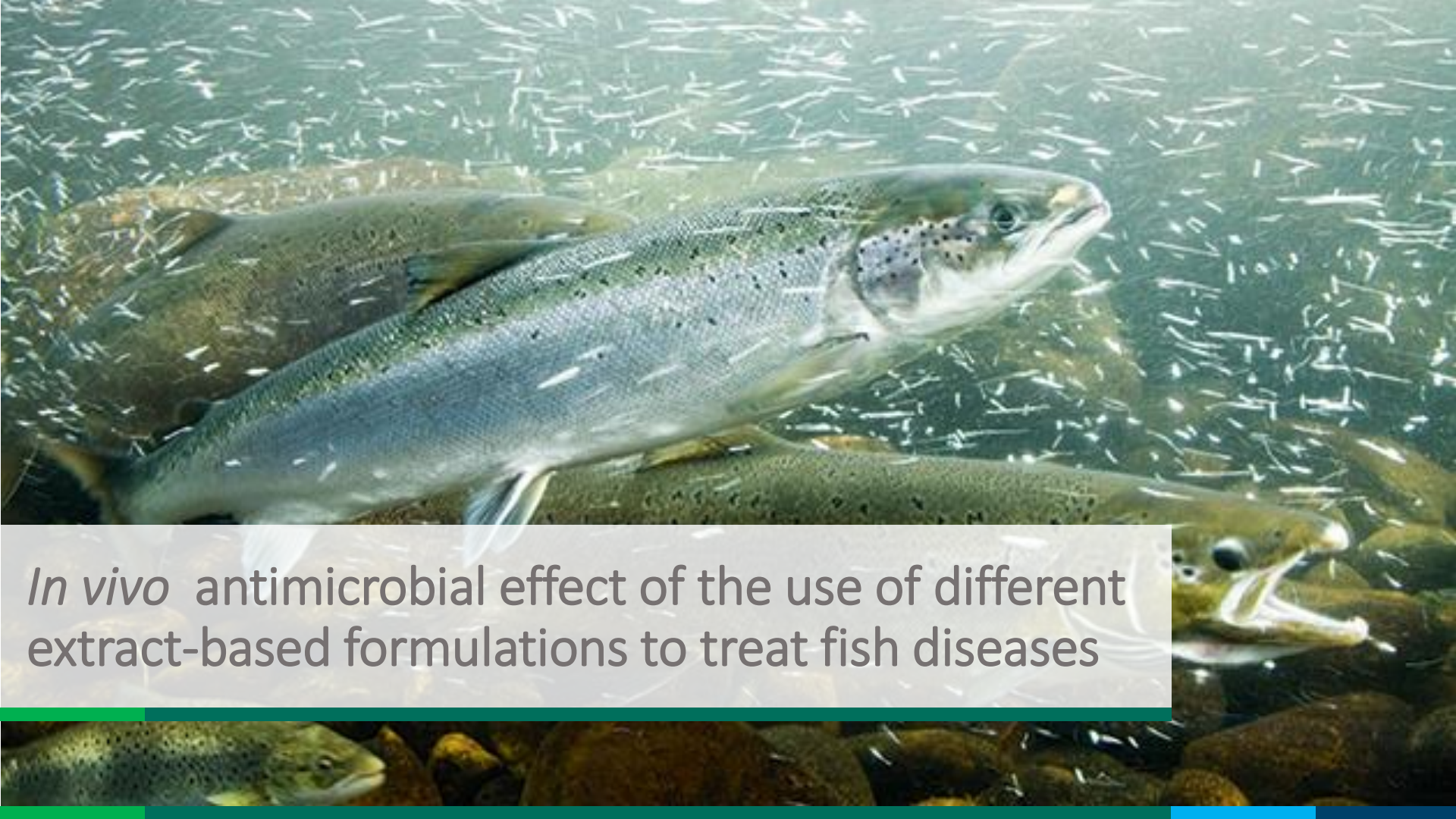
Days post-challenge	N. perurans challenged			Control
	Tank 1	Tank 2	Tank 3	Tank 4
0	0	0	0	0
6	0.8	0.8	1	0
13	1.2	1.2	0.8	0
20	1.6	1.4	1.8	0
29	1.9	1.9	2.2	*

* Suspected contamination

Progression of AGD score



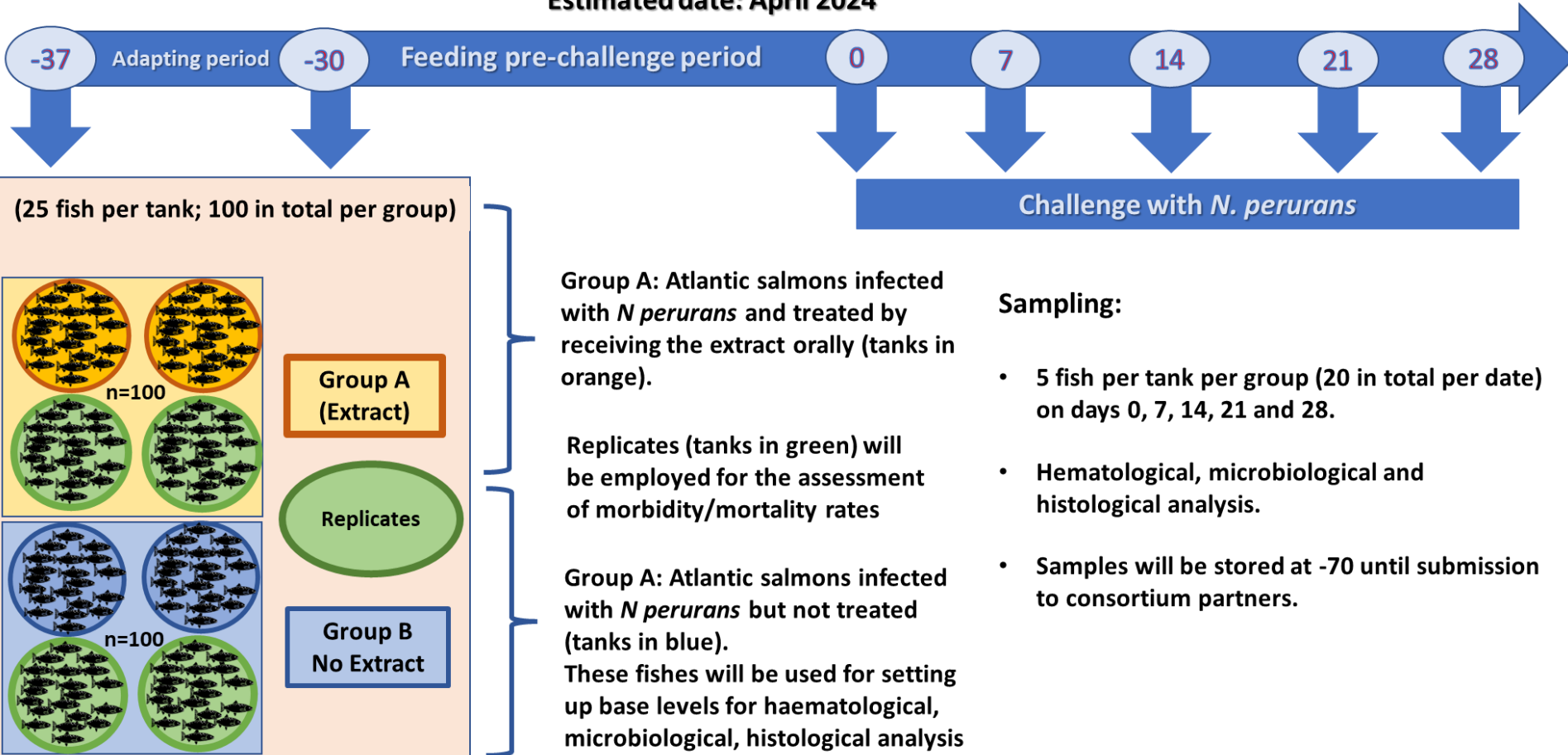
AGD SCORE CARD (score worst gill arch)	
Score	Description
0	No sign of infection and healthy red colour
1	1 white spot, light scarring or undefined necrotic streaking
2	2-3 spots/small mucus patch
3	Established thickened mucus patch or spot groupings up to 20% of gill area
4	Established lesions covering up to 50% of gill area
5	Extensive lesions covering most of the gill surface (50%+)



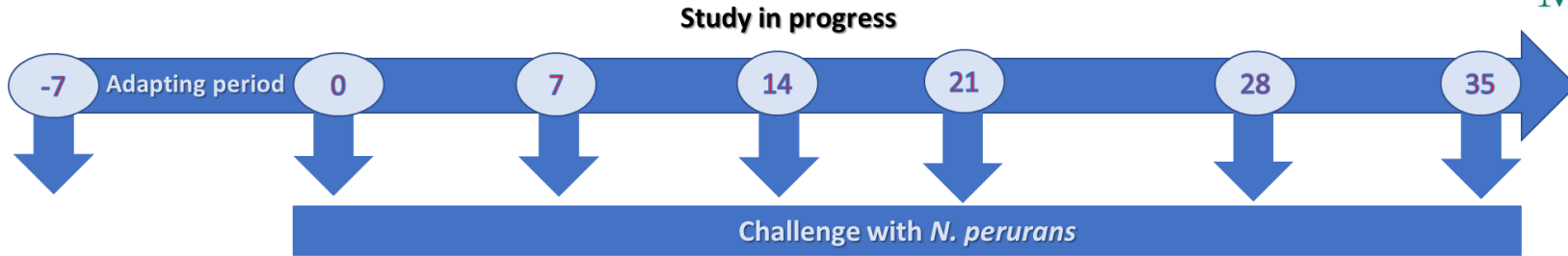
In vivo antimicrobial effect of the use of different extract-based formulations to treat fish diseases

Feeding Trial + Challenge with *N. perurans*

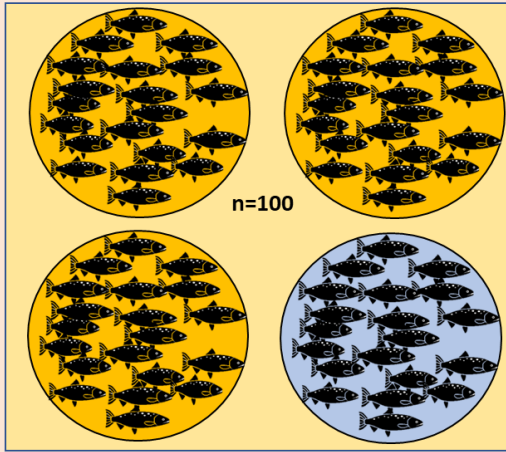
Estimated date: April 2024



Validation of Amoebic Gill Disease Challenge Model in Atlantic salmon by bath immersion



(25 animals per tank; 25 control and 75 challenged; 100 in total)



Study objectives:

- Validate the protocol and the virulence of the strain
- Fish will be treated once they have developed a gill score of 1 to reflect when treatments would be done in the field
- Define the times to be expected to find clinical signs and rates of morbidity and mortality

Future approach



- Although this study only focused on amoebicidal activity, the main effect of the polyphenols is due to antioxidant activities.
- Therefore, the amoebicidal activity seen in this study and the possibility of improvement in fish health due to their antioxidant activity may enable these NeoGiANT extracts to be used as a viable, non-toxic alternative for treating AGD. *In vivo* studies are currently underway to confirm this.
- **The application of functional feed ingredients represents a great opportunity to advance fish growth and health, boost the immune systems, and induce physiological benefits beyond those provided by traditional feeds.**

Future approach



- Considering the effect *in vitro*, we hypothesise that the effect *in vivo* should be higher due to the immunological effect of the polyphenols
- Feeding could mean an alternative to treatment decreasing the risk of AMR
- Using grape extract to treat the AGD could reduce the antimicrobial residues

Acknowledgement



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Marina Di Fonzo



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Maria Celeiro
Anniek Bus
Tommy Van Limbergen



Thanks for your attention!!!



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