



ParaFishControl

New treatments against sparicotylosis

**ParaFishControl Workshop “Mediterranean Fish Parasite
Management Strategies”**

Porto, 10th September 2019

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Table of Contents

1. Challenge and impact in the industry
2. Our approach – Our team
3. Our proposed solution
4. Our solution vs currently employed solutions
5. Expected benefits for the industry
6. Status and next steps
7. Conclusions



Challenge and Impact

➤ Challenge:

no effective, no environmentally, no consumer-safe **synthetic or natural** compound

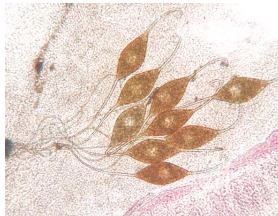
formalin baths (Italy; soon to be abandoned in the rest of EU countries)

bath treatments are unpractical, labour-intensive or unmanageable under certain circumstances

rare registered parasiticides/ unavailable in aquaculture medicine

recurrent, seasonal proliferations in cages

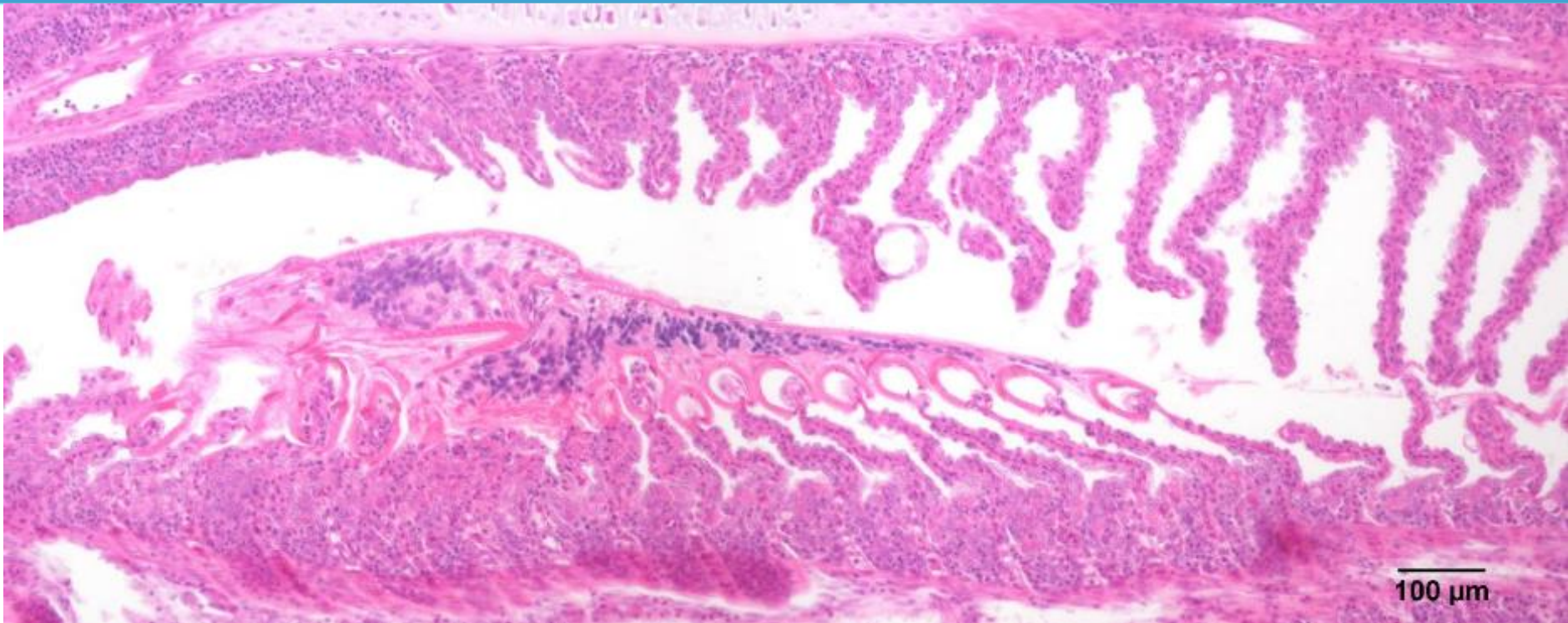
increase of >0.4 of FCR (>50,000 tons excessively spent)



➤ Impact:

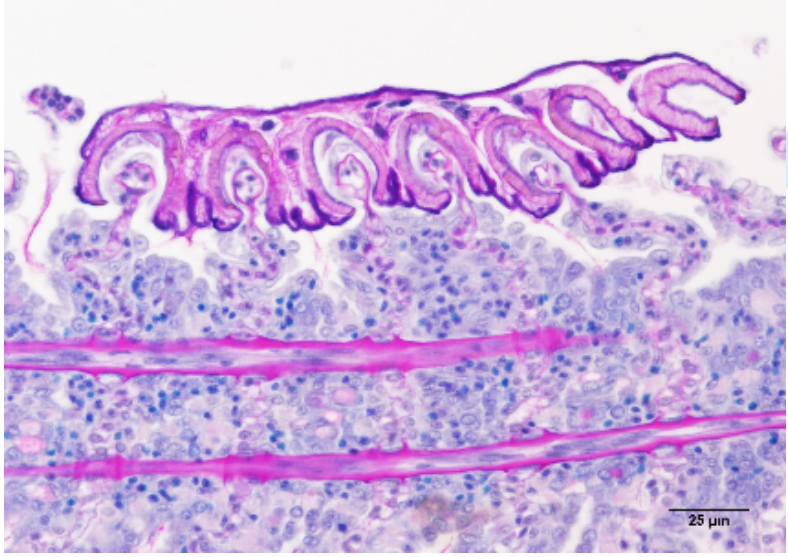
grow-out loss (parasites) from **1 to 10%** of harvest size, with an annual cost of **\$1.05 to \$9.58** billion

- direct mortalities
- decreases in growth performance
- feed conversion and product quality
- low reproduction efficacy
- increased susceptibility to other diseases
- negative public image of aquaculture



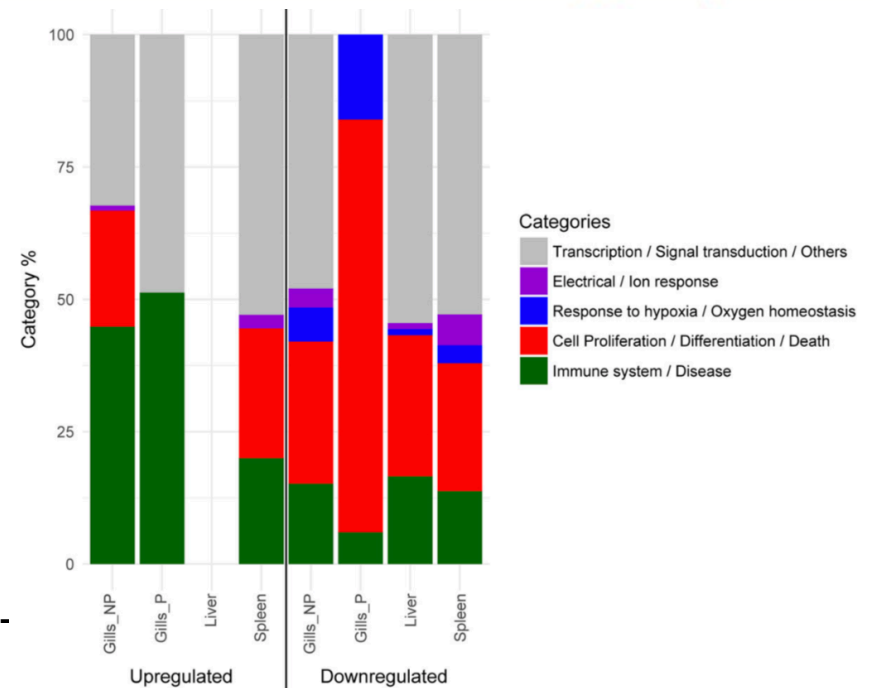
lamellar shortening,
clubbing and synechia,
proliferation of the epithelial tissue,
fusion of the secondary lamellae,
increase in chloride cells,
necrosis,
deletion of filaments

anaemia, sloughing of epithelial cells, SBI



Transcriptomic data:

- **local silencing effect** of the parasite in the infected gills!
- non-infected gill tissues of parasitised fish had strong **immune response** and **apoptosis-related genes**
- strong systemic reaction in the spleen (compared to gills or liver): inhibition of apoptosis, autophagy, platelet activation, signalling and aggregation, and inflammasome
- hypoxia-related genes were down-regulated in gills and spleen



Piazzon et al. BMC Genomics (2019) 20:200
<https://doi.org/10.1186/s12864-019-5581-9>

BMC Genomics

RESEARCH ARTICLE

Open Access

Acting locally - affecting globally: RNA sequencing of gilthead sea bream with a mild *Sparicotyle chrysophrii* infection reveals effects on apoptosis, immune and hypoxia related genes

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Our approach and our team

- **Brief description of the proposed solution**

1. collection of live parasites
2. LC testing of the different synthetic and natural compounds
3. LC in *Sparus aurata* fibroblas cell line (SAF-1) viability testing

- **Team involved in the development**

AITS-CSIC & Andromeda, Spain
IOR, Croatia

Our proposed solution (I)

bring live infected sea bream into the laboratory experimental recirculation tank (AITS-CSIC) and keep for two weeks for increasing the intensity

collect the live parasite: 10 adult specimens into 6-well plates with the different **synthetic compounds** (*bithionate sodium, bitoscanate, coumarin, diallyl sulphate, n, n-diethyl-m-toluamide, 4-hexylresorcinol, monocrotiline and pyrethrins 50%*)

test preliminary 4 different concentrations (1 mM, 0.1 mM, 0.01 mM, 1 µM)

monitor the survival under stereomicroscope at 1 h, 4 h, 12 h and 24 h (endpoint - when no contracting movements after probing with a needle longer than 7" were observed)

scale down new LC, so the LC100 was reached **within 4 h** (triplicate, 6 specimens per replica, testing seven concentrations per compound)

Our proposed solution (II)

Develop the same assay, using different **natural compounds**:
camphor,
cedrol,
curcumin,
eucalyptol,
garlicin 80%,
(+)-trans-chrysanthemic acid



Our proposed solution (III)

Potential effect on the host:

evaluate the viability of sea bream fibroblast (SAF-1) cells using 0.1, 1 and 10 μM concentrations

assessed cell vitality % after 24, 48 and 72 h



Expected benefits for the industry

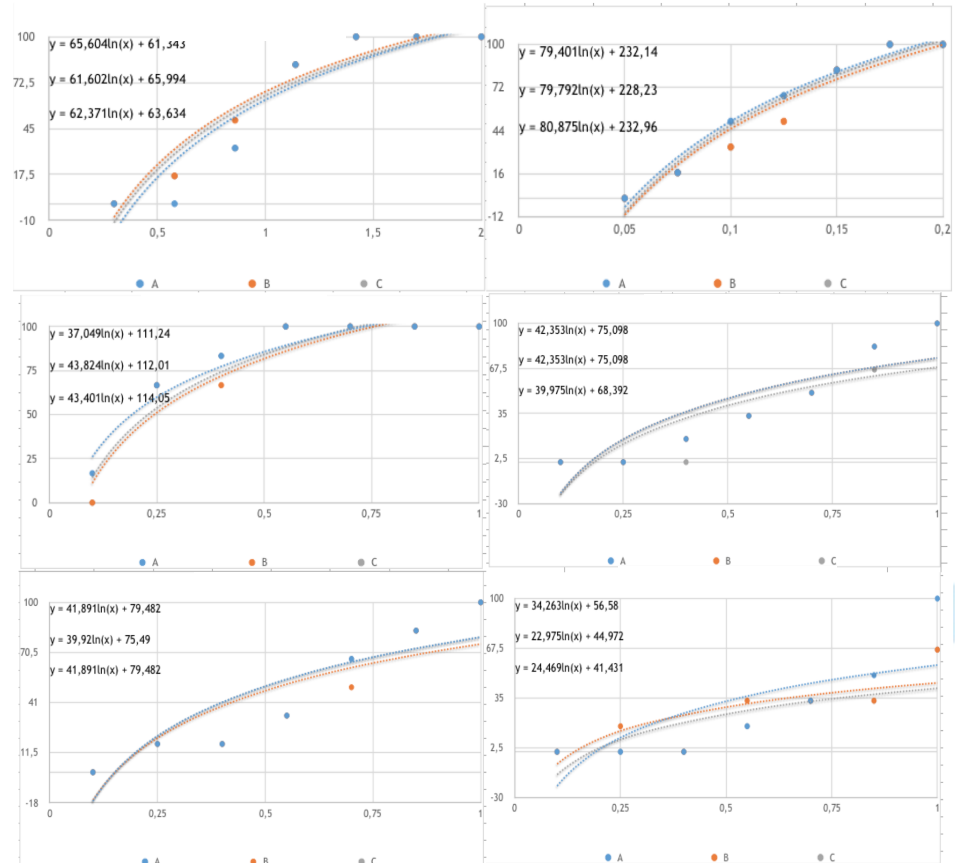
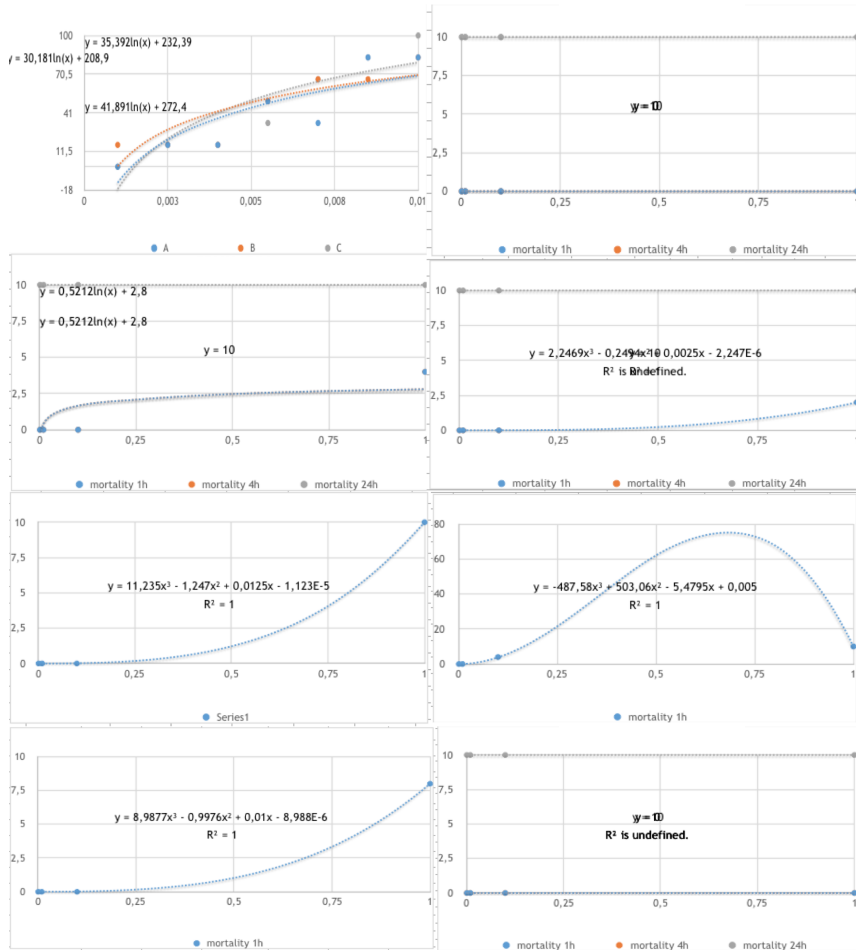
after performing validated and standardised assay, **rank the “best of” compound**

criteria:

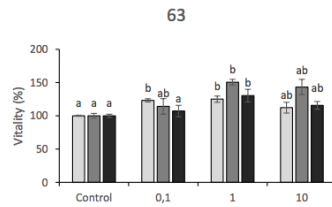
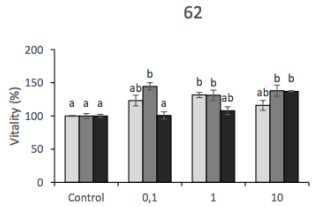
1. LC100 at 4 h post-application
2. toxicity to the host
3. pharmacokinetics study (availability and metabolites in the host tissues)
4. potential resistance (expression of ABC transporters in the parasite)
5. assessment of impact on the environment (literature data)
6. economic moment

Current status and next steps

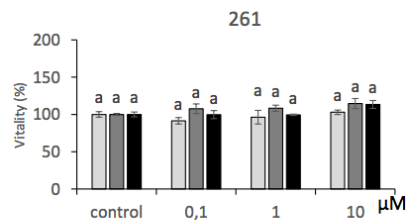
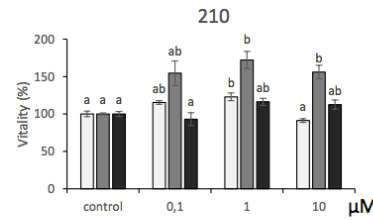
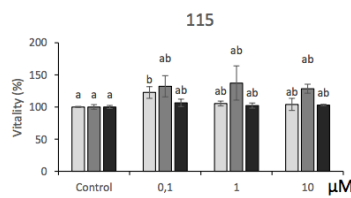
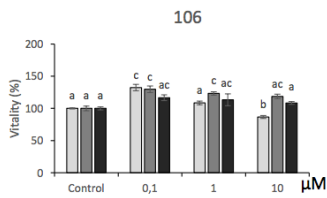
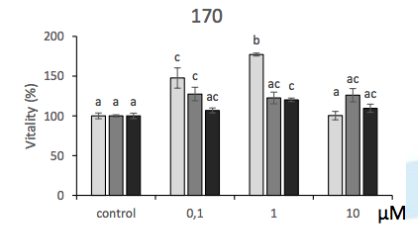
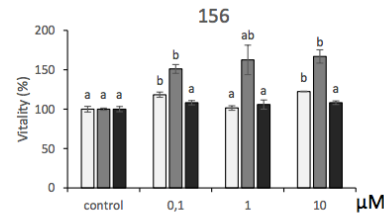
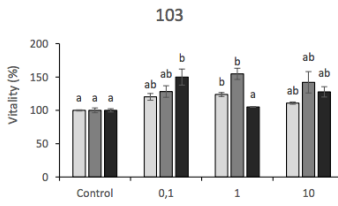
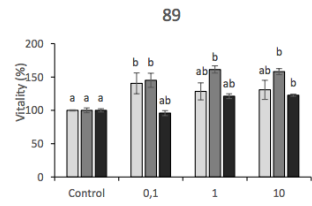
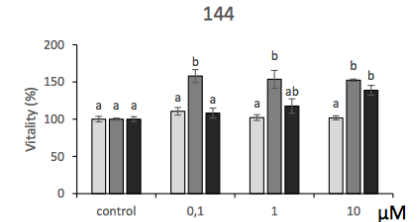
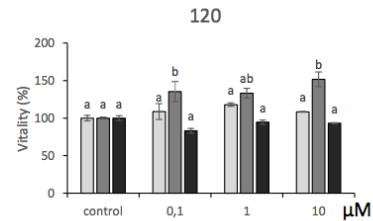
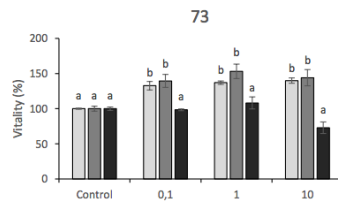
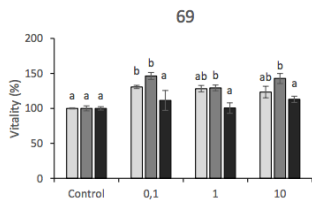
LC50



Current status and next steps



SAF-1 vitality %



□ 24 h ■ 48h ■ 72h

□ 24h ■ 48h ■ 72h

Conclusions

best synthetic has LC50 **20x lower** compared to the natural

although standardised and validate, time consuming approach!!!!

after succeeding to assemble & annotate *Sparicotyle* genome: search for “**chokepoint**” genes (known for 10 nematodes) - chemogenomic screening from public drug DBs - id of the best compound - LC assay

A "chokepoint reaction" is a reaction that either uniquely consumes a specific substrate or uniquely produces a specific product in a metabolic network, and is also balanced by a reaction that respectively produces or consumes that substrate (that is, a chokepoint reaction that produces X must be balanced by a reaction that consumes X, or else X would be a dead-end metabolite).

Thank You



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