ParaFishControl

Alternative treatments for *Ceratothoa oestroides*

ParaFishControl Workshop "Mediterranean Fish Parasite Management Strategies"

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Challenge and Impact

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➢ Challenge:

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

management measures:

changing of the cage nets,

removal of aggregating-wild fish populations,

manual extirpation of parasite from the fish during vaccination,

size-sorting or similar farm activities

pyrethroid deltamethrin,

feed-administered emamectin benzoate

difluobenzuron

➢ 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

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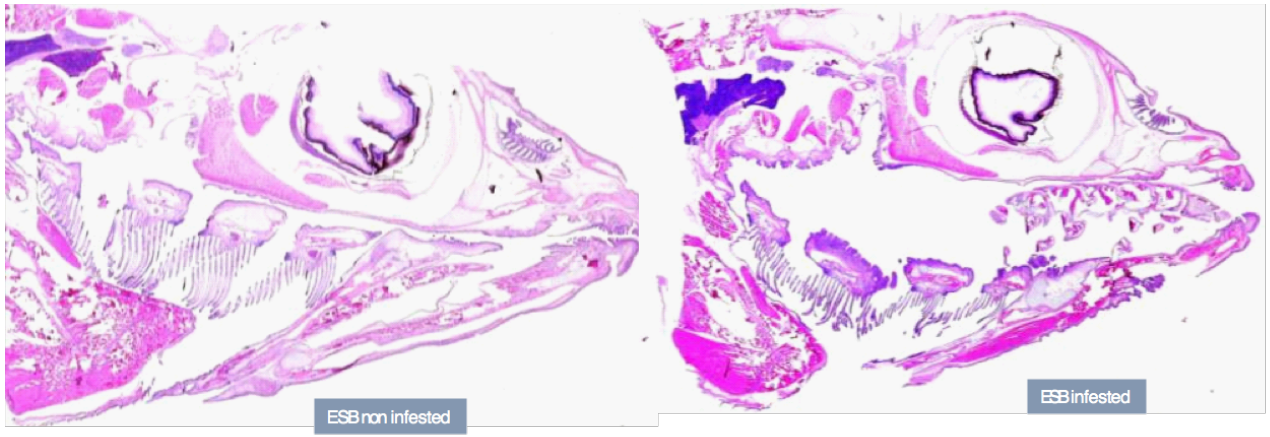
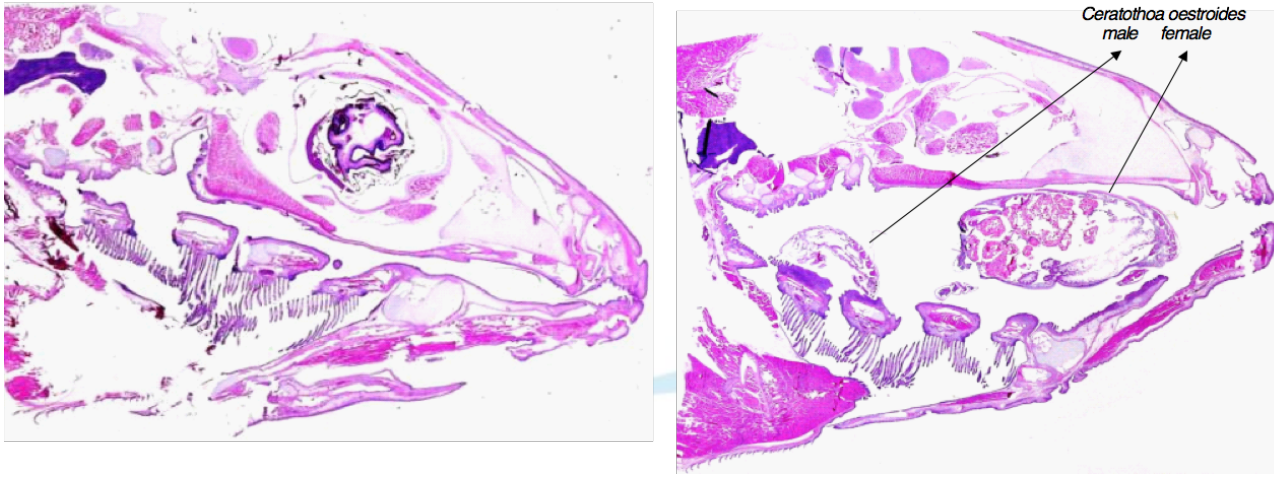
tissues damage at the parasitation site,
at systematic level: growth defects,
decrease in mean host weight and size,
depressed fecundity,
increased mortalities



sea bream (Sparus aurata)
sea bass (Dicentrarchus labrax
meagre (Argyrosomus regius)



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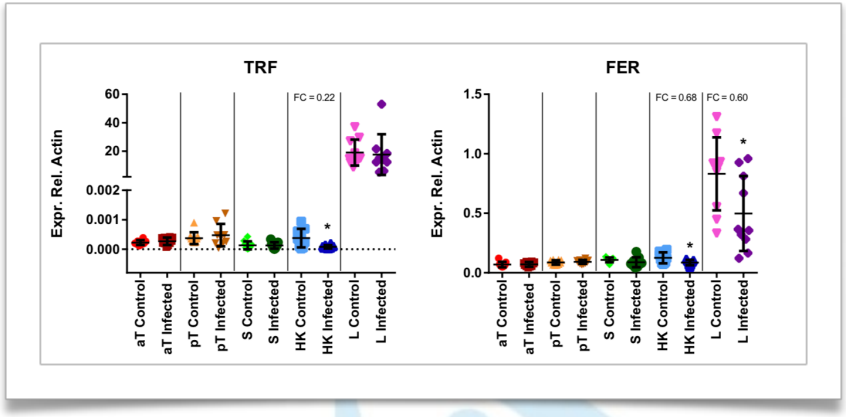
gen expression data:

- local and systematic effect of the parasite!

- B (IgM, IgT, IgD)
- T (CD3z, CD4, CD8)
- MHC I & II
- cytokines (IL6, IL8, IL1b, IL4/13A1, IL17a/f, IL10, TNFa)
- starvation (IGF1, GHR1 & 2)
- apoptosis (CAS3, CAS6)
- wound heeling (MMP9)
- ion transport & metabolism (transferrin, ferritin)



paper in prep Piazzon et al.

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Our approach and our team

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- **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**

IOR, Croatia

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Our proposed solution (I)

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bring live female *C. oestroides* into the laboratory experimental in vitro system and keep until hatching of pulli is observed

collect the live parasite: 10 adult specimens into 6-well plates with the different **synthetic compounds** (*bitoscanate, closantel, diallyl sulphate, doramectin, monocrotiline and moxidecin*)

test preliminary 10 different concentrations, from 0.01 to 0.1 M, or from 0.1 to 1M, depending on the chemical

monitor the survival under stereomiscroscope at 0.5 h, 1 h and 2 h (endpoint - no pleopods movements in the pulli observed)

scale down new LC, so the LC50 was reached **within 2 h** (triplicate, 10 specimens per replica, testing seven concentrations per compound)

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Our proposed solution (II)

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

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Our proposed solution (III)

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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

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Expected benefits for the industry

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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

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Current status and next steps

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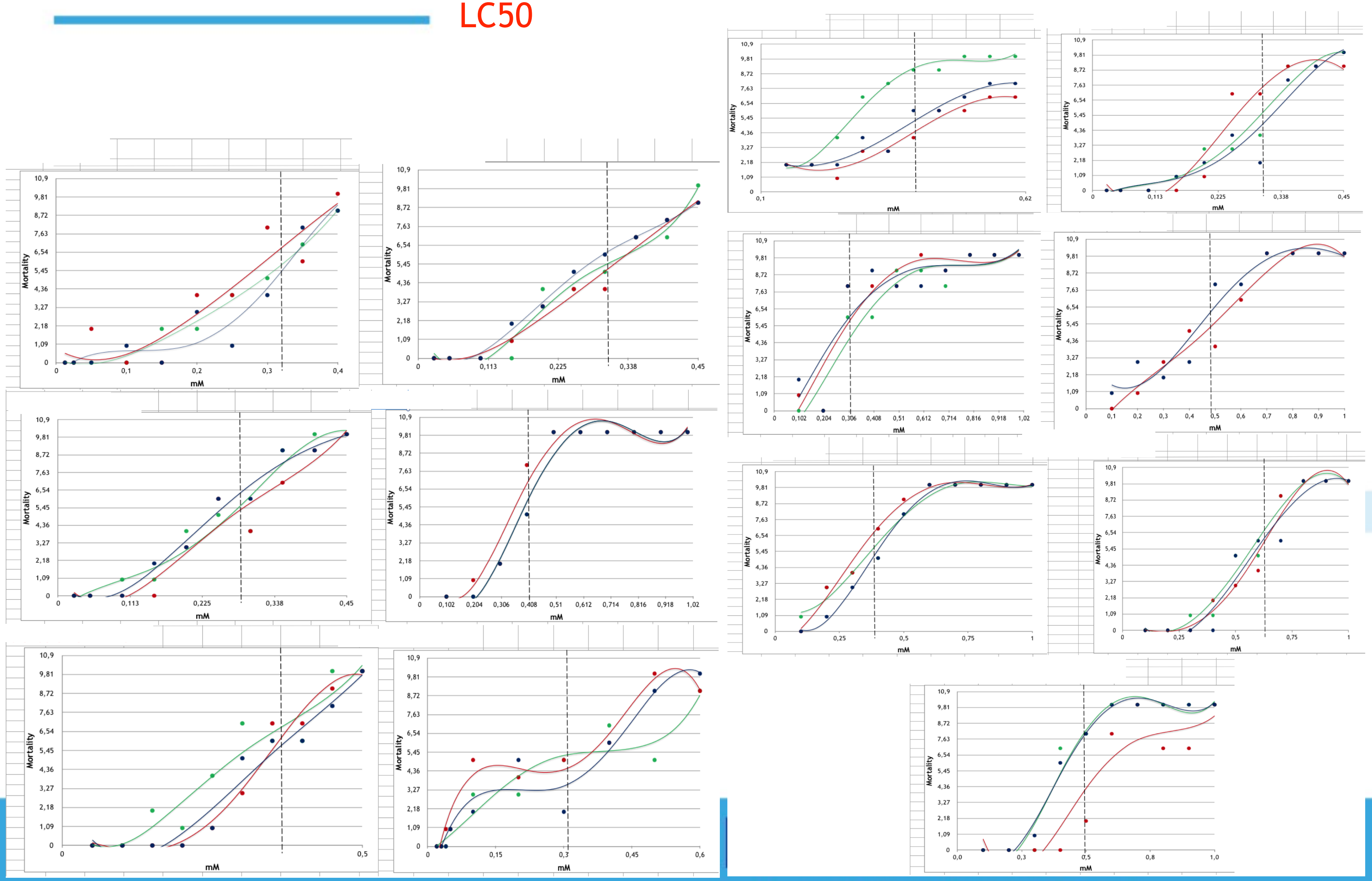
LC50



Current status and next steps

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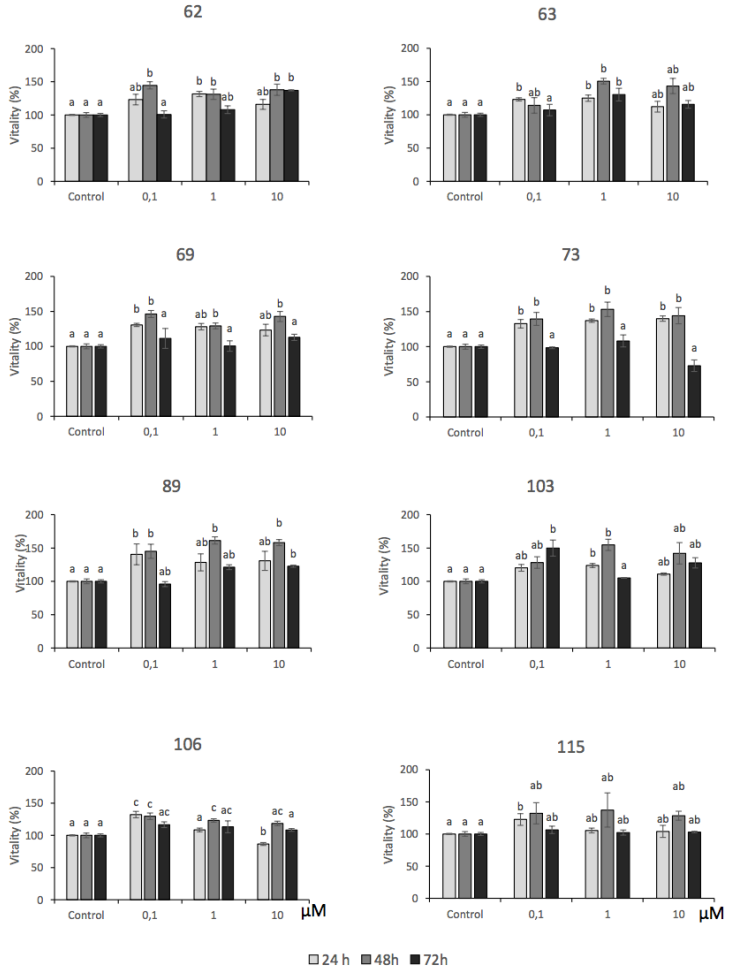
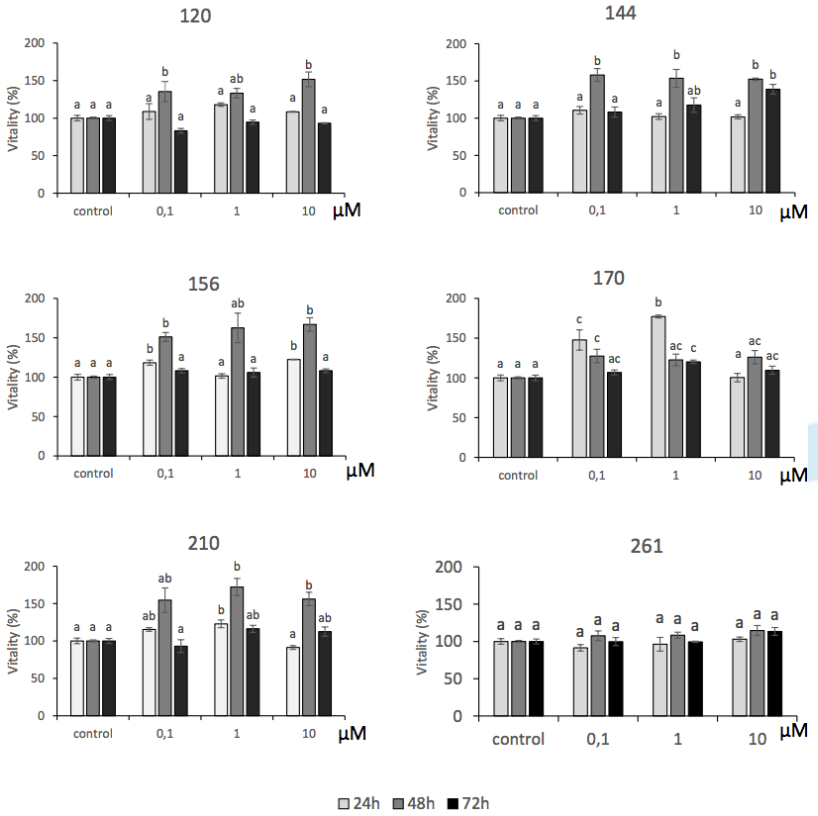


SAF-1 vitality %



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Conclusions

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interestingly, synthetic and natural compounds had LC50 in same decimal range

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

unfortunately no genome assembled & annotated as in case of *Sparicotyle*

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Thank You

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