ParaFishControl

New treatments for parasitic diseases in European aquaculture farms
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ParaFishControl Final Conference
"Innovative Strategies to Control Parasites in Aquaculture Farms"
Brussels, 11th March 2020

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Fish species within Parafishcontrol

Saprolegniosis: a threat to aquaculture and natural fish stocks

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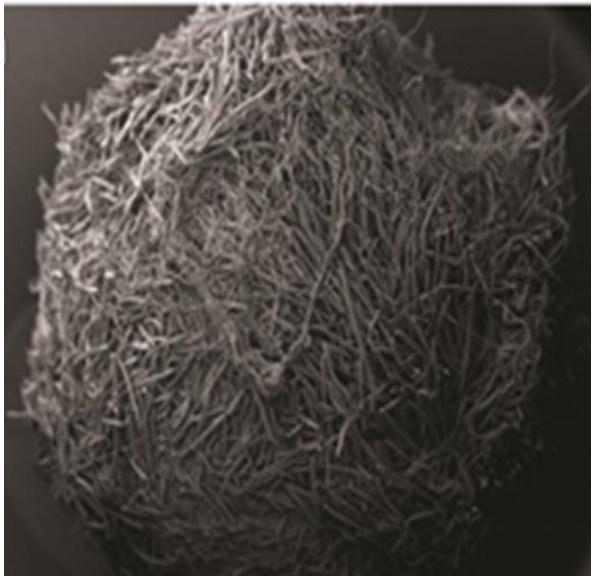
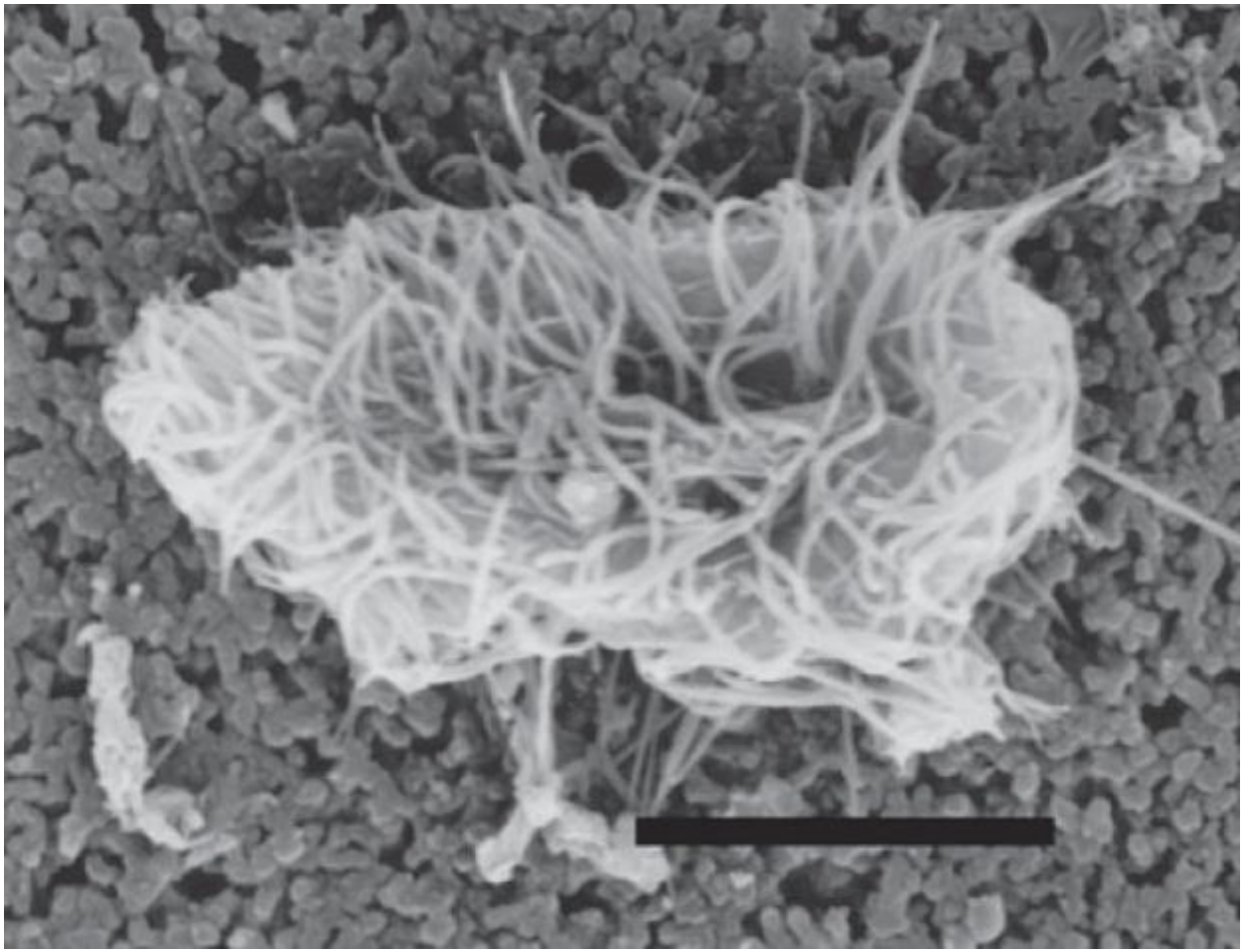


Caused by the oomycete *Saprolegnia* spp.
Compounds tested by P13 UNIBO

Scuticociliate Philasterides



Testing compounds against Ich by P9 KU, P8 INIA and P10 MTA

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Tested against infective stages (theronts) of
Ichthyophthirius multifiliis

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Sparicotyle by P6 HCMR and P19 Skretting

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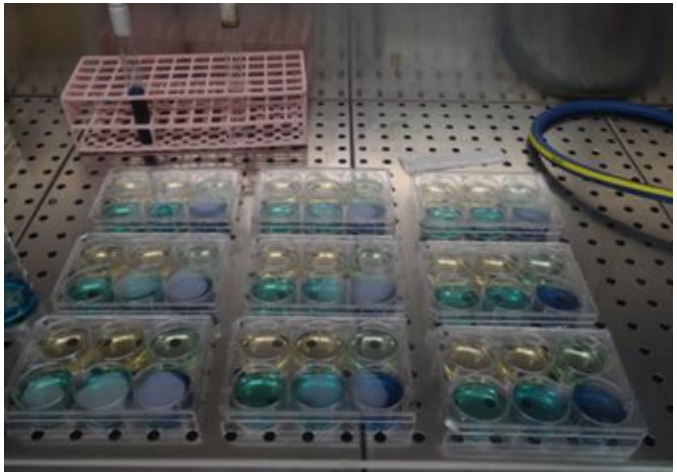
IOR P7: Testing new compounds against Ceratothoa oestroides pulli

UNIBO Methodology

Protocol I: screening in agar (MIC)



Protocol II: screening in water (MLC)

Tested strains

- reference strain of *Saprolegnia parasitica* (CBS 223.65 provided by CSIC-RJB Madrid, Spain) isolated in Holland from northern pike (*Esox lucius*)
- field strain of *S. parasitica* (ITT 320/15/20) isolated in Italy from brown trout (*Salmo trutta fario*)
- field strain of *Saprolegnia delica* (ITT 290/15/15) isolated in Italy from rainbow trout (*Oncorhynchus mykiss*)

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Tested compounds / concentrations

Tested molecules

acetic acid, benzoic acid, boric acid, iodoacetic acid, lactic acid, oxalic acid, tartaric acid, hydrogen peroxide, sodium percarbonate

malachite green used as a reference compound

copper sulfate added because widely used to control saprolegniosis in aquacultured fish

Commercial products

Actidrox®, De Marco, Italy

Detarox AP®, Perdomini, Italy

Virkon S®, Dupont, UK

Tested concentrations

0.1; 1, 5; 10; 50;100; 250; 500; 1000 and 5000 ppm

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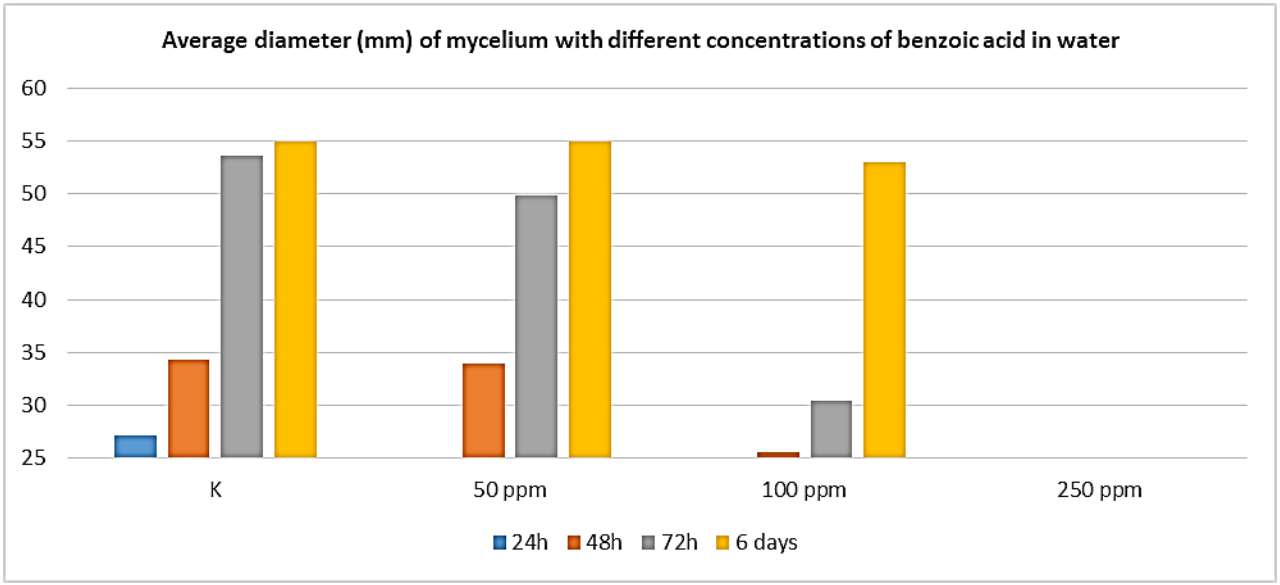
Example showing effects of benzoic acid in water on mycelium



Tested compounds / concentrations

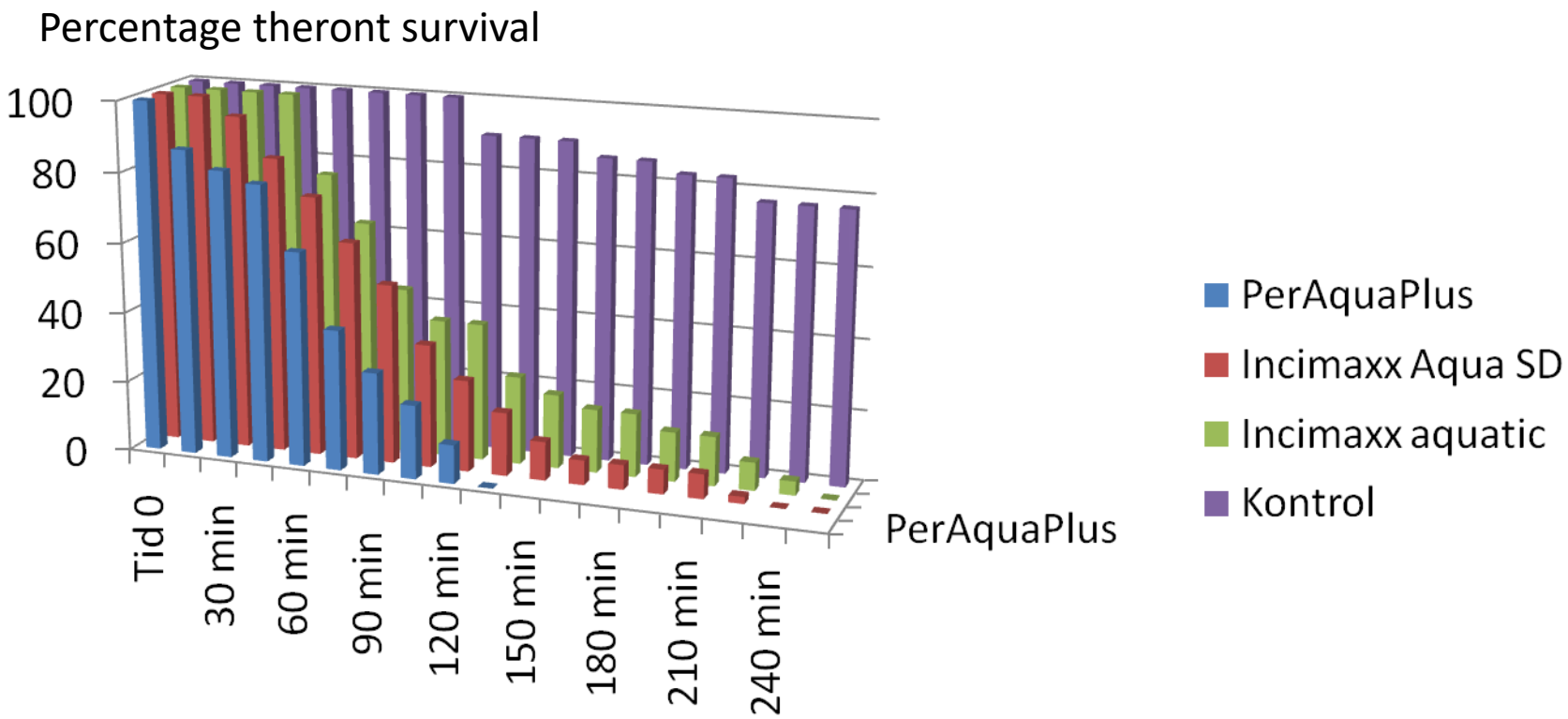
2',4'-DIHYDROXYCHALCONE
7-HYDROXYFLAVONE
CAMPHOR (1R)
DIALLYL SULFIDE
ESCULETIN
EUCALYPTOL
PALMATINE CHLORIDE
PIPERINE
PLUMBAGIN
SCLAREOLIDE
UMBELLIFERONE
USNIC ACID

COMPOUND	MIC (mM)
263 Zinc pyritione	0.01
67 Butoconazole	0.1 (only for 320 strain)
99 Clotrimazole	0.1
91 ciclopiroxolamine	0.1
100 -5-chloro-8-hydroxyquinolone (Cloxyquin)	0.1
132 econazole nitrate	0.1
288 Sulconazole nitrate	0.1
114 Dequalinium chloride	0.1
310 Triclosan	0.1
300 tetrametiylthiuram disulfide (thiram)	0.25
68 Butyl4-hydroxybenzoate (butil paraben)	0.25
95 Climbazole	0.25
169 Hexetidine	0.25 (only for CBS strain)
314 Undecylenic acid	0.25
67 Butoconazole	0.25
65 Bronopol	0.25 (only for 290 strain)

Compounds from ZF-S tested in vitro against Ich by P9 KU and P10 MTA

Compound	Stock	Screening	max effective dilution	tested dilutions
Biotin	10 mM	0.5-0.0001mM of stock	100	25-100
Chrysanthemic acid,	10 mM	0.5-0.0001mM of stock	100	25-100
Nitarsone VETRANAL	08 mM	0.5-0.0001mM of stock	100	25-100
Eucalyptol	10 mM	0.5-0.0001mM of stock	100	25-100
Ronidazole	10 mM	0.5-0.0001mM of stock	100	25-100
Secnidazole	10 mM	0.5-0.0001mM of stock	100	25-100
Psoraldine	10 mM	0.5-0.0001mM of stock	100	25-100
Tinidazole	10 mM	0.5-0.0001mM of stock	200	50-200
Dimetridazole	10 mM	0.5-0.0001mM of stock	200	50-200
Metronidazole	10 mM	0.5-0.0001mM of stock	200	50-200
Nifursol	10 mM	0.5-0.0001mM of stock	750	100-750
Resveratrol	10 mM	0.5-0.0001mM of stock	750	100-750
Conessine	10 mM	0.5-0.0001mM of stock	10000	2000-10000
Sanguinarine	10 mM	0.5-0.0001mM of stock	15000	3000-15000
Tomatine	10 mM	0.5-0.0001mM of stock	40000	22000-40000

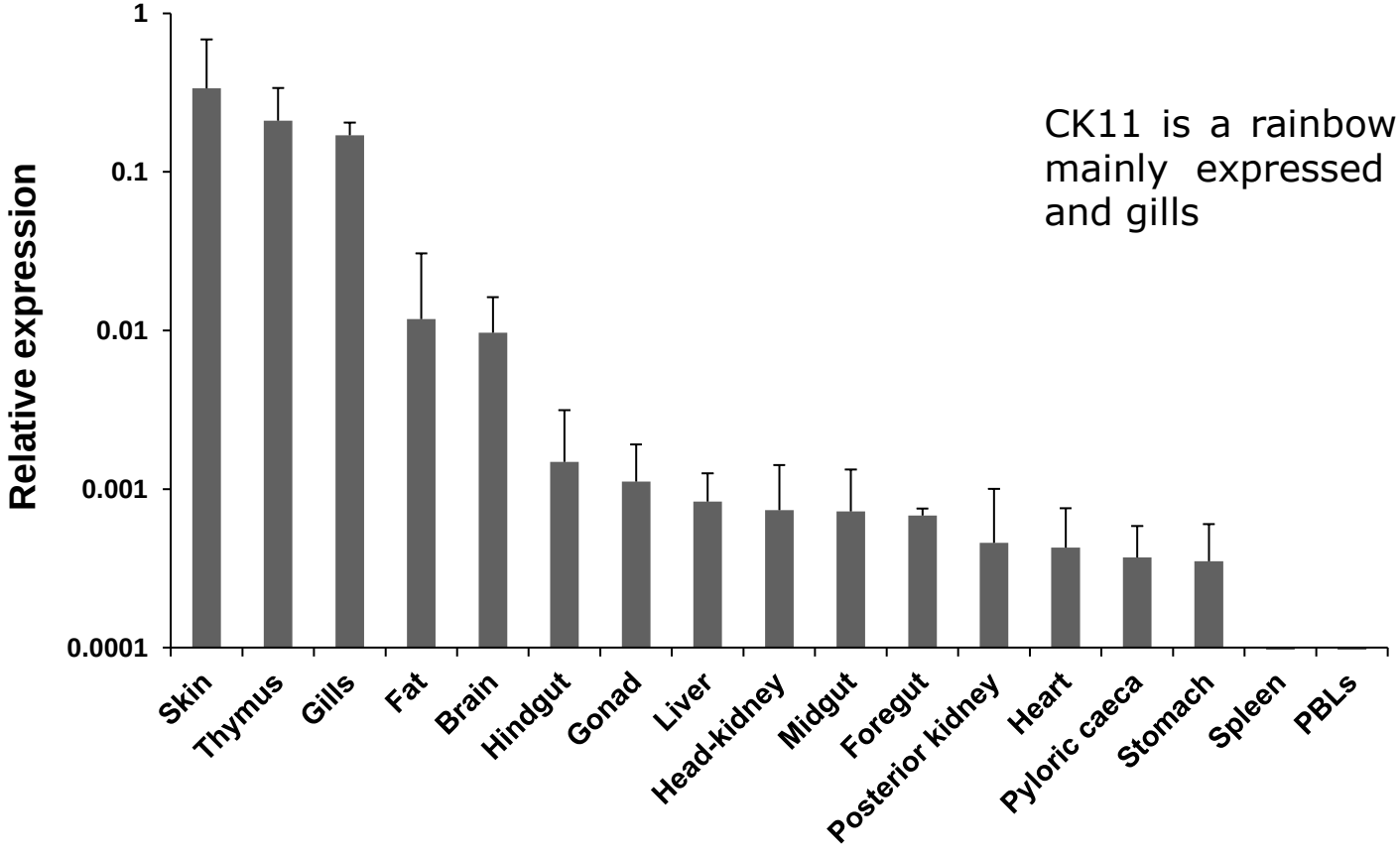
Peracetic acid:
Effects on theronts 0.08 mg/l



Field tests using peracetic acid conducted in rainbow trout farms

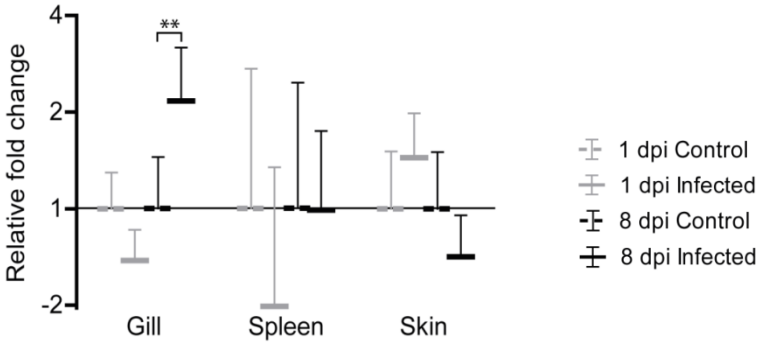
- Both traditional earth pond systems and partly recirculated systems tested – total 5
- One pulse: 0.5 to 1.0 ml per cubic meter water
- Effective but reinfection occurs
- 6-8 pulses per day
- Twice a week
- Effect lasts for a week

P8 INIA testing antiparasitic activity of rainbow trout chemokines

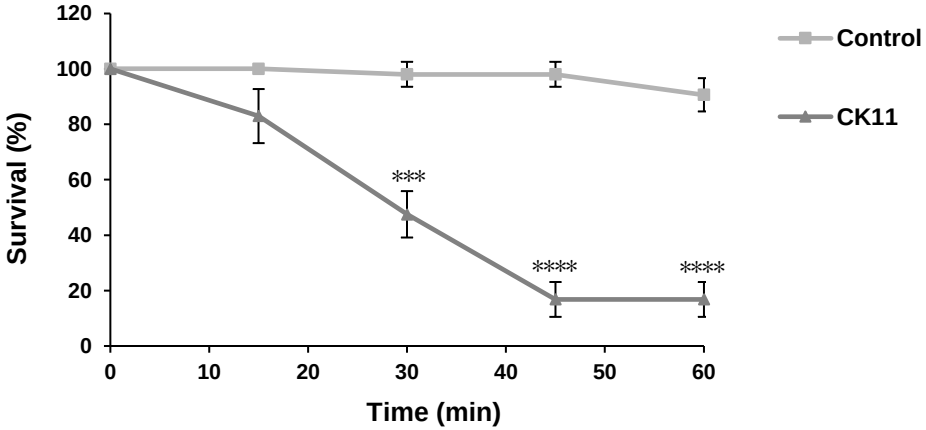


CK11 is a rainbow trout chemokine mainly expressed in skin, thymus and gills

Effect of CK11 on Ich (P8 and P9)



CK11 transcription is induced in gills in response to Ich

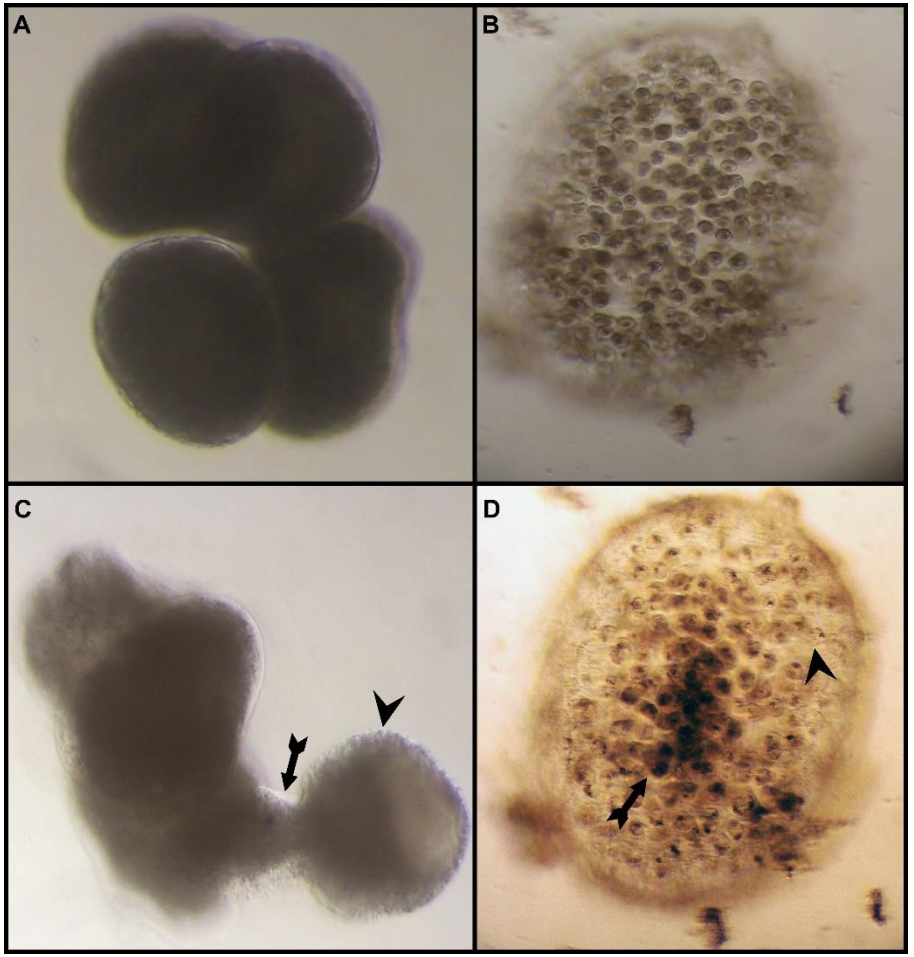


CK11 can affect the viability of Ich theronts

P29 KNAW and P9 KU showed that also bacterial compounds are effective against ICH

Pseudomonas lipopeptides solubilize ICH membrane constituents

P9: Also aqueous extracts of herbs such as garlic, oregano and thyme eliminate ICH parasites



P11 USC Philasterides: testing compounds

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Products to be tested	**74**

Products already tested by our group

- **0106: Curcumine**
- **0125: Dimetridazole**
- **0207: Metronidazole**
- **0273: Resveratrol**
- **0274: Romidazole**
- **0037: Artemisinin**
- **0301: Timidazole**

Experimental work

-1. Test toxicity in living ciliates at **100 µM. 37 products were toxic**

-2. Among the 37 products, a second selection was made based on toxicity for animals (based on literature review), toxicity for RAW 264.7 cells and toxicity for *Philasterides* (tested in the lab).

-3. **Nine products** were selected for a more detailed evaluation

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Products of natural origen tested against *Philasterides*, but toxic for RAW cells MTT/Propidium iodide (IP)

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2'-4' dihydroxychalcone	002	17,49 µM	+	+
Conessine	102	17,61 µM	+	+
Piperine	249	67,13 µM	+	+
Plumbagin	251	28,12 µM	+	+
Tomatine	306	33,73 µM	+	+
Usnic Acid	315	68,25 µM	+	+

Products used as food additives

Product	Code	IC50 24 h	Toxicity *Philasterides*	Toxicity RAW/MTT
Butyl 4-hydroxybenzoate	68	67,55 µM	+	-
4'-hexylresorcinol	170	66,59 µM	+	+
Sclareolide	280	46,05 µM	+	-

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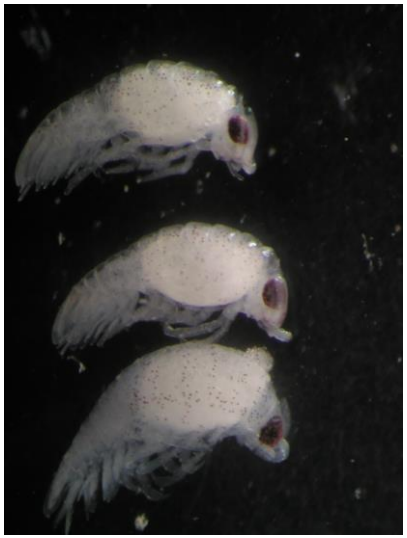
P19 SKRE and P6 HCMR

Effect of functional feeds against *Sparicotyle chrysophrii* in sea bream

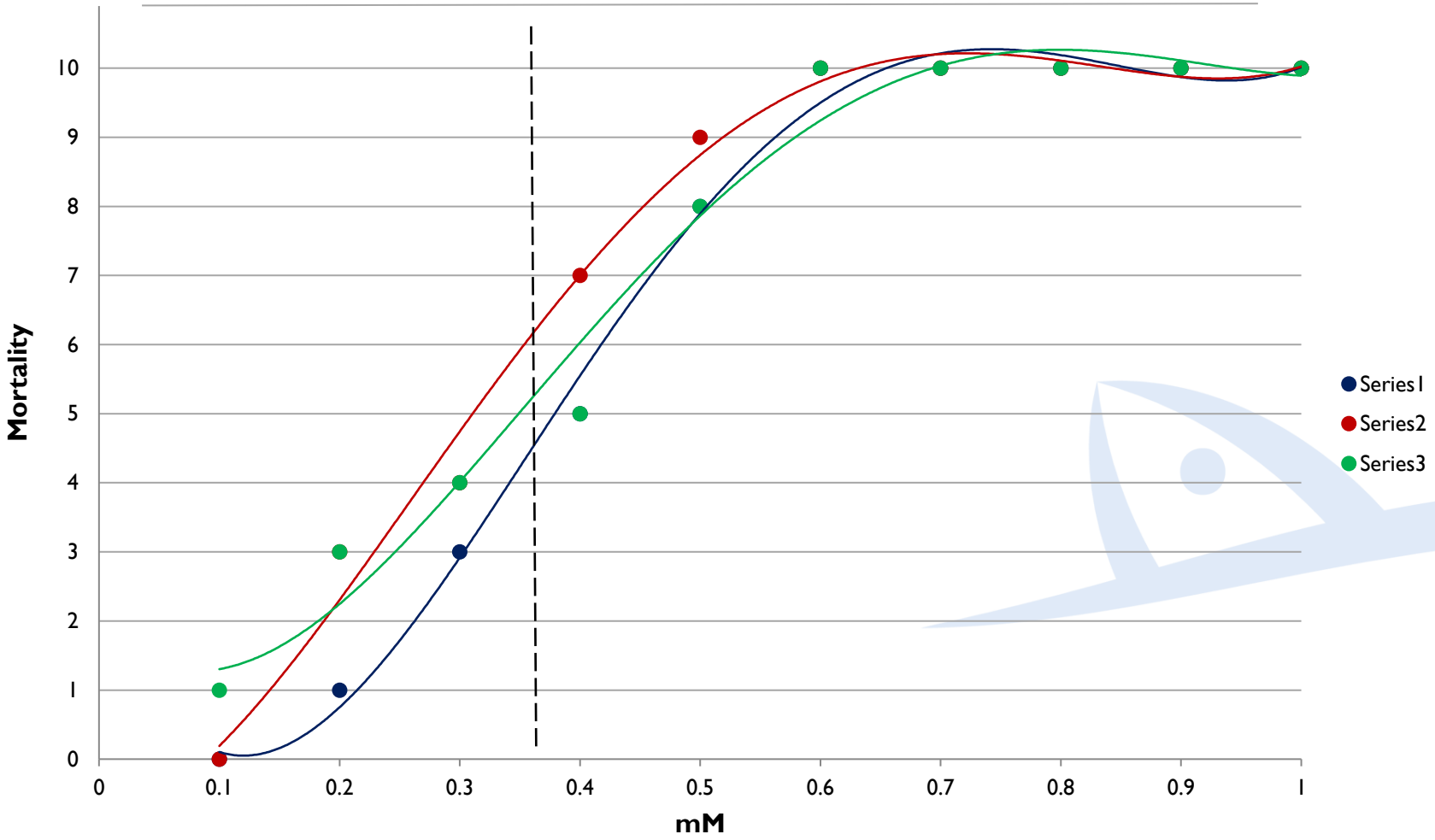
- Cohabitation challenge model: Experimental cages inside concrete tank. Flow-through open system. Donor infected fish from affected farm introduced in tank outside cages (1:1)
- May to August 2017: Temperature from 23.5°C to 28.7°C
- Naïve sea bream: 30 g: 60 fish/cage
- 3 treatments in triplicates; 1 control and 2 experimental diets
- Experimental diets based on combination of natural ingredients with anti-parasitic effect and extra nutritional support for the fish

Testing compounds for effects on *Ceratothoa* pulli in vitro



Compound	LD50 mM
EUCALYPTOL	0,35
GARLICIN 80%	0,57
AURAPTENE	0,36
BITOSCANATE	0,29
CAMPHOR	0,28
(+)-TRANS-CHRYSANTHEMIC ACID	0,44
CLOSANTEL	0,28
DIALLYL SULFID	0,26
CEDROL	0,29
DORAMECTIN	0,37
MONOCROTALINE	0,32
MOXIDECIN	0,33
CURCUMIN	0,44

Example using eucalyptol against *Ceratothoa*



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Conclusion

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- A wide range of natural and synthetic compounds were tested for effects against the main European fish parasites
- Many are effective but most of these are toxic to fish
- Some will be investigated further and may be licensed
- Some compounds are non-toxic and environmentally friendly
- Efforts are under way to produce the novel compounds in large scale to the benefit of the aquaculture industry

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Thank You

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