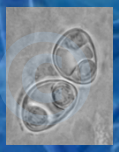
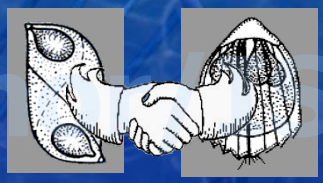


On the dynamics of mixed myxozoan infections and potential mechanisms of within-host interactions



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Initiation of this study

Little is known about the course and the dynamics of mixed myxozoan infections in the same host organ and in relation to the development of the host

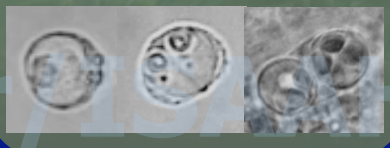
⇒ Study mixed infections of coelozoic myxozoans infecting the excretory system of fish

Gadus morhua

- *Zschokkella hildae* Auerbach, 1910
- *Gadimyxa atlantica* Køie *et al.*, 2007

Salmo trutta

- *Tetracapsuloides bryosalmonae* Canning et al. 1999
- *Sphaerospora truttae* Fischer-Scherl et al. 1986
- *Chloromyxum schurovi* Shul'man & Ieshko 2003

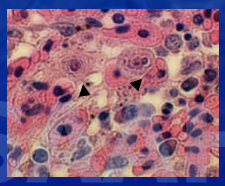


Aims of the study

Monitor the different myxozoan species in a population of fish during development of fish from smallest fry to market size fish.

- Detect date/period of initial infection
- Prevalence and semi-quantitative intensity of infection of different myxozoan parasites during development of the hosts.
- Location of the different stages of parasite
- Possible parasite-parasite interactions

Detection of myxozoan developmental stages is problematic due to their size, number and cryptic nature.



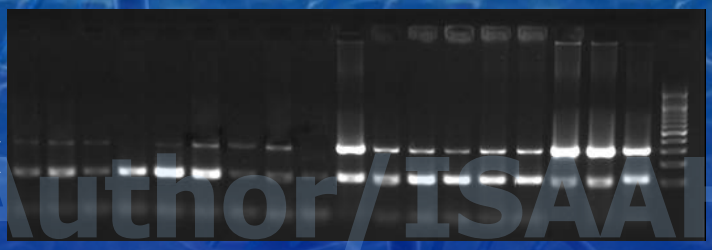
Molecular Methods:

Multiplex PCR
(Double label) *in situ* hybridisation

G. morhua study

Prevalence and semi-quantitative intensity of infection

Multiplex PCR KIDNEY



0 + cod kidney site A

0 + cod kidney site C

0 + cod kidney site B

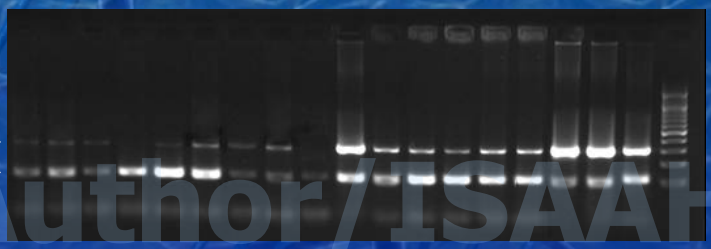
FRY (90 days)

- Fry become infected with both myxozoans at very early age
- Infection with *G. atlantica* is more intense

G. morhua study

Prevalence and semi-quantitative intensity of infection

Multiplex PCR KIDNEY



0 ++ cod kidney

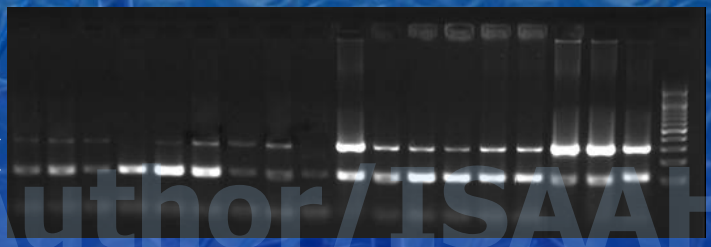
0++ cod (6 months)

- Intensity of infection with *G. atlantica* and *Z. hildae* increases
- *G. atlantica* is the predominant species
- 23% of 0++ fish showed increased quantity of PCR products of *Z. hildae*

G. morhua study

Prevalence and semi-quantitative intensity of infection

Multiplex PCR KIDNEY



1+ cod kidney

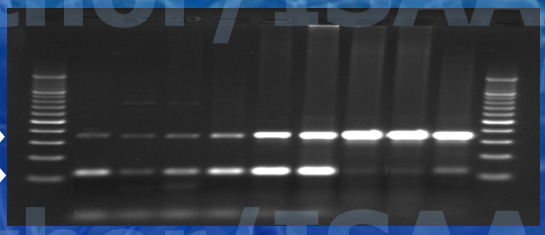
1+ cod (18-19 months)

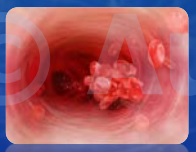
- Intensity of infection with *G. atlantica* decreases
- *Z. hildae* is (by far) the predominant species

G. morhua study

Prevalence and semi-quantitative intensity of infection

Multiplex PCR BLOOD





BLOOD SAMPLES

- Both species produce blood stages
- *G. atlantica* is present in 0++ cod, absent (weak bands) in 1+ cod.
- Intensity of infection of *Z. hildae* increases considerably in 1+ cod, once *G. atlantica* has disappeared from the blood.

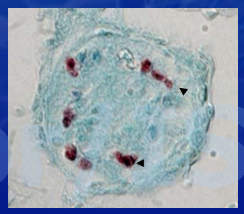
G. morhua study

Localisation - Double label *in situ* hybridisation

G. atlantica: purple signal. ***Z. hildae:*** blue signal.

FRY (90 days)

- Hardly any *Z. hildae* stages detectable, infection is dominated by *G. atlantica*.
- Predominantly intratubular stages: prior to or in the process of spore formation
- Small number or pluricellular interstitial stages (17.5-41.2 µm)
- Ver small (6-15.5 µm diameter), bicellular intraglomerular stages



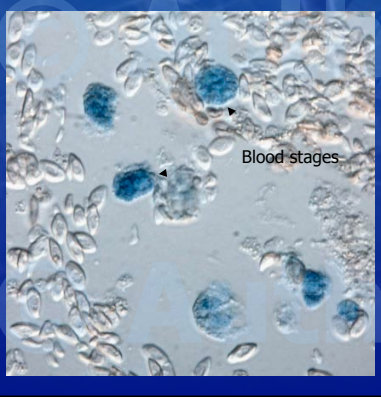
Intraglomerular stages

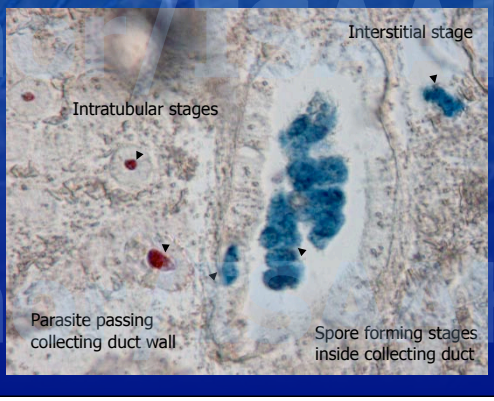
G. morhua study

Localisation - Double label *in situ* hybridisation

0++ cod (6 months)

- Heavy invasion of *Z. hildae*: large numbers of pluricellular blood stages (16.4-35 µm) inside blood vessels. Numerous interstitial stages (15-160 µm).
- *Z. hildae* spore-forming stages occurred predominantly in the collecting ducts and ureters. Invasion of collecting ducts occurred via the epithelium.
- *G. atlantica* was present in smaller numbers than in 0+ cod and occurred in the form of intratubular, spore forming stages.

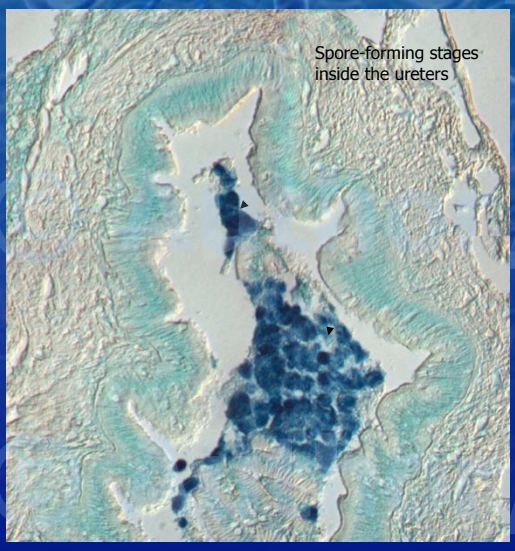




G. atlantica: purple signal. ***Z. hildae:*** blue signal.

G. morhua study

Localisation - Double label *in situ* hybridisation



1+ cod (18-19 months)

- Only few intratubular remains of *G. atlantica* infection
- *Z. hildae* is (by far) the predominant species
- *Z. hildae* blood stages and interstitial stages occur as in 0++ cod but the number of spore-forming stages in the collecting ducts and ureters has increased considerably, sometimes filling them completely and causing slouthing of the epithelium.

G. atlantica: purple signal.

Z. hildae: blue signal.

G. morhua study

Conclusions

Spatial and temporal separation of the infection with *G. atlantica* and *Z. hildae* in cod during their development despite simultaneous infection of the smallest fry with both species

- *Reciprocally varying prevalences with one species increasing in prevalence while another decreases, despite parallel transmission rates of both, suggest within-host competition (Richie, 1988).*
- *Within-host competition could also explain the spatial separation of the two myxozoans in the excretory system of the kidney, as antagonistic interactions between parasitoids is often mediated by niche separation (Pedersen, 2004).*
- *Potential mechanisms (Mideo, 2009): 1) Exploitation competition, 2) Immune-mediated competition, 3) Interference competition*
- *Interference competition is commonly known for Plasmodium in human blood in mixed infections and may be mediated by cross-reactive antibodies. Usually, the suppressed species rebounds after the other species has abated, and shows a prolonged infection (Richie, 1988).*

G. morhua study

Follow-on ideas from cod study

- Study the course of single-species infections of *G. atlantica* and *Z. hildae* in cod

→ **PROBLEM: Unknown invertebrate host of *Z. hildae***

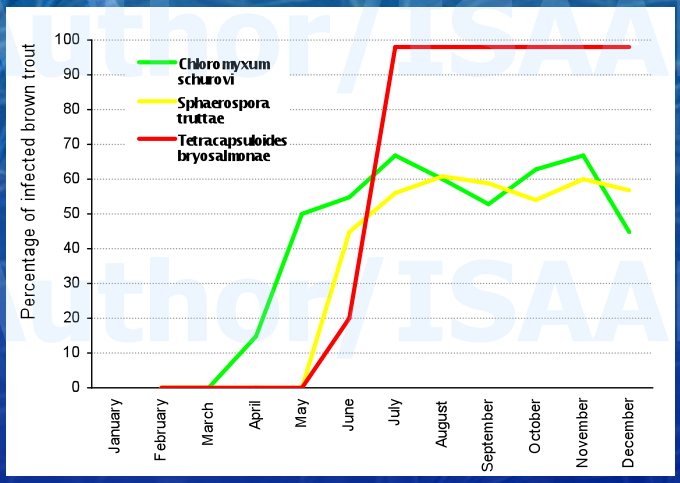
- Blood is first and probably most important site of encounter → *in vitro* studies of blood stages to study the effect of one species on the other

→ **PROBLEM: Differentiation of blood stages of the two species is not possible**

S. trutta

S. trutta study

Multiplex PCR KIDNEY

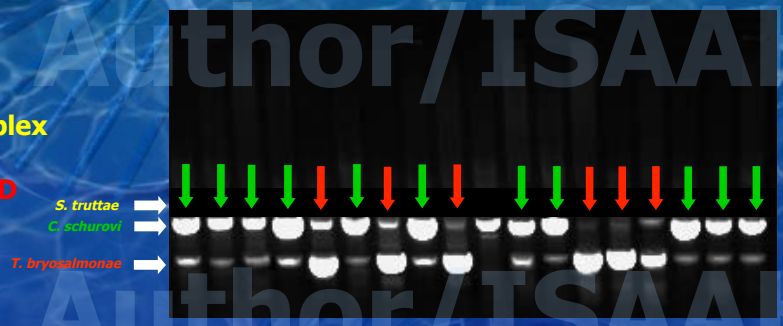


- Difference to *G. morhua* system: Different time points of initial invasion of the kidney myxozoans: *C. schurovi* invades brown trout 2 months earlier than *S. truttae* and *T. bryosalmonae*

⇒ concentrate study on data from July to December.

S. trutta study

Multiplex PCR BLOOD

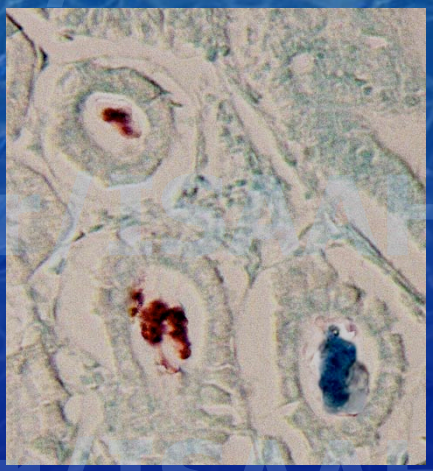


- The quantity of PCR product of *S. truttae* showed no clear pattern of dependency of the quantity of PCR products of the other two species.
- Clear reciprocal variation of intensity of infection of *C. schurovi* and *T. bryosalmonae* in the blood.
- Although the invasion of brown trout with *C. schurovi* takes place 2 months before that of *T. bryosalmonae*, the presence of *T. bryosalmonae* seems to cause a reduction in the number of *C. schurovi* blood stages

S. trutta study

- After the first year brown trout acquire immunity to *T. bryosalmonae* whereas the number of spore-forming stages of *C. schurovi* increases considerably. → *This suggests a similar course of infection as in the case of G. atlantica/Z. hildae in G. morhua.*
- Niche separation of *T. bryosalmonae* and *C. schurovi* in the kidney?

→ *T. bryosalmonae and C. schurovi both occur in the renal tubules but mixed infection of the same tubule or tubule section (histology) was never detected.*



S. trutta study

Ongoing work

- Study course of infection with *T. bryosalmonae* and with *C. schurovi* independently and as a mixed infection in SPF brown trout fry using spores from infected bryozoans *Fredericella sultana* and oligochaetes *Eiseniella tetraedra.*
- Successfully isolated blood stages using a kit for isolation of Malaria parasites from human blood.

→ *Offers a variety of opportunities; one aim is the study of myxozoan parasite-parasite interaction in vitro and the potential isolation of mediators from supernatants.*

Thanks for your attention

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