



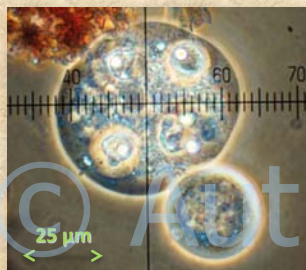
Histopathological features of the infection by the myxosporean *Zschokkella hildae* in the kidney of Atlantic cod

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Introduction

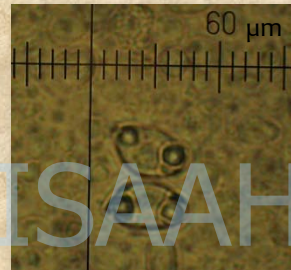
- Atlantic cod is a common host for myxosporeans, few reported as pathogens
eg. *Myxobolus aeglefini* Auerbach, 1906 (Kabata, 1957)
- *Zschokkella hildae* Auerbach, 1910 is a common myxozoan parasite of Atlantic cod; an invertebrate host is unknown to date
- The morphology, phylogeny and infection dynamics have been reported in detail by Holzer *et al*, 2010
- It is located in the urinary system: collecting ducts, ureters and urinary bladder
- In cultured cod, double infections with *Gadimyxa* spp. Køie, 2007 are common (Holzer, 2010)



Pansporoblasts



Z.hildae myxospore diagram from Lom & Dyková, 2006



Myxospores

Objectives

To assess the impact of *Zschokkella hildae* infection on farmed Atlantic cod (*Gadus morhua*) using histopathology



Materials & Methods

Fish conditions

- 200 healthy, 1+ farmed fish from the West coast of Scotland and North of England
- Mixed for 12 months in a tank-based research facility
- Seawater flow-through system, filtered to 60µm
- Ambient temperature, seasonal range 6-16 °C

Histology

- Tissue fixation in 4% NBF
- Tissue stains : H&E, Cason's trichrome and Gram's



Single-round PCR assay

- From formalin-fixed paraffin-embedded tissues (Crumlish, 2007; Santos, 2008)
- Specific primers targeted to *Z.hildae* 18S rDNA sequences (Holzer, 2010)

ISH

- Double-label in situ hybridization (Holzer, 2010)
- Simultaneous detection of both *Z.hildae* & *G.atlantica* (Holzer, 2010)

Materials & Methods

Urinary tract histology sections

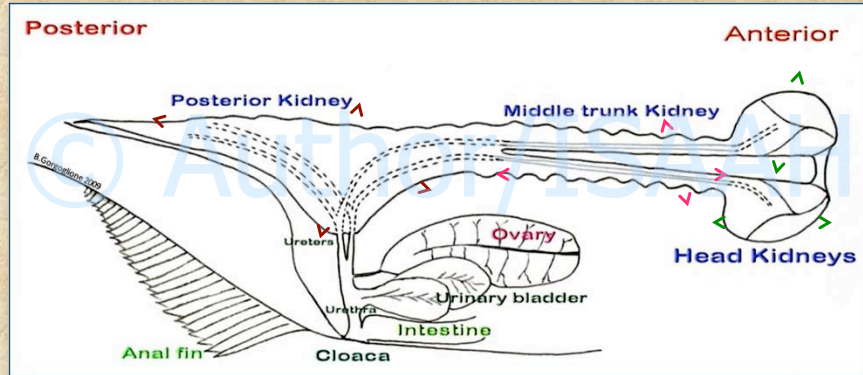
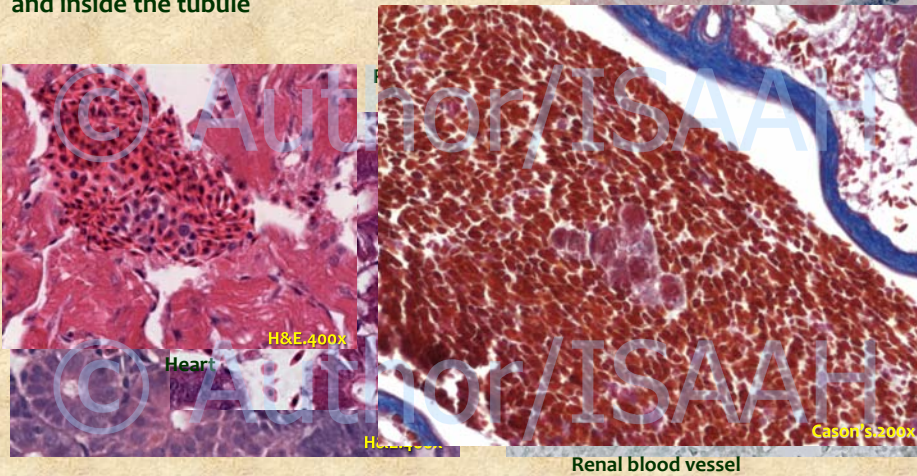
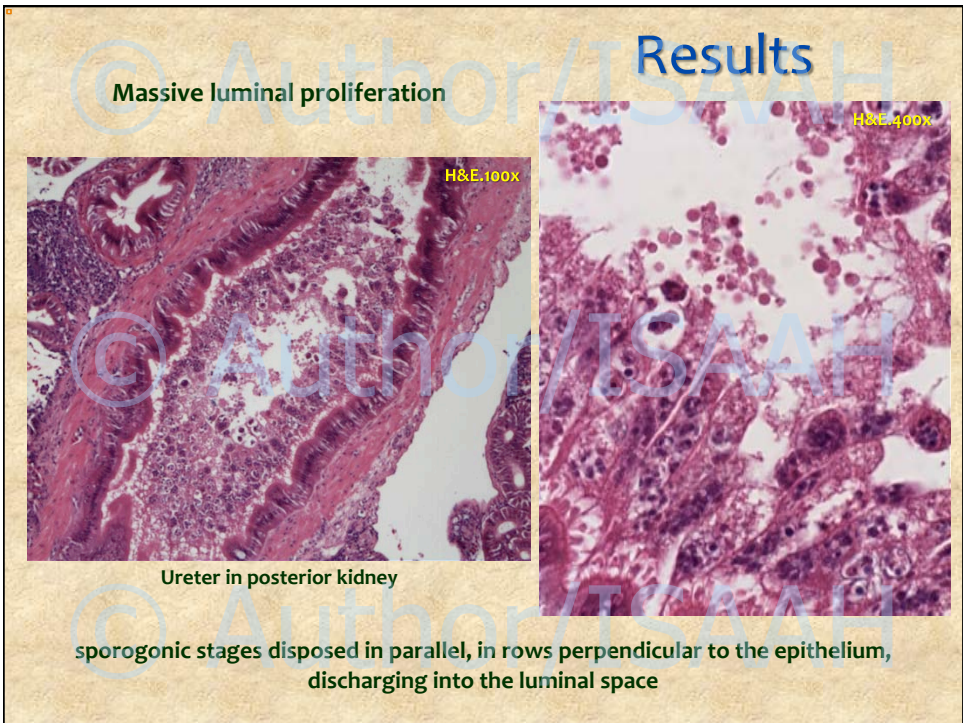
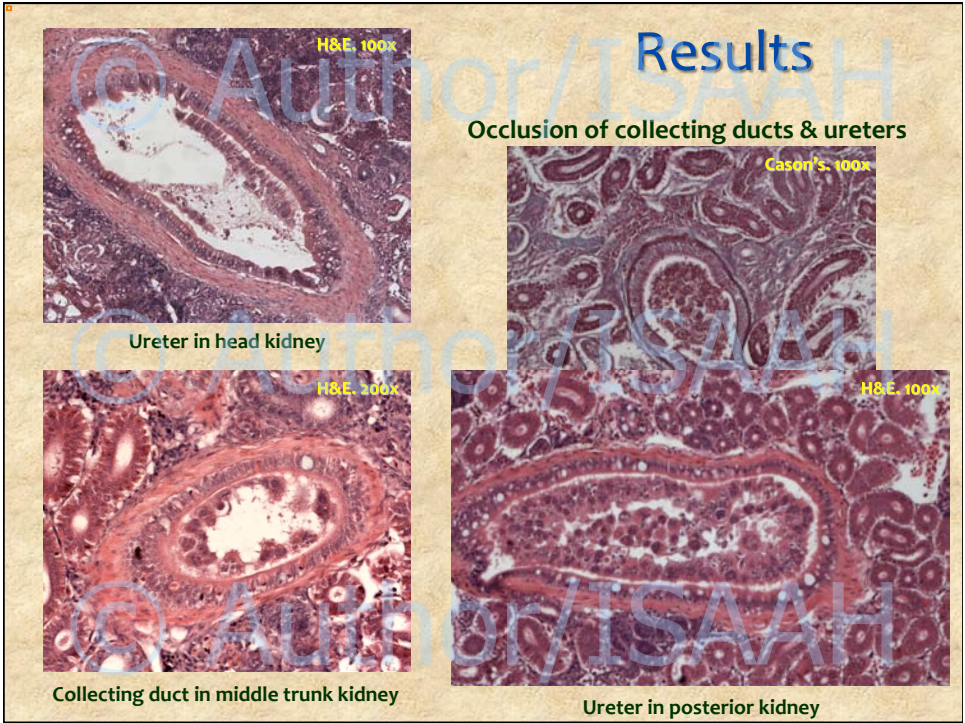


Diagram of Atlantic cod urinary system segments

Results

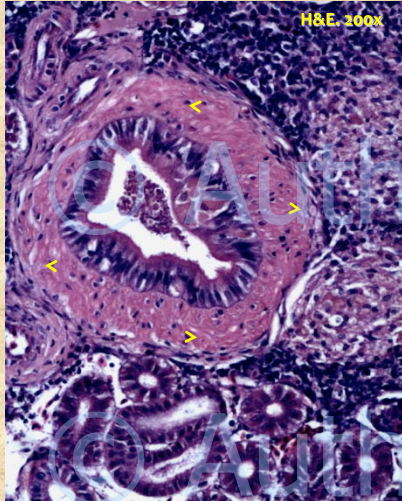
Pre-sporogonic stages detected in the blood, migrating in the interstitial tissue traversing the tubular epithelium and inside the tubule



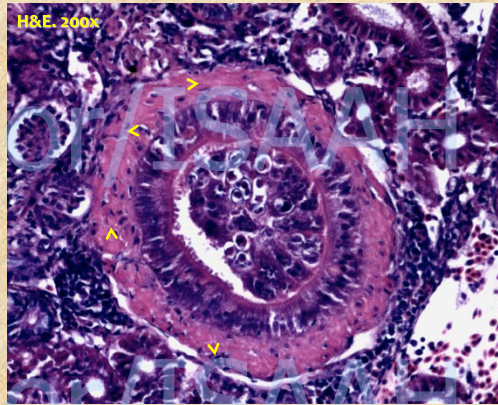


Results

Hyperplasia of supporting-connective tissue around infected collecting ducts



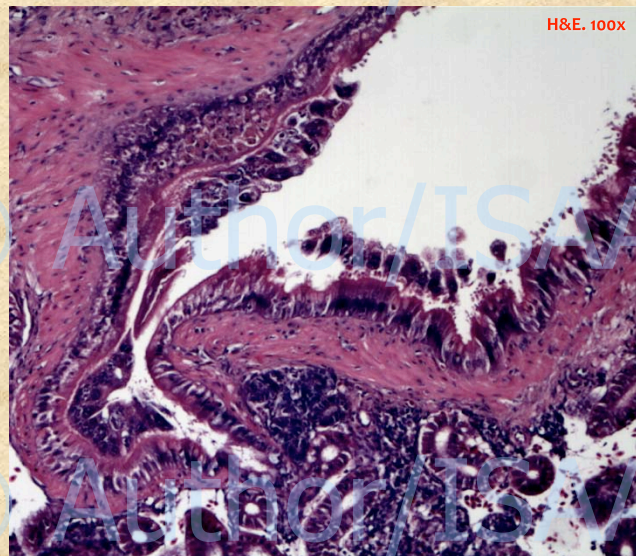
Collecting duct in posterior kidney



Collecting duct in posterior kidney

Results

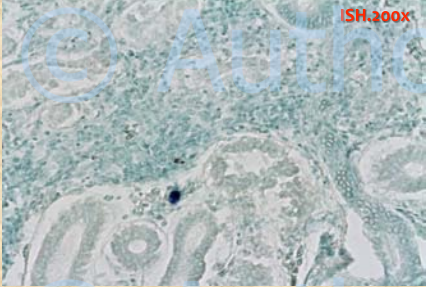
Sub-epithelial eosinophil granulocyte infiltrations



Ureter in posterior kidney

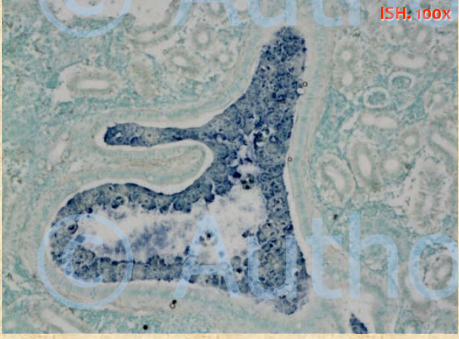
Results

ISH and PCR confirmed *Z.hildae* identity

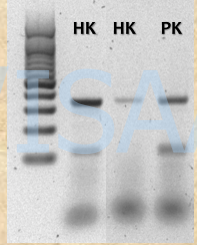


ISH, 200x

Interstitial migration in posterior kidney



ISH, 100x



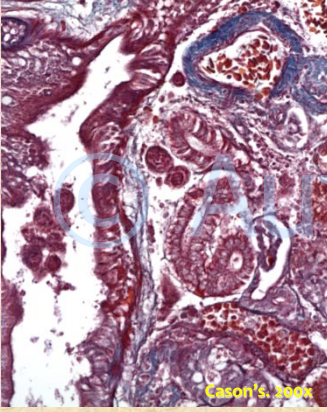
Sample	<i>Z.hildae</i> (336 bp)	<i>G.atlantica</i> (104 bp)
HK	+	-
PK	+	+

...as well *G.atlantica* lower presence

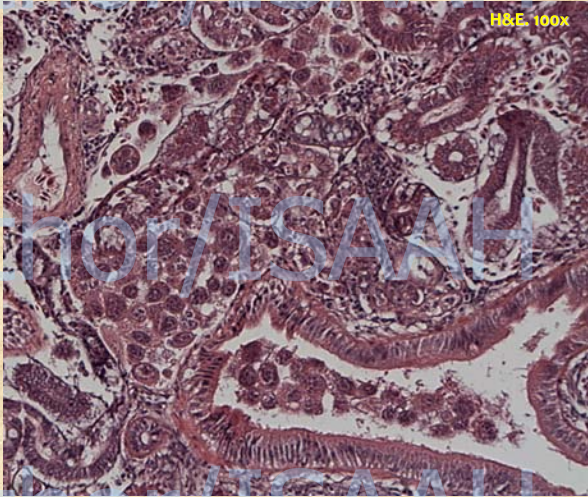
Z. Hildae sporogonic plasmodia in large clusters occluding the ureter lumen

Results

Histozoic extrasporogonic stages in posterior kidney



Cason's, 350x

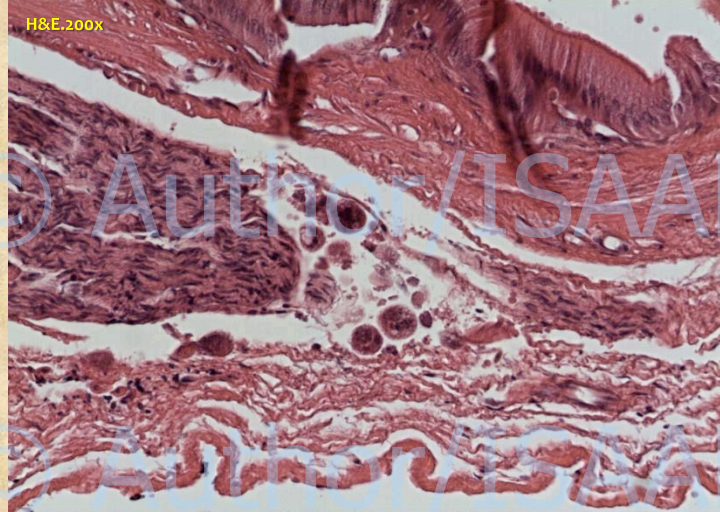


H&E, 100x

Zschokkella hildae extrasporogonic plasmodia inside the collecting duct and in the interstitial tissue

Results

Extrasporogonic stages in the urinary bladder connective tissue, between the external serous and the epithelial basal membrane

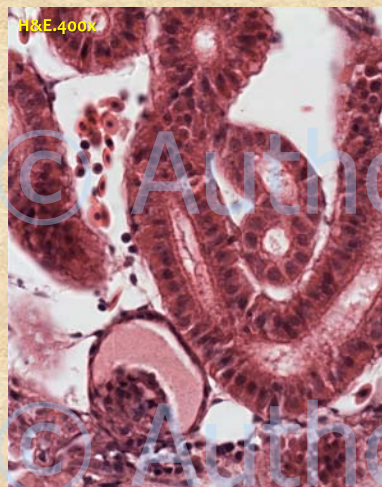


however, no associated tissue damage !

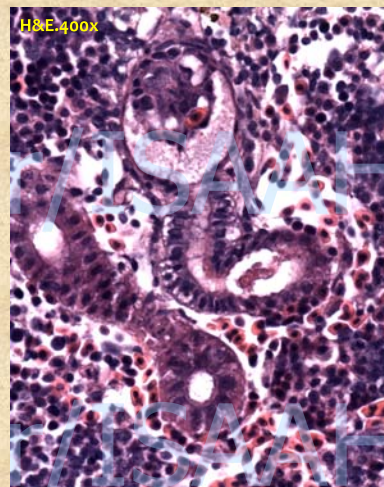
Results

Glomerular oedema

> Due to the occlusion ?

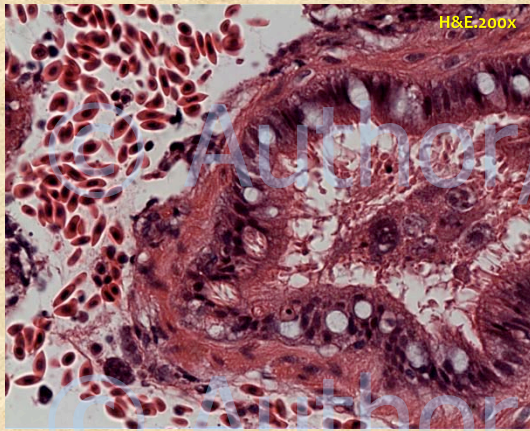


Posterior kidney



Head kidney

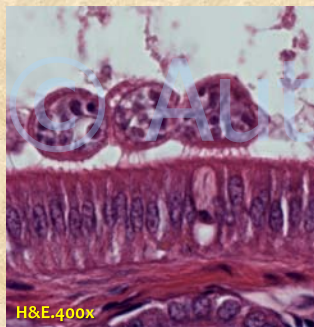
© Author/ISAAC Results
Rodlet cell invasions associated with plasmodia



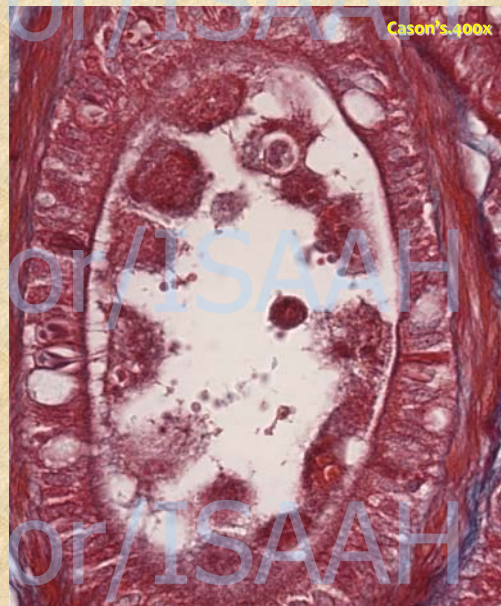
RC in the collecting duct epithelium, posterior kidney



© Author/ISAAC Results
Discharging rodlet cells attached directly to large plasmodia

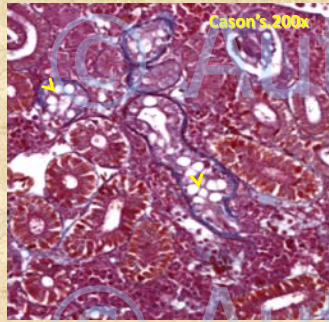


Detail from ureter in the posterior kidney



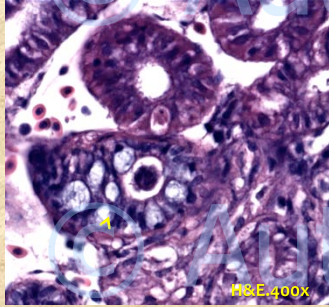
Collecting duct in the head kidney

Results



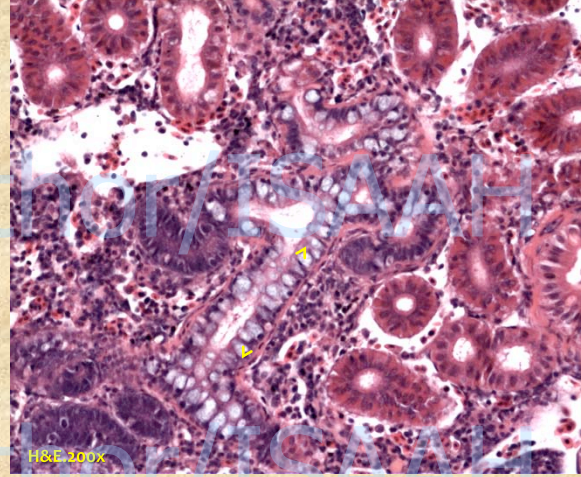
Cason's 200x

Head kidney



H&E 400x

Hydropic vacuolation of the tubular epithelial cells



H&E 300x

Posterior kidney

Mesonephron degeneration/regeneration

Conclusions

- *Z.hildae* 100% prevalence
- Dynamic of host infection
- First evidence of *Z. hildae* histozoic extrasporogonic plasmodia
- Parasites directly attacked by Rodlet cells
- Mesonephron degeneration/regeneration
- No evident external signs of disease

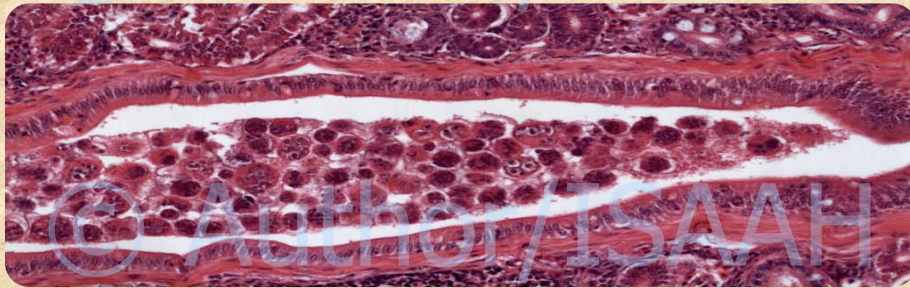
Impact

- No clinical signs of disease
- Minimal tissue damage:
 - degeneration of tubules and collecting ducts most significant

But:

The mesonephron regeneration may explain the lack of evident gross impact on host

- Rodlet cells seem to be the most important weapon against *Z. hildae*
- No clear evidence of inflammatory or immune response despite...



Aknowledgements

- **Dr Astrid Holzer**
- **Dr Rodney Wootten**
- **Dr Andrew Tildesley**
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