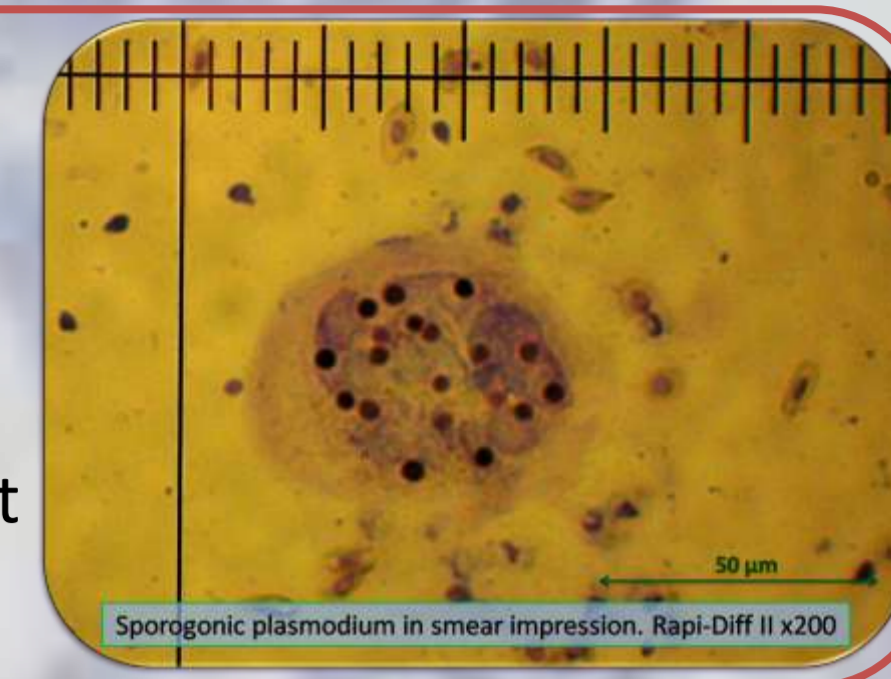


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## Introduction

Quantitative methods to assess infection levels are needed for many purposes and require to be standardised to allow accurate comparisons between groups of infected fish. The techniques commonly used were initially adapted and tested. Tissue maceration followed by mesh filtration and sedimentation are the best means of collecting myxospores for identification and morphological studies. Squash preparations, smear impressions and fresh preparations were also successful, but blood and tissue contamination were the most important obstacles to a clear microscopic examination, although these were improved with the use of Neutral red 0.1 % and Rapi-Diff II stain. Although these are all valid methods for collecting and separating myxosporean parasites, they present difficulties when trying to develop a standardised method for urinary tract parasites and were too time consuming for analysis of large samples.



## Objective

This study describes and evaluates 3 methods to assess the infection level of myxosporean parasites living in the urinary system of Atlantic cod (*Gadus morhua* L.). *Zschokkella hildae* (Auerbach, 1910), described mainly from the urinary tract of gadoids from North European seas and the Atlantic Ocean, is common in 1 to 3 year old cod, tending to decrease with host age (Karasev, 1996). Double infections with *Gadimyxa* spp. (Køie, Karlsbakk & Nylund, 2007) are common in gadoids (Køie, 2007 & 2008).

## Standard kidney histological sections

Standardised longitudinal kidney sections can be used to count the percentage of infected renal tubule in cross sections, and the number of parasites in the interstitial tissue or glomeruli. This technique was optimised on young cod, where the entire LS of the kidney can be achieved in a single section to estimate the percentage of renal tubules infected by *Gadimyxa atlantica*.

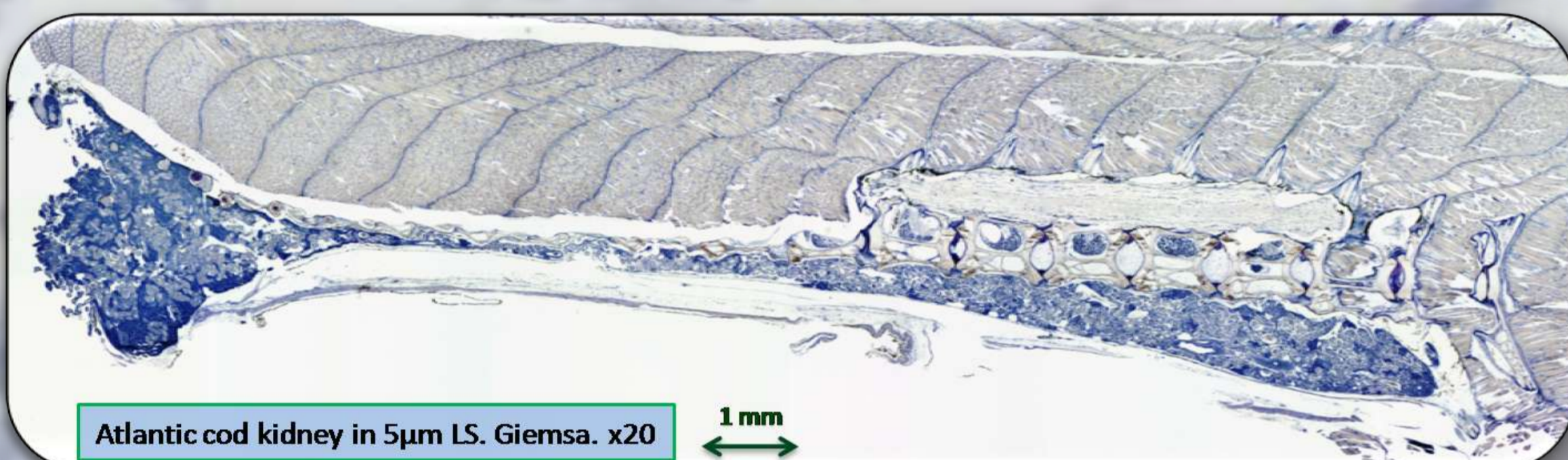
Procedure:

- Whole fry fixed in 10% Neutral Buffered Formalin (NBF)
- Fixed samples trimmed prior to blocking by removal of the head and the caudal region

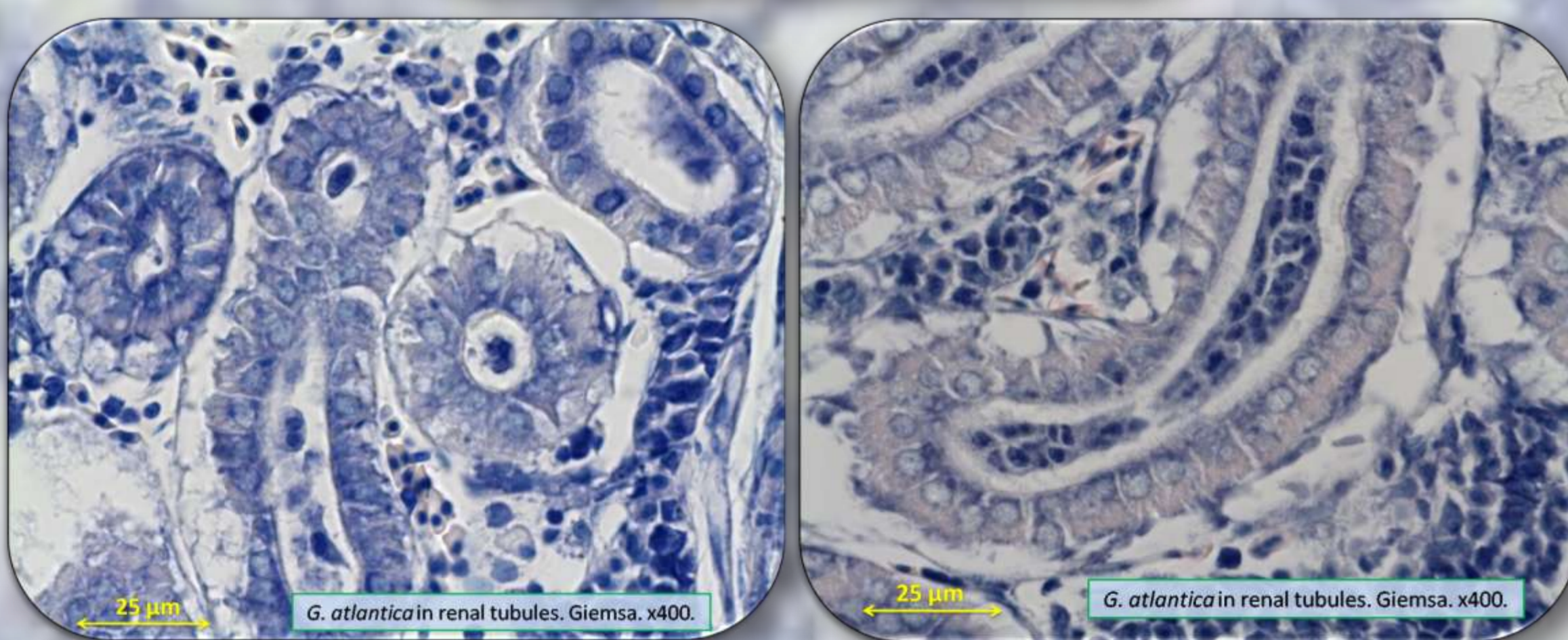
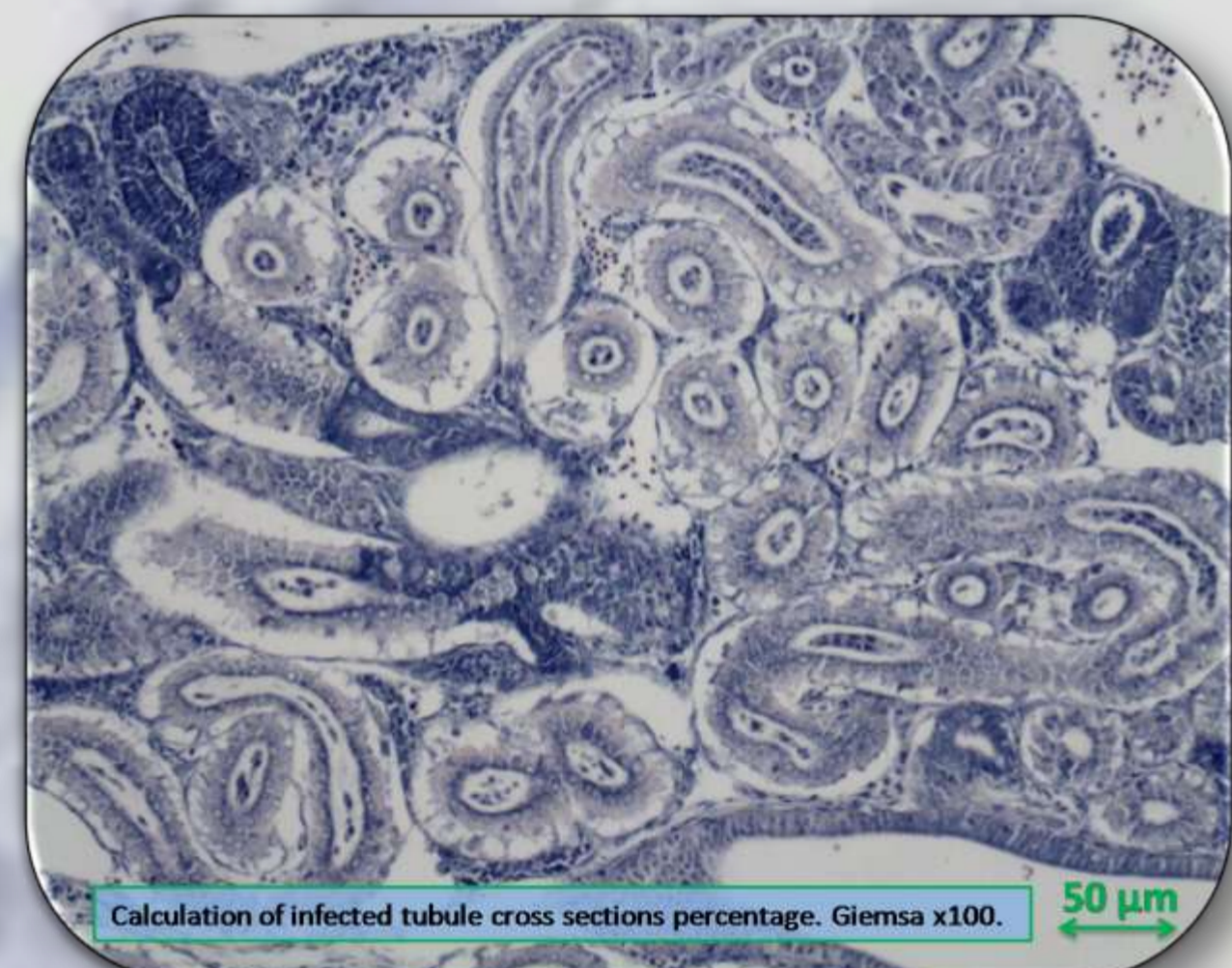


Cut made following the line of the spine to expose the maximum kidney face along the midline.

- Giemsa stain for discrimination of parasite from host cells



- All renal tubule cross sections counted as either infected or uninfected, using a hand held counter
- Two adjacent kidney sections per fish counted
- Recording parasites with obvious, clear nuclear staining; the number of parasite cells in each tubule is not taken into account



Advantages:

- Used routine histological techniques
- Parasite distribution in situ observed
- Delayed & repeat counting possible
- No contamination
- Suitable for any standardised section of kidney even for larger fish

Disadvantages:

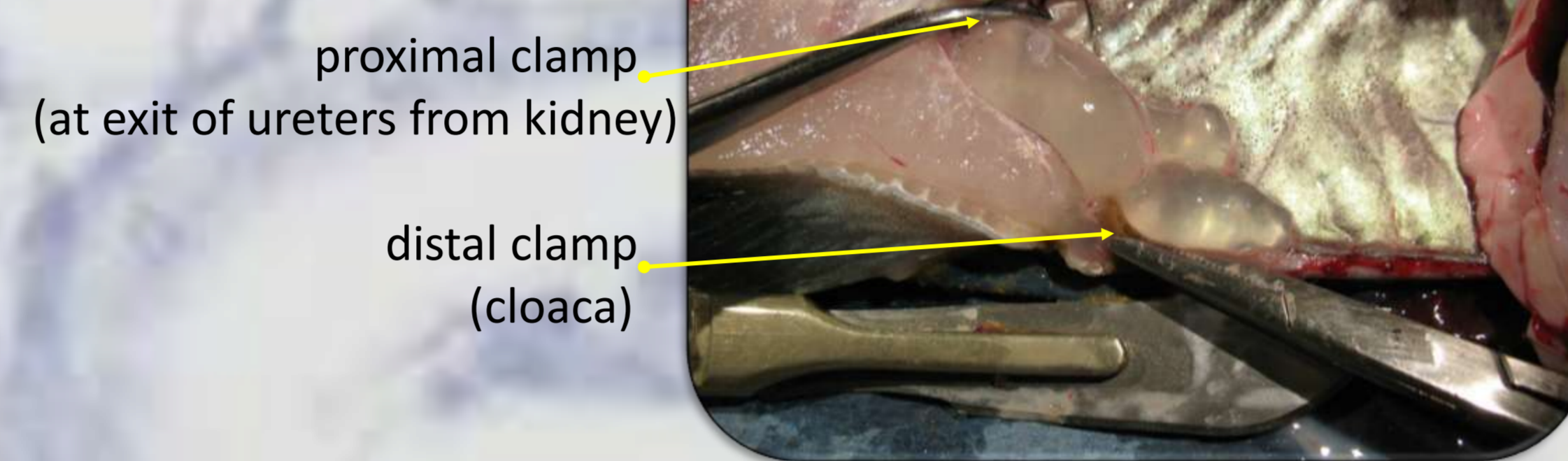
- Time consuming technique
- Suitable only for parasites living in the kidney parenchyma
- Not suitable for parasites in collecting ducts, ureters or urinary bladder e.g. *Z. hildae*

## Distal urinary tract flushing

The distal urinary tract, including the extra-renal portions of both ureters, urinary bladder and urethra, is dissected out and flushed with a known volume of PBS; different parasite life stages are easily counted at the microscope using a haemocytometer. The technique was optimised on 28-33 cm 1+ Atlantic cod.

Procedure:

- Dissection along the mid line and evisceration
- Distal urinary tract clamped in two points with autostatic forceps:



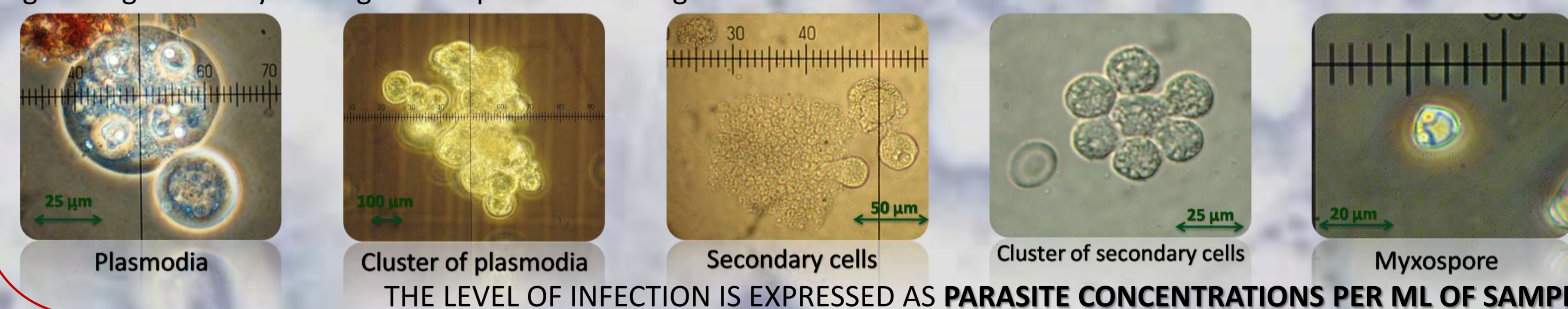
- Peritoneal membrane incision allowing the urinary tract ablation
- Urine drained (10-30 sec) into a suitable vessel, e.g. bijoux, after removal of the proximal clamp



- Flushing by fine needle syringe through the urethra, with 1ml PBS
- Bladder gently squeezed to extract the remaining fluid

## Sample analysis

- Final samples fixed in a known volume of 10% NBF
- Haemocytometer counting: 3 aliquots per each sample examined at x400 magnifications in phase contrast. It may be necessary to adjust the final parasite density appropriate for use in a haemocytometer. This can be achieved by further dilutions or centrifugation followed by re-suspensions. The haemocytometer allows for counting of different myxosporean life-stages, e.g. during this study 5 categories of parasite life stages in Atlantic cod were counted:



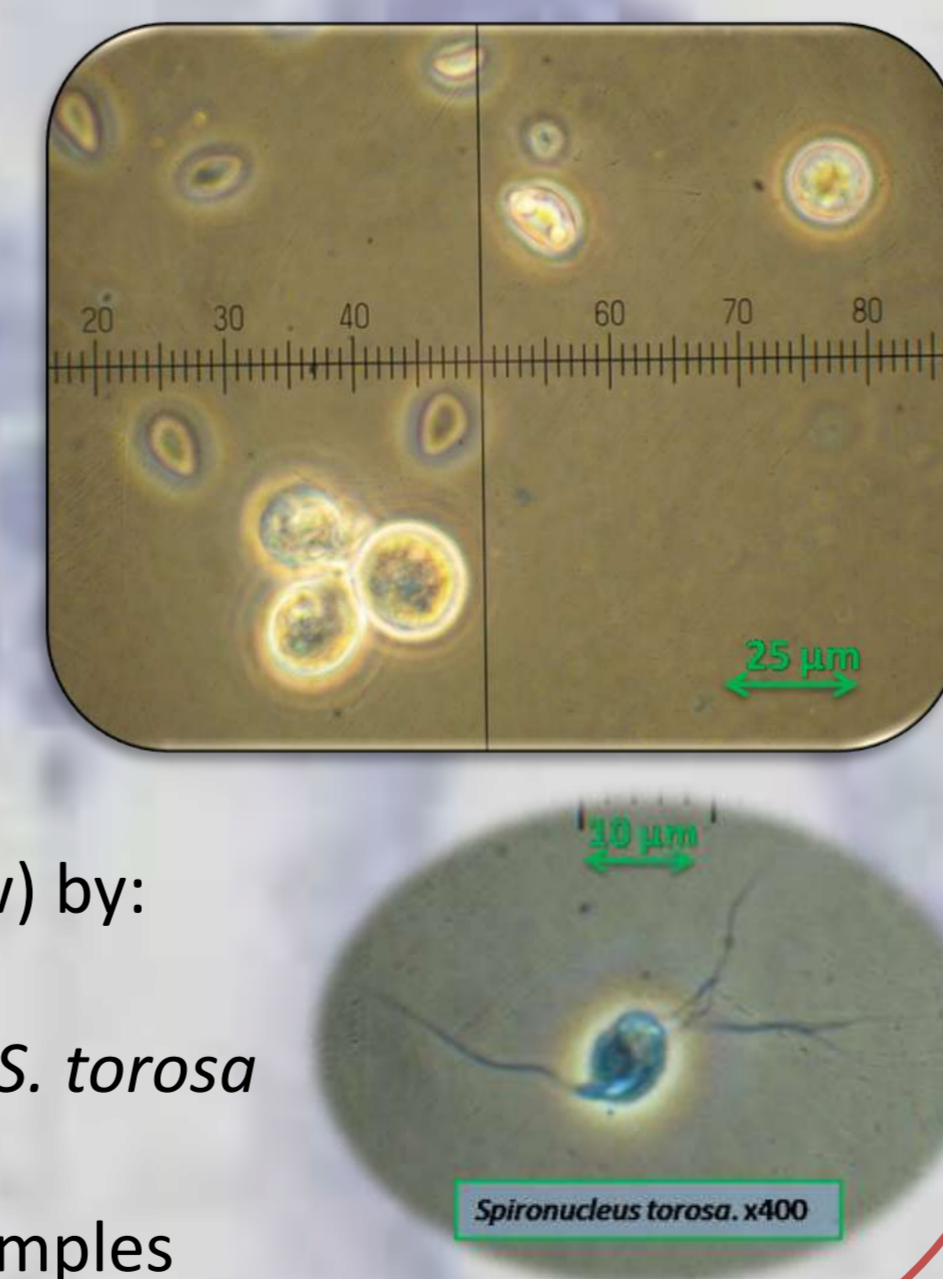
THE LEVEL OF INFECTION IS EXPRESSED AS PARASITE CONCENTRATIONS PER ML OF SAMPLE

Advantages:

- Good yield
- Standard dilutions achieved
- Parasite integrity retained
- Clear microscope visualisation
- Safe storage without parasite loss or degeneration
- Relatively fast technique

Disadvantages:

- Sample contamination (usually low) by:
  - Blood
  - Intestinal parasites e.g. in cod by *S. torosa*
- Occasionally faeces and sperm
- Crystals may form in fixed urine samples
- Requires some skill; not easy to carry out

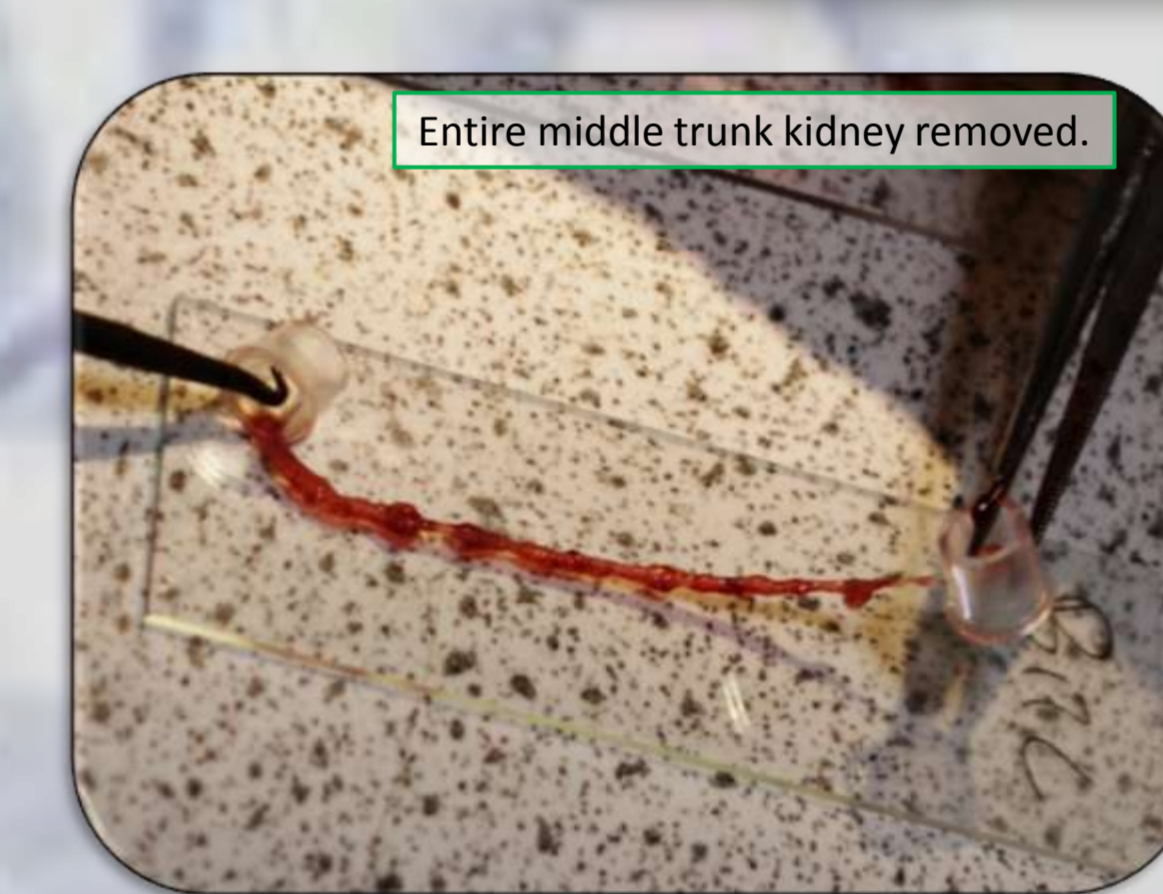
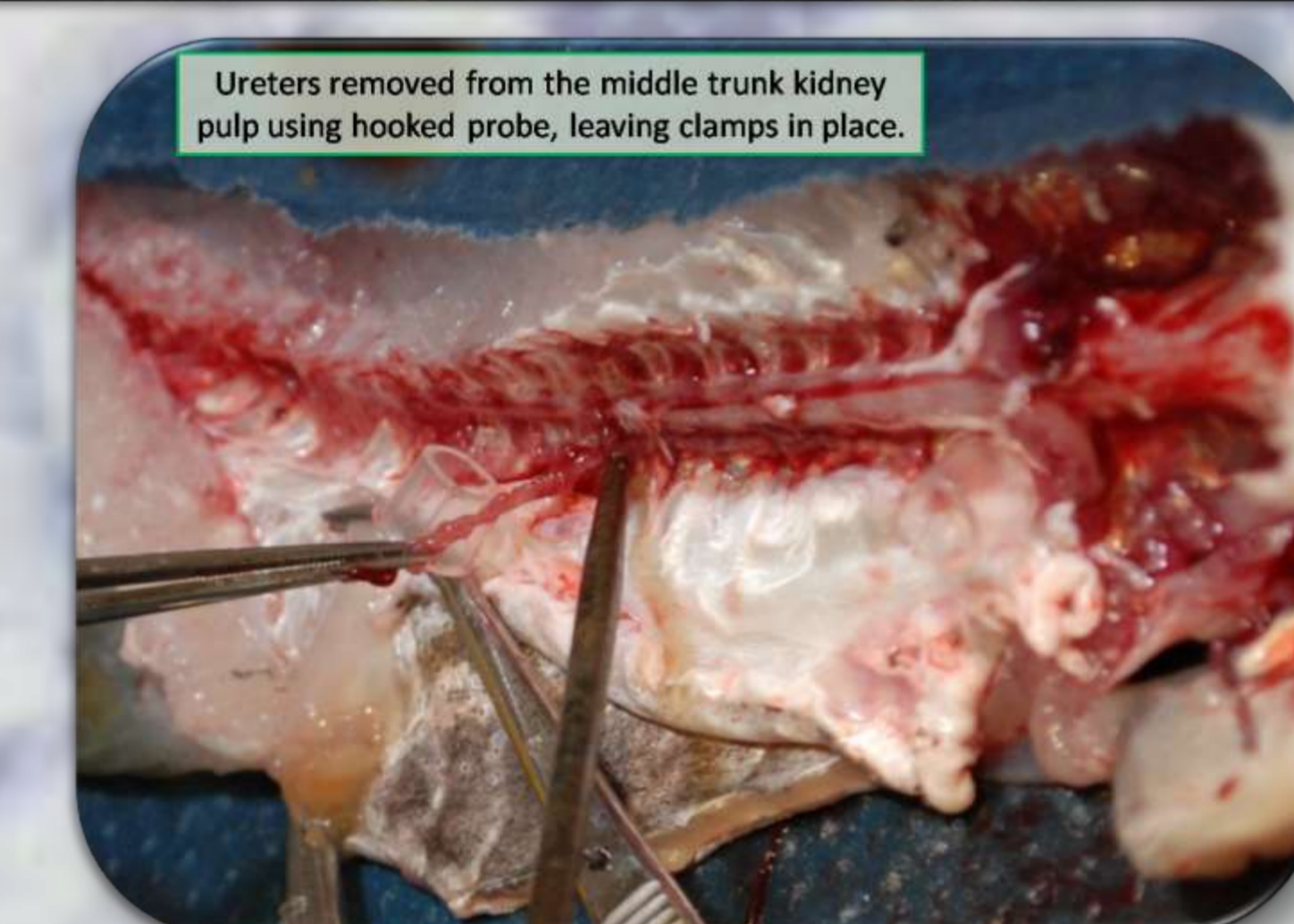


## Parasite dislodgement from ureter

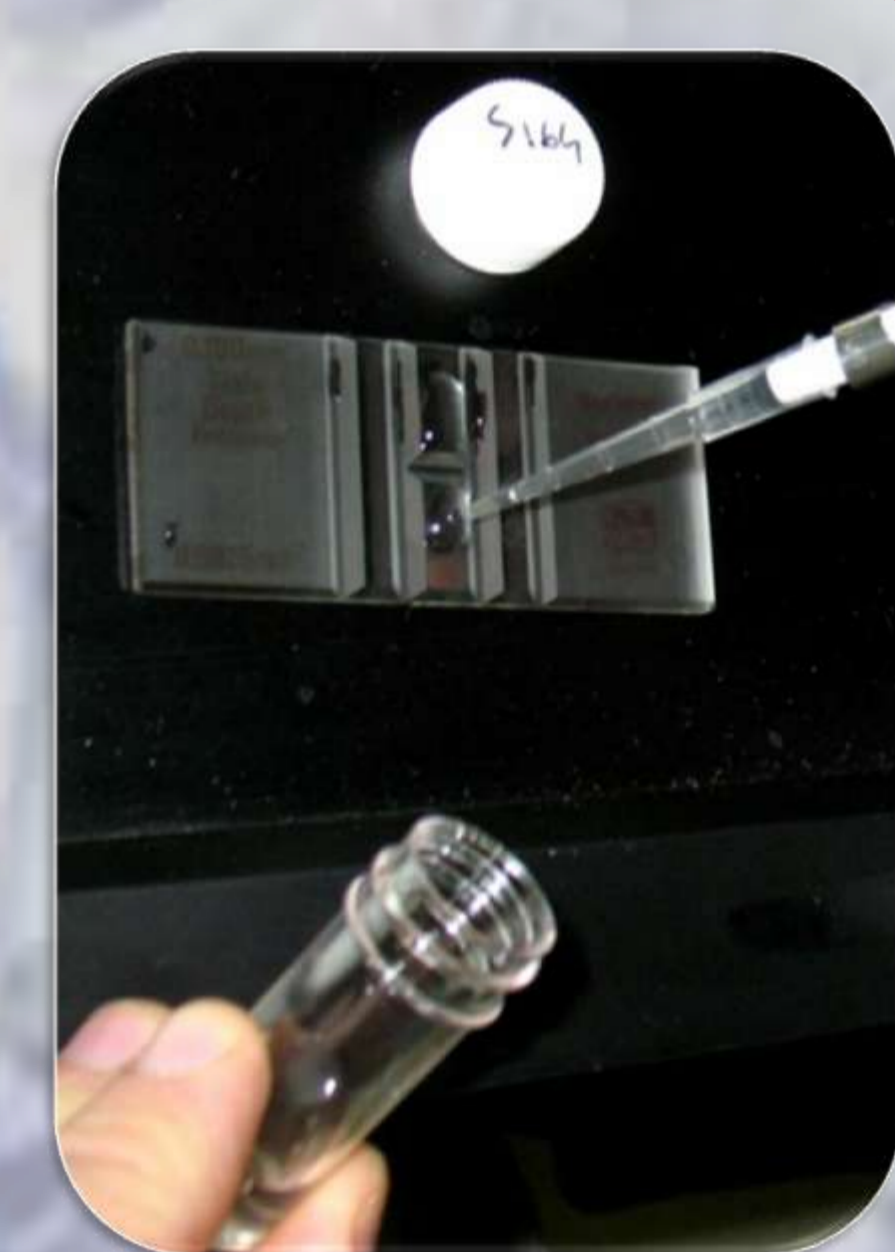
A standardised segment of ureter from the middle trunk kidney is dissected out and parasites extracted in a known volume of PBS; different parasite stages can be counted using a haemocytometer.

Procedure:

- Dissection, evisceration and ablation of swim bladder and head kidney
- Ureters clamped proximally and distally



- Ureter section sliced longitudinally and flushed into a bijoux using 1ml PBS
- Magnetic stirring: 30 min at minimum rate

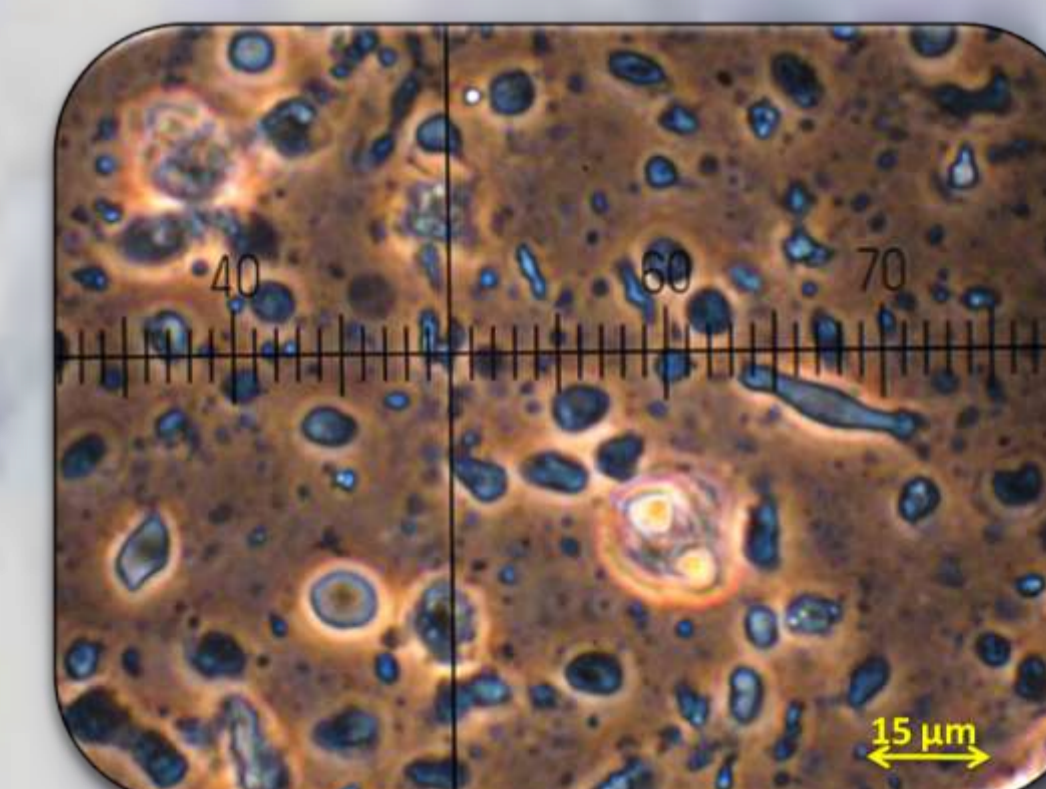


Advantages:

- Good yield
- Standard dilutions achieved
- Parasite integrity retained
- Safe storage without parasite loss or degeneration

Disadvantages:

- Time consuming
- Sometimes hard to recognise smaller parasite life stages amongst blood and other cellular contaminants
- Rapid post-mortem kidney parenchyma degeneration
- Meticulous dissection and evisceration required to achieve ureter precise sections



## Conclusions

Quantitative methods to estimate myxosporean burden were found to be useful for evaluating treatment benefits. The method using histology also showed the parasite distribution in the kidney parenchyma. The "Distal urinary tract flushing" was found to be the most suitable technique of the methods used to collect and count myxozoan parasites from the ureter of cod.

## References

- Karasev A.B., Mitenev V.K. & Shulman B.S. (1996) Ecological peculiarities of the parasite fauna of cod and pollock in the vicinity of the Kislaya Inlet tidal power plant, western Murman (the Barents Sea). *Sarsia* **80** (4), 307-312.
- Køie M. (2008) The parasite fauna of *Arctogadus glacialis* (Peters) (Gadidae) from western and eastern Greenland. *Polar Biol* **31**, 1017-1021.
- Køie M., Karlsbakk E. & Nylund A. (2007) A new Genus *Gadimyxa* with three new species (Myxozoa, Parvicapsulidae) parasitic in marine fish (Gadidae) and the two-host life cycle of *Gadimyxa atlantica* N. Sp. *J. Parasitol* **93** (6), 1459-1467.

## Further information

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