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Diseases of Asian seabass or Barramundi, *Lates calcarifer* Bloch

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Aims of present study

- “ Study the major diseases impacting the culture of *L. calcarifer* to improve diagnosis & control



Asian seabass or barramundi, *Lates calcarifer* Bloch as an aquaculture fish species

- “ growing importance in South East Asia & Australia
- “ a warm water fish species; water temperature needs to be above 20°C
- “ a euryhaline fish species; habitats from full strength seawater at 30 parts per thousand (ppt) to freshwater.
- “ Farmed in freshwater ponds or marine sea cages

Hatchery culture of *L. calcarifer*

- “ Eggs hatch in <1 day
- “ fed live feed as rotifers & artemia from 2 days old.
- “ weaned onto an artificial feed at ~3 weeks old.
- “ Very specialized and labour intensive due to the need for live feed production.
- “ larvae rearing stage and the broodstock gonadal maturation phase need to be carried out in saline water.

Grow-out of *L. calcarifer*

- “ Typically grows to 350g in six months & 2 kg in 2 years
- “ Grow-out cages size typically squares of between 2-4m wide & 2m deep
- “ Large circular cages 10-15m diameter & 10m deep

Background on research

- “ 12 years experience as fish pathologist & aquatic animal health veterinarian in Singapore
- “ Familiar with the range of diseases seen in cultured fish species including *L. calcarifer*
- “ Some diseases not well described

Materials used in research

- “ L. calcarifer fish samples from
 - . Singapore where I used to work
 - . Fisheries from WA . not many barra farms here!
 - . An established Indonesian farm
 - . Sampled over a 3 month period in 2008 from 16 nurseries in Vietnam



Original thoughts on scale loss

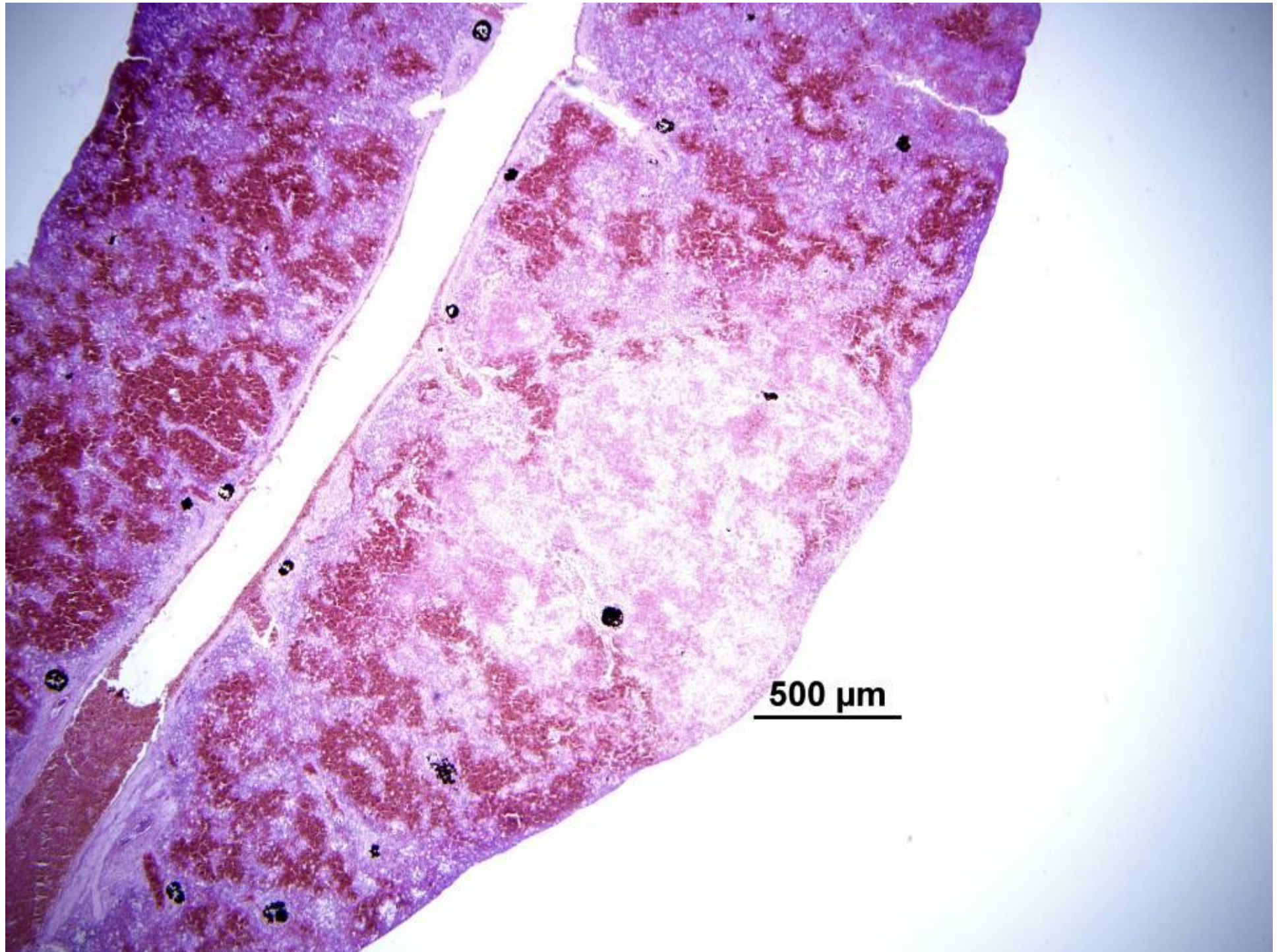
- “ Bacterial disease; Vibrio species & filamentous bacteria are ubiquitous
- “ Management factor related as often disease outbreaks follow net change
- “ 2006: some cases had pathology suggesting a viral aetiology
- “ Scale drop described in Langkawi/Penang

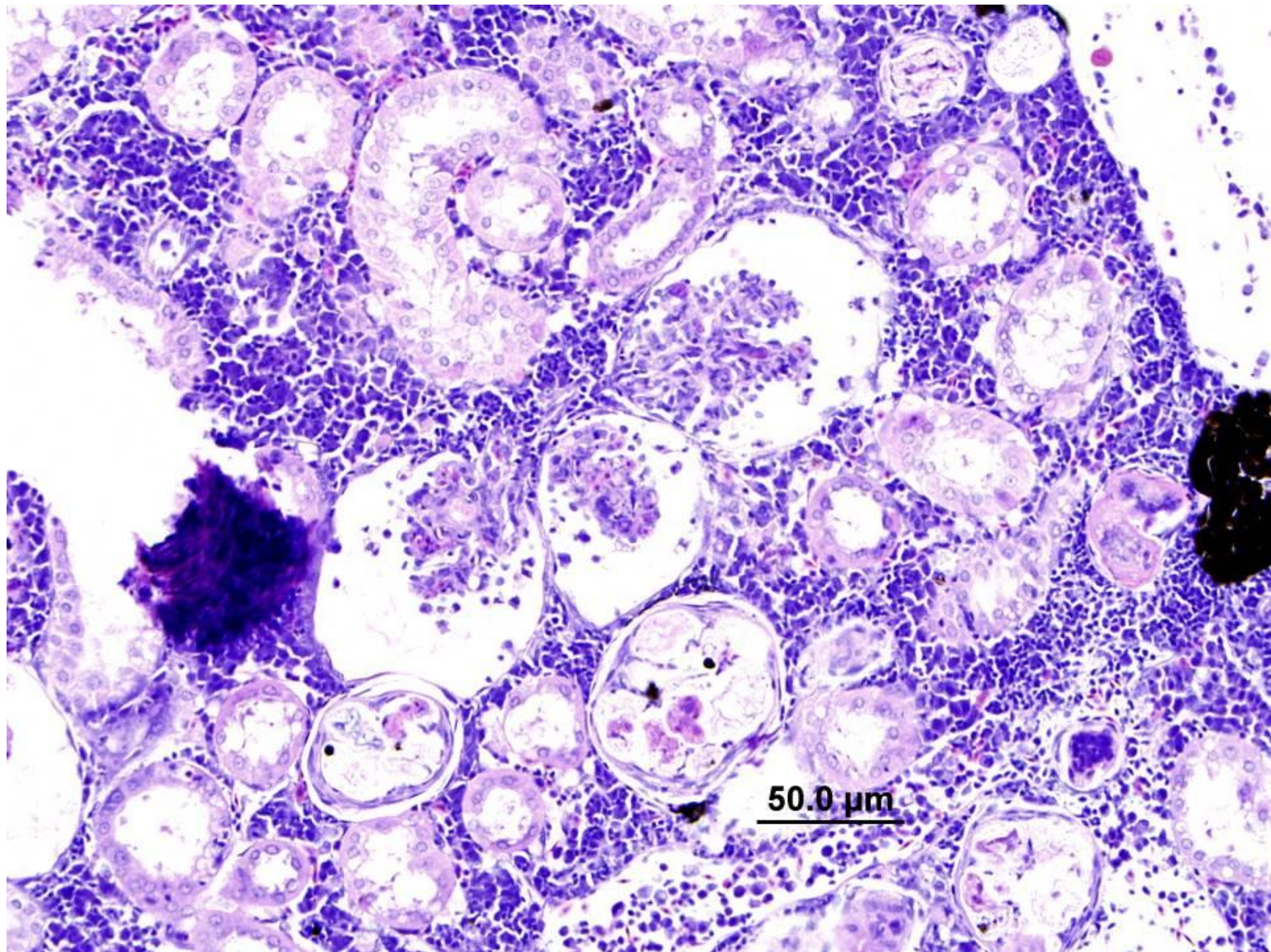
Scale drop in *L. calcarifer* (2006 case)

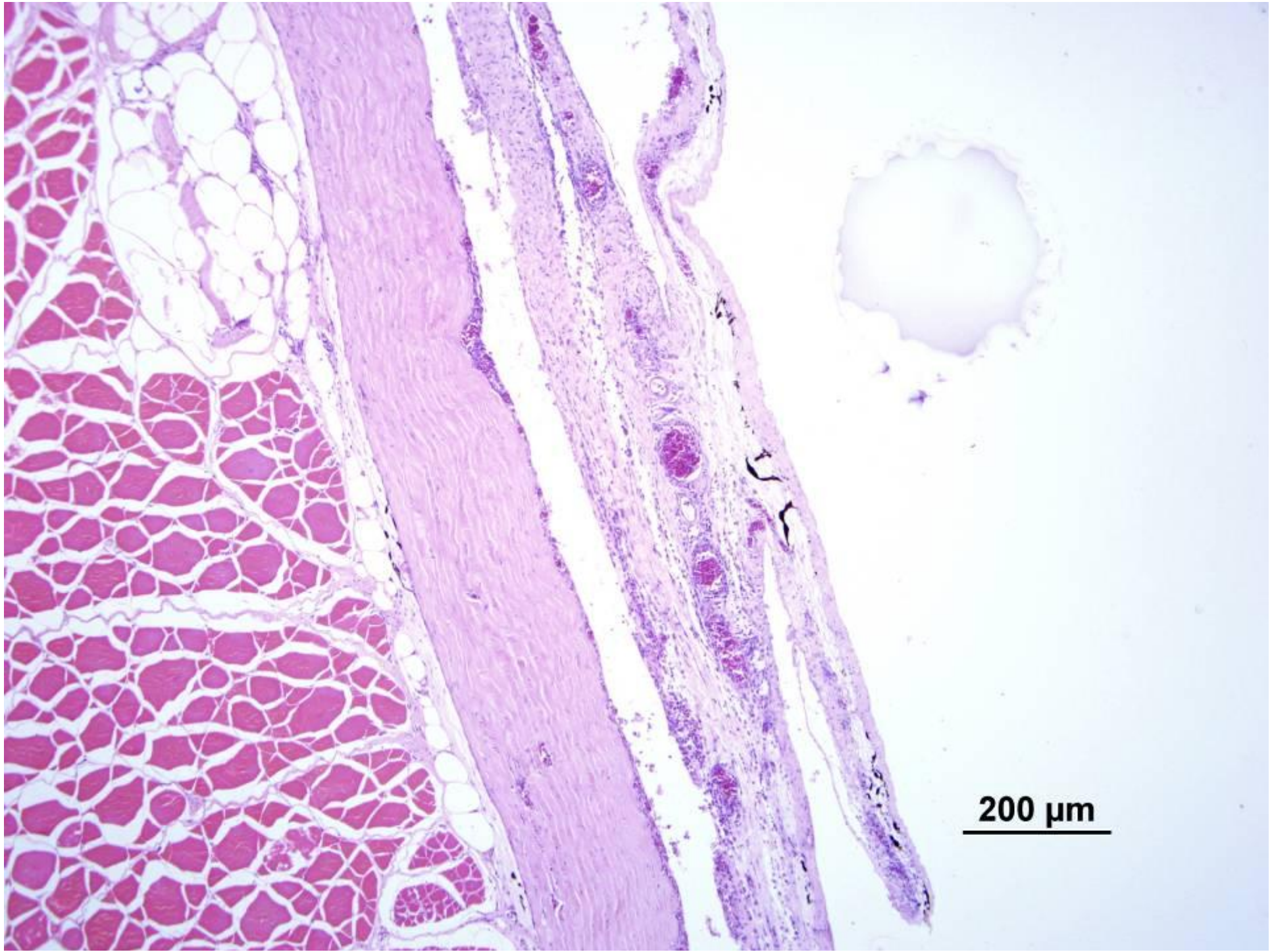


Histology of Scale drop:

- “ focal areas of cell deaths/infarcts in major organs associated with blood vessel inflammation & damage
- “ Low numbers of inclusion bodies
- “ Hypothesis: Blood vessel damage could cause scale loss
- “ Pathology suggests viral aetiology
- “ early TEM attempts could not demonstrate virus
- “ 2009 samples showed few viruses
- “ Virus size was different from other *L. calcarifer* iridovirus

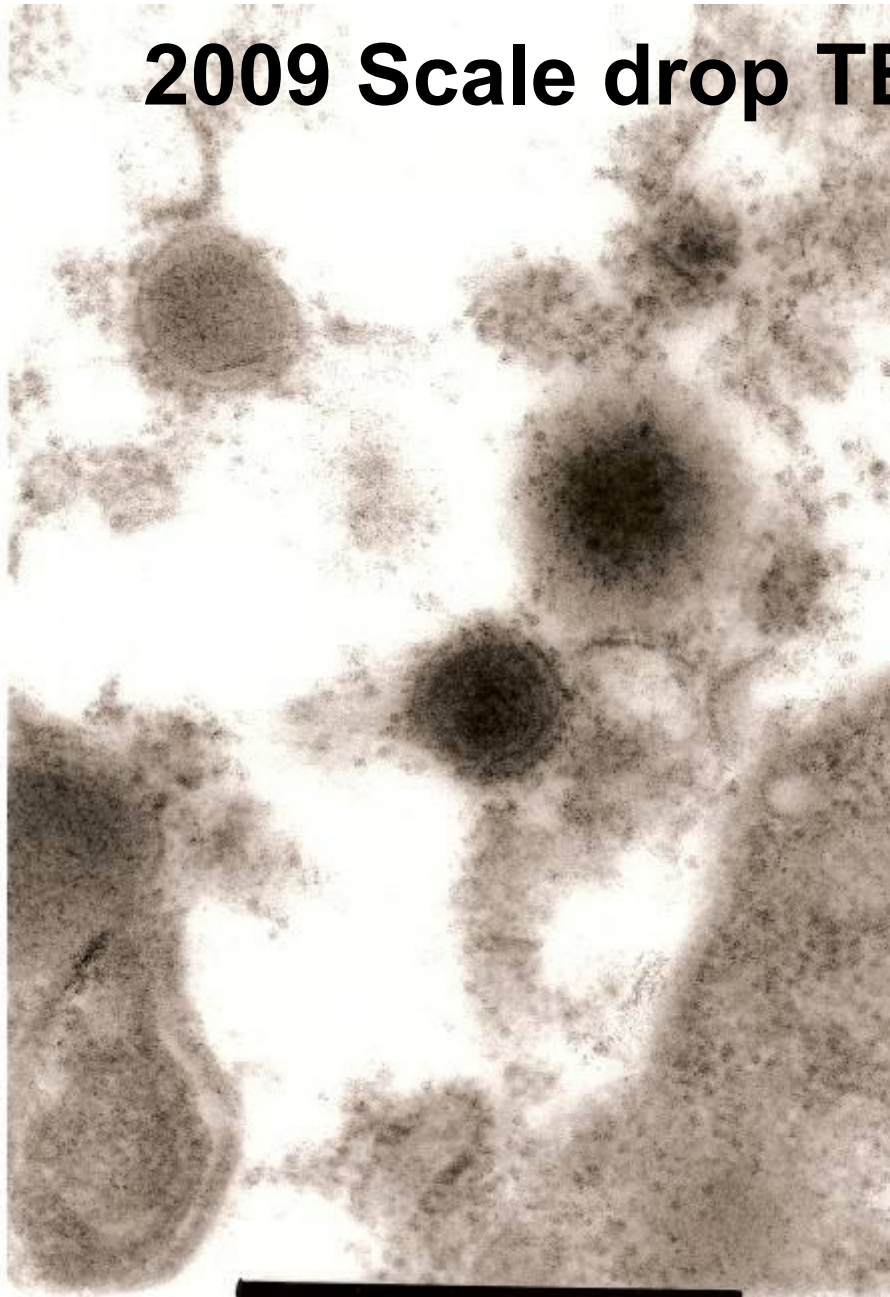




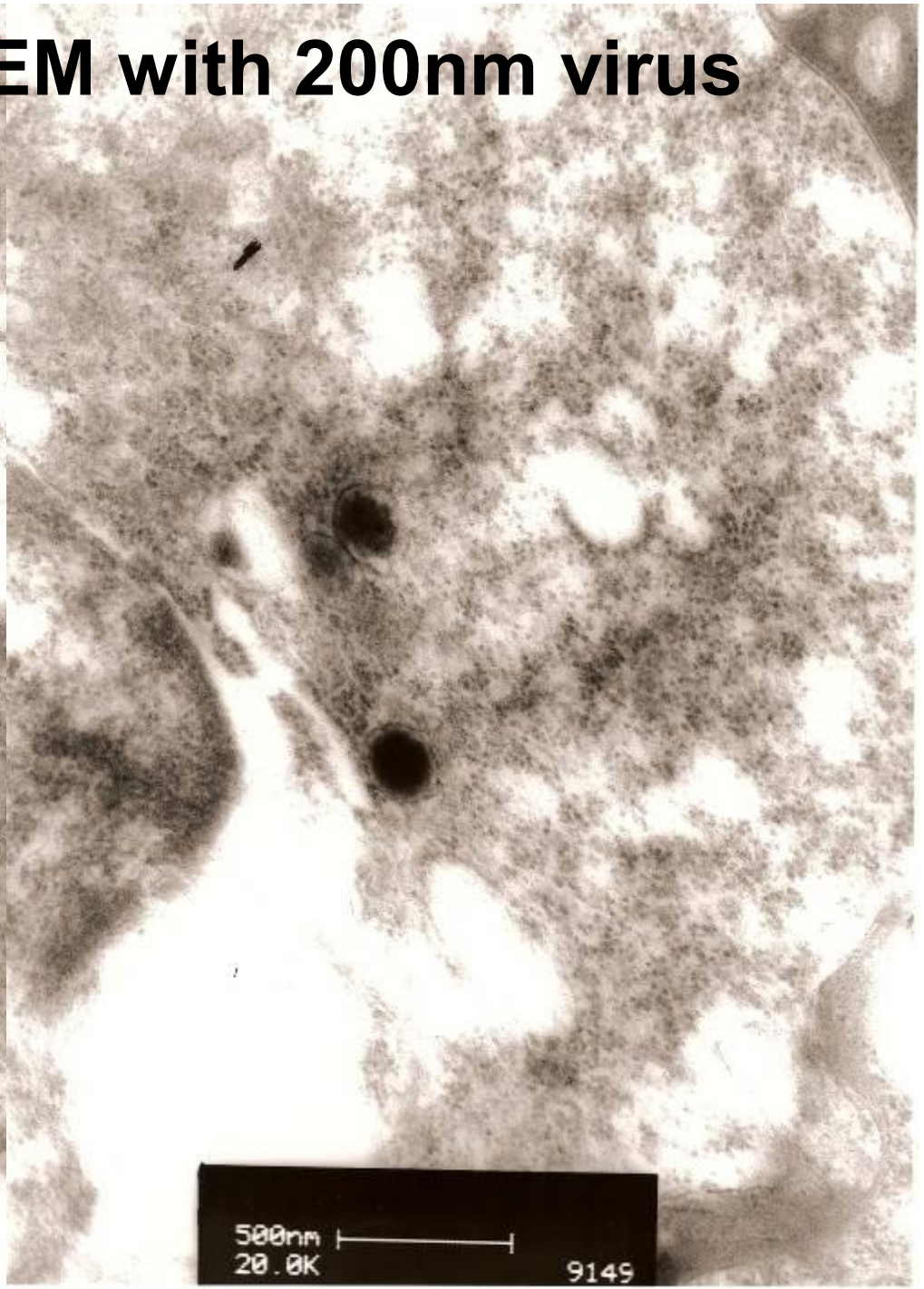


200 μm

2009 Scale drop TEM with 200nm virus



200nm
37.0K
9154

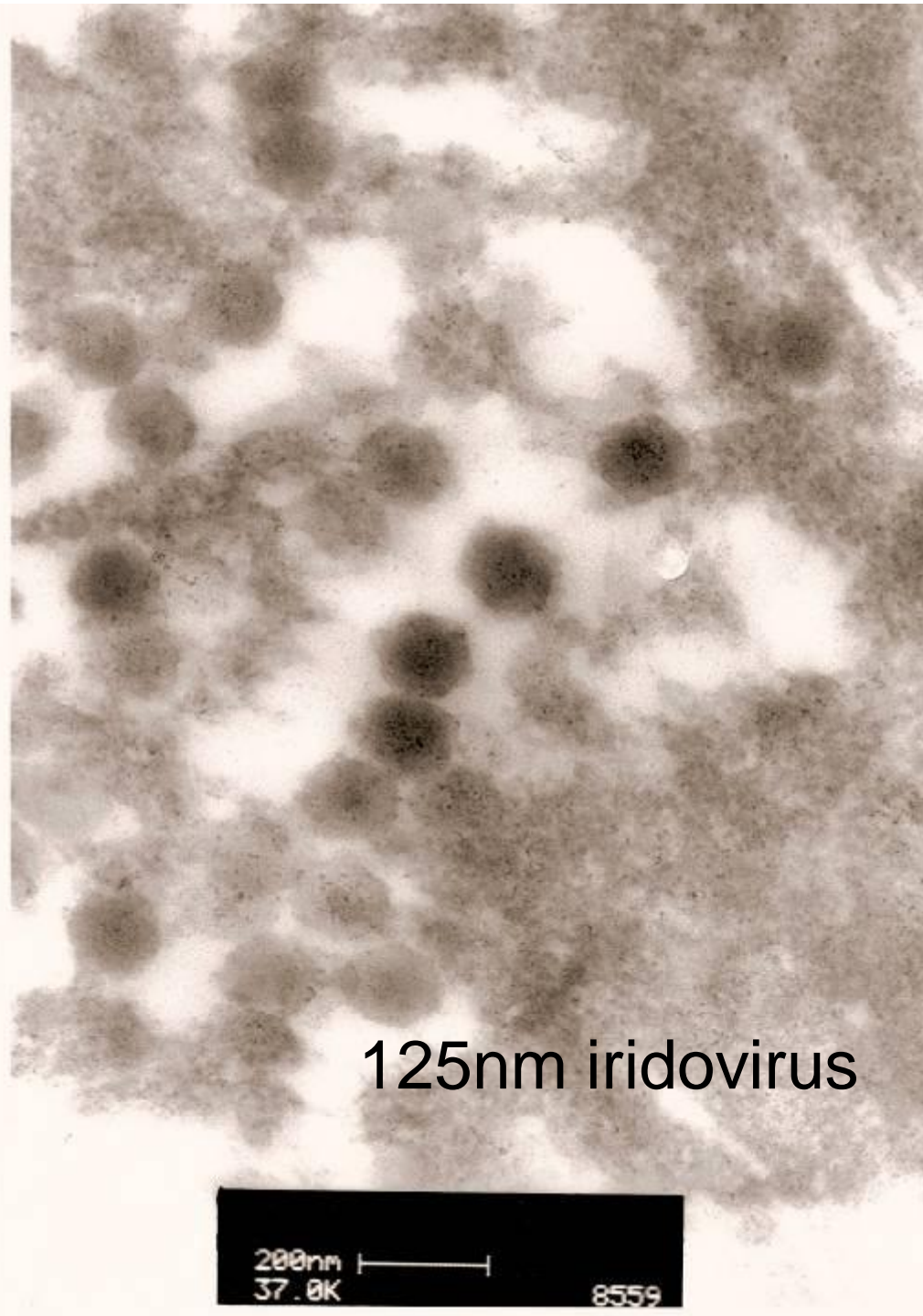


500nm
20.0K
9149

Wanted to compare my scale drop virus to other
L calcarifer iridovirus cases I have already
encountered.

Systemic iridovirus in *L. calcarifer* from Singapore

- “ Not often observed in Singapore
- “ No Singapore materials for TEM but wax blocks available
- “ Dug out wax block tissues for TEM



- “ Glutaraldehyde fixed tissues are optimal for TEM
- “ Formalin fixed wax tissues do not preserve ultrastructure well
- “ Difficult to get good resolution at higher magnifications

Systemic iridovirus in *L. calcarifer* from Indonesia

“Managed to obtain samples from an established farm in Indonesia

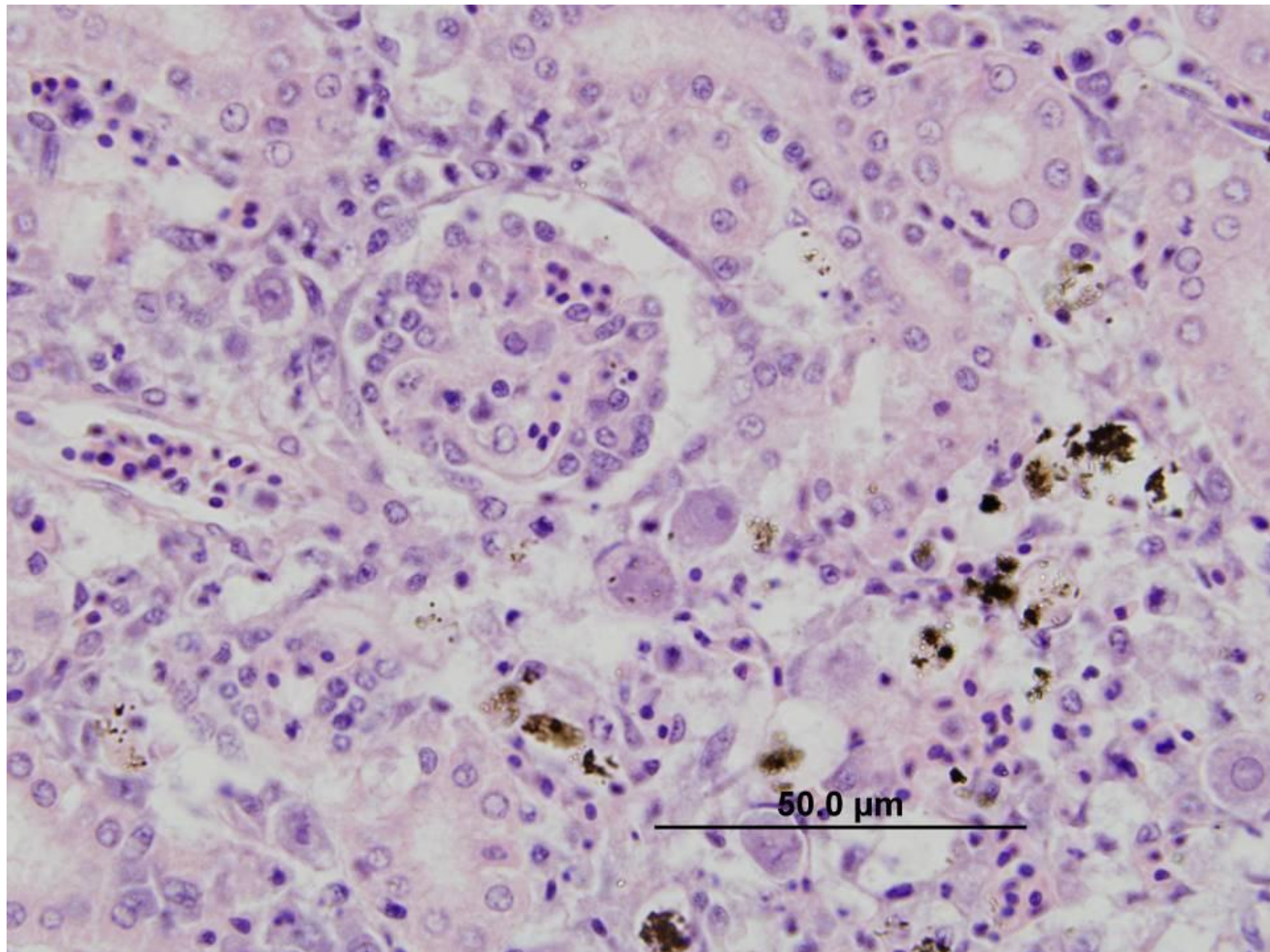
“5 tonnes production of mainly 3kg fish every week

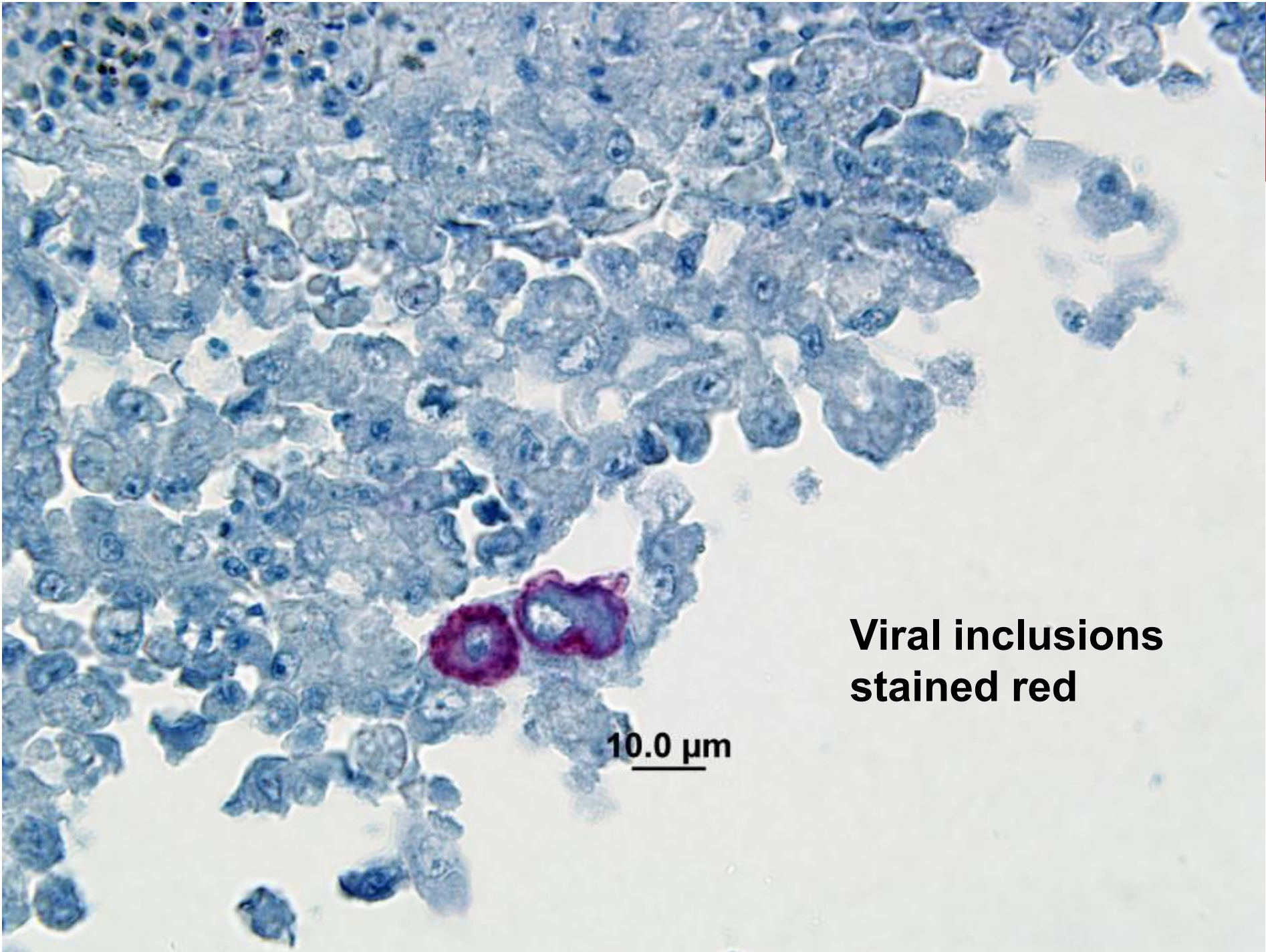
“Earlier, farm had depended on PCR & clinical signs to diagnose disease

“Histology examination showed iridovirus as a background disease on farm, more widespread than initially thought

Systemic iridovirus in *L. calcarifer* from Indonesia

- “ In younger fish <3m/o, presence of large numbers of inclusion bodies makes it relatively easier to diagnose
- “ Positive staining with immunohistochemistry using a monoclonal antibody developed to Red Sea bream iridovirus
- “ Sequencing of PCR product suggests relation to other systemic iridviruses





**Viral inclusions
stained red**

10.0 μm

Learning points with immunohistochemistry on fish tissues

- “ Many reagents have limited shelf life
- “ Especially labeled antibodies
- “ Fish tissues different so that when adapting techniques need to optimise
- “ Antigen retrieval using microwave had to be reduced to avoid making fish tissues drop off slides

Systemic iridovirus TEM

“Pathology, immunohistochemistry & PCR suggests a systemic iridovirus infection in fish from Indonesia

“TEM could not definitively demonstrate virus

“Time of sampling is critical in looking for pathogen. Too early, virus not formed. Too late, no more pathogen.

“Re-examine samples that have just arrived this week

Further work

- “ Use Red sea bream iridovirus monoclonal antibody immunohistochemistry on scale drop cases
- “ Use DNA probe developed from PCR product of *L calcarifer* systemic iridovirus (Indonesia) on scale drop cases
- “ Virion size of systemic iridovirus different from scale-drop virus
- “ Try get scale drop samples for PCR and sequencing to compare with 125nm *L. calcarifer* iridovirus (Singapore)



“One of 16 Nurseries in Vietnam where fish samples were taken

“Imported fingerlings are held in fibreglass or cement tanks until sold to grow-out farms

History & Clinical signs of *L. calcarifer* sampled from Vietnam

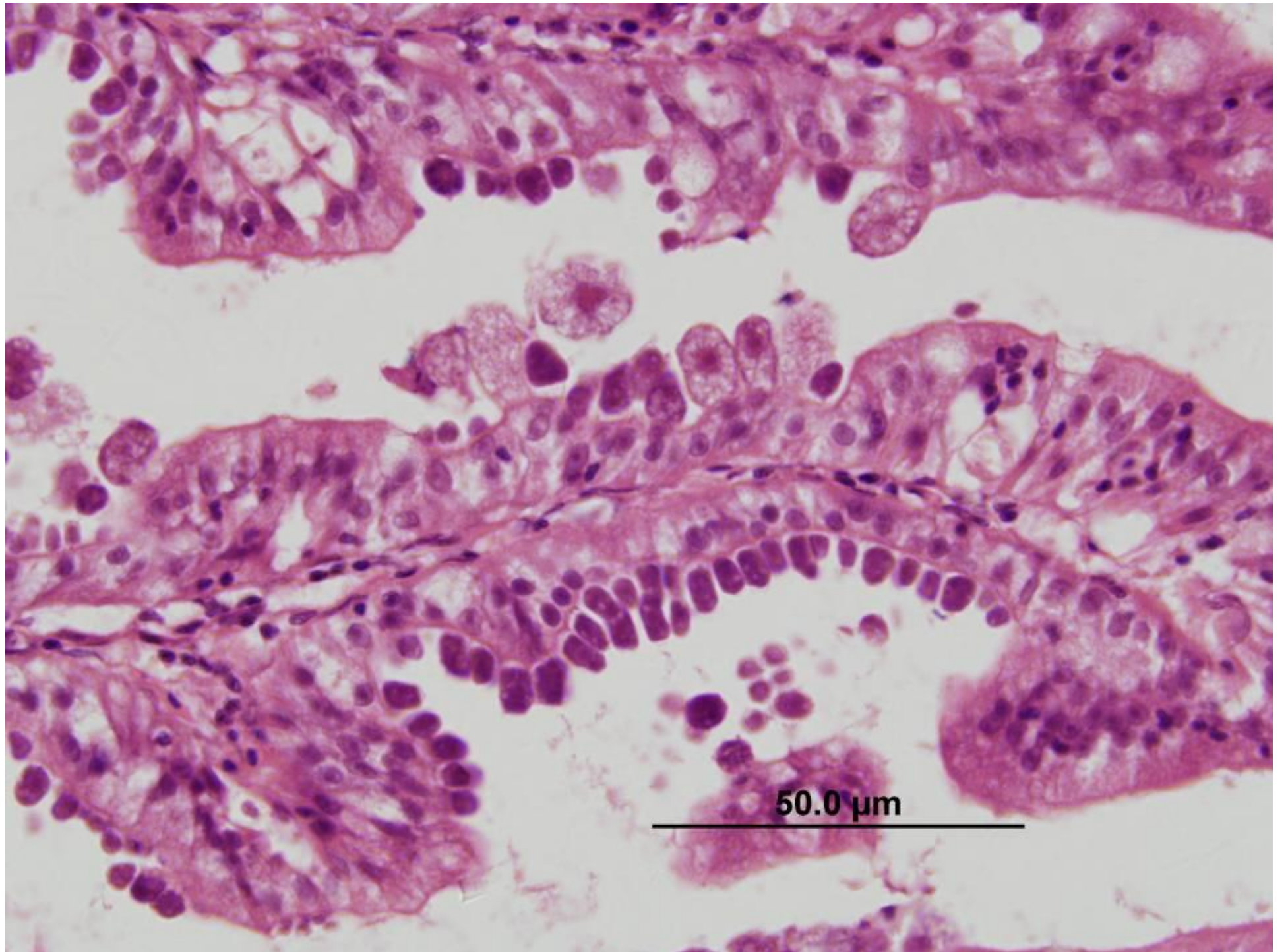
- “ Low grade mortality
- “ Lethargic
- “ Tail rot, skin ulcers, scale loss
- “ Loss of appetite

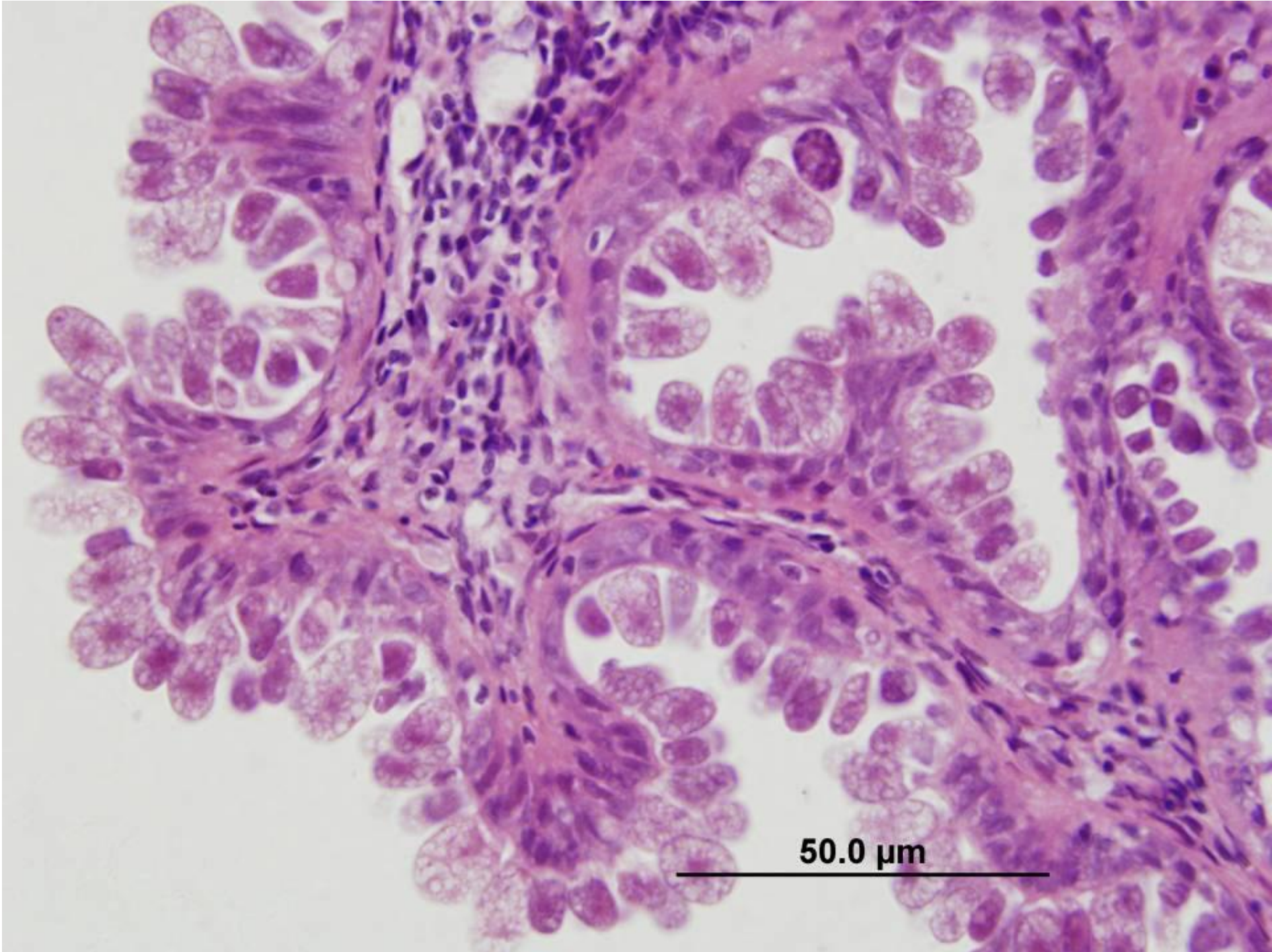
Histologic & TEM observations

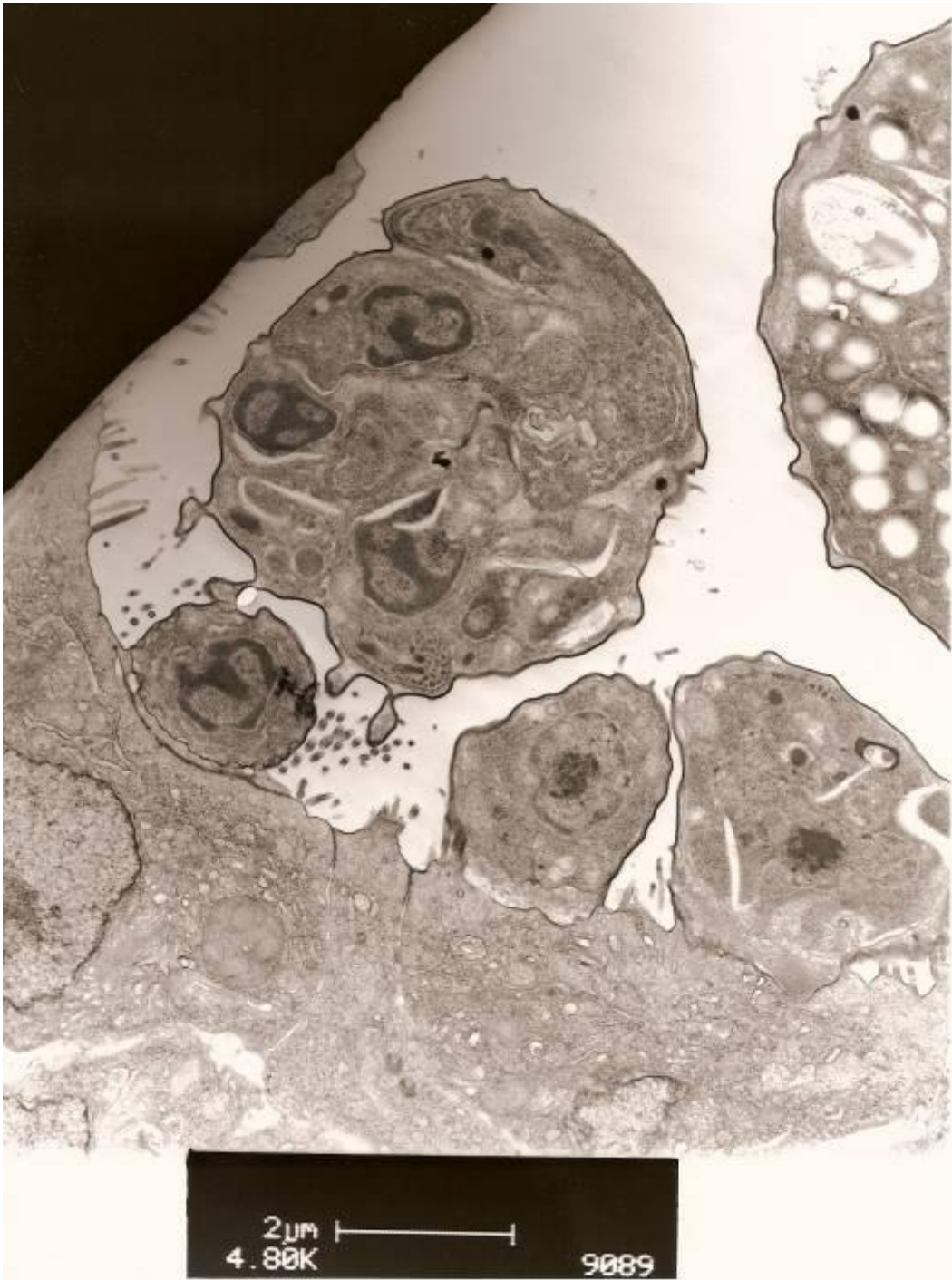
“Intestinal protozoa infection often heavy & associated with degeneration/necrosis of gut

“TEM images resemble cryptosporidium

“PCR shows DNA sequence close to other cryptosporidium







Learning points of PCR on fixed tissues

- “ Fish tissues need to be decalcified with an acid prior to sectioning to make histology slides
- “ Poor success with PCR using acid treated decalcified tissues
- “ Cryptosporidia case was detected by PCR in spare formalin fixed tissues over a year old but not in wax block tissues

Further research on Cryptosporidia

- “ Developing of in-situ hybridization DNA probes using PCR products
- “ Immunohistochemistry using commercially available antibodies

Learning points on research in fish

- “ Information available from research in better known animal species often useful
- “ Materials generated from PCR can be useful to develop DNA probes to detect same or similar pathogens
- “ Published materials show successful outcomes only and do not show the pain taken to achieve it!

Acknowledgements

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