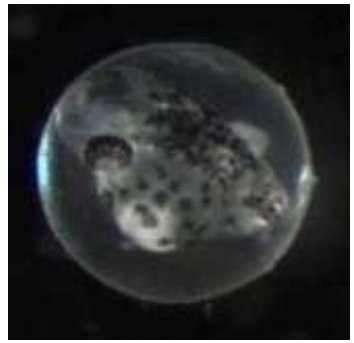
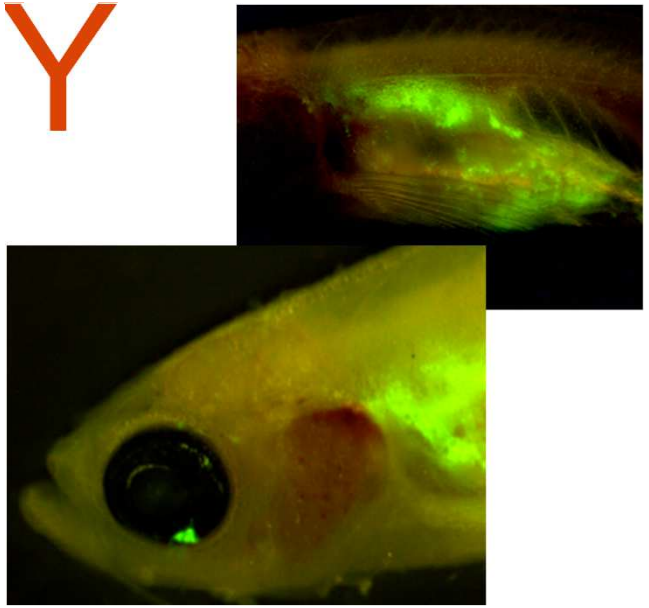
INFECTIOUS DISEASES OF LABORATORY ZEBRAFISH





Justin L. Sanders

Department of Biomedical Sciences

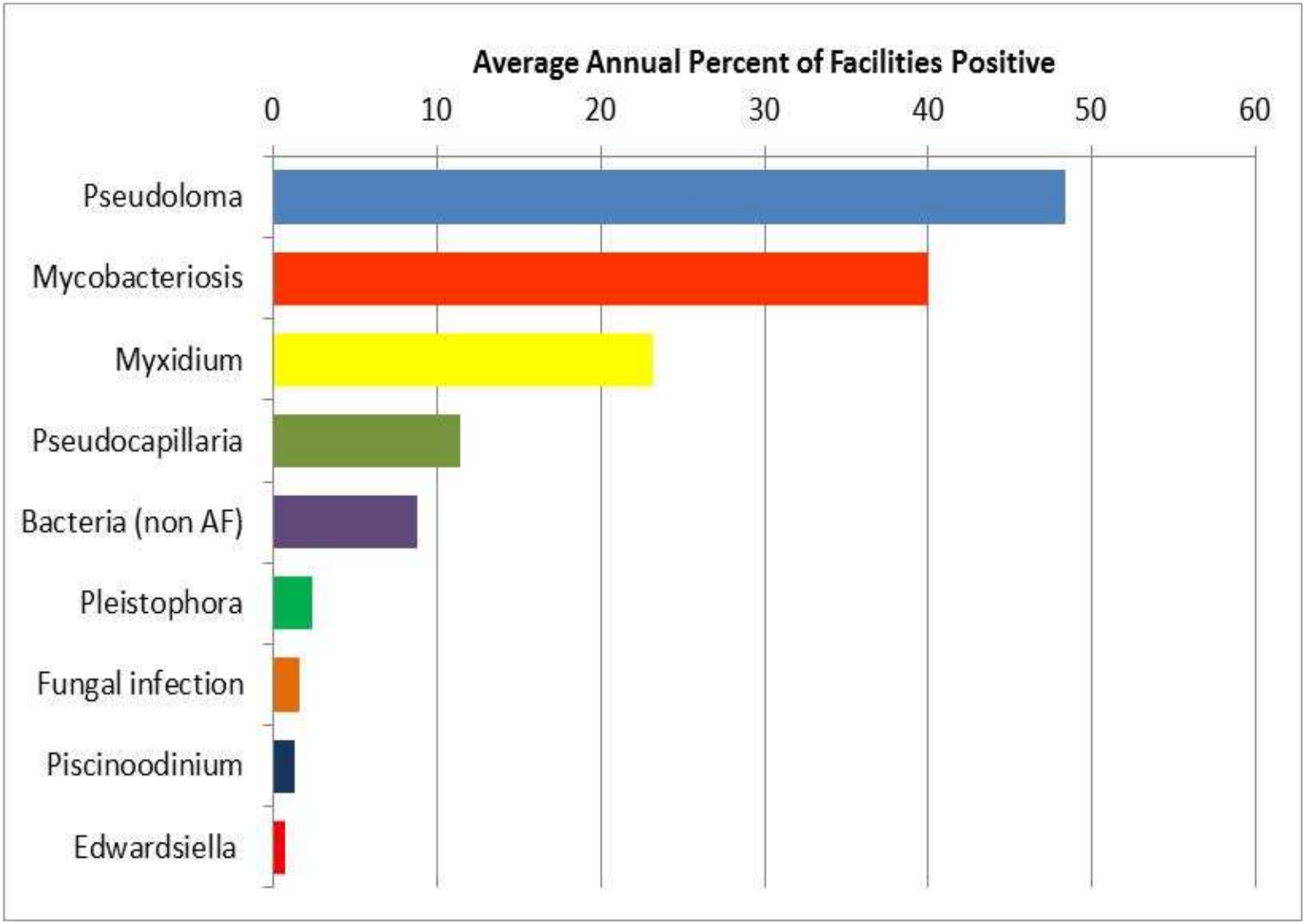
Oregon State University

Infectious Diseases – ZIRC Diagnostic Service Summaries 2006-2014

About 7,000 fish and 100 facilities

- *Pseudoloma*: 48% labs, 16% fish
- Mycobacteriosis: 40% labs, 6% fish
- *Edwardsiella ictaluri:* 3 labs in 2011.
- *Pseudocapillaria:* 11% labs, 1.3% fish
- Gut Hyperplasia/Neoplasia: 12% labs, 2.8% fish
- *Myxidium*: 19% labs, 2.4% fish
- *Piscinoodinium* (max 2 %), *Pleistophora* (max 7%)

Pathogens in Zebrafish Facilities 2006-2015: – ca 10,000 fish, 100 laboratories



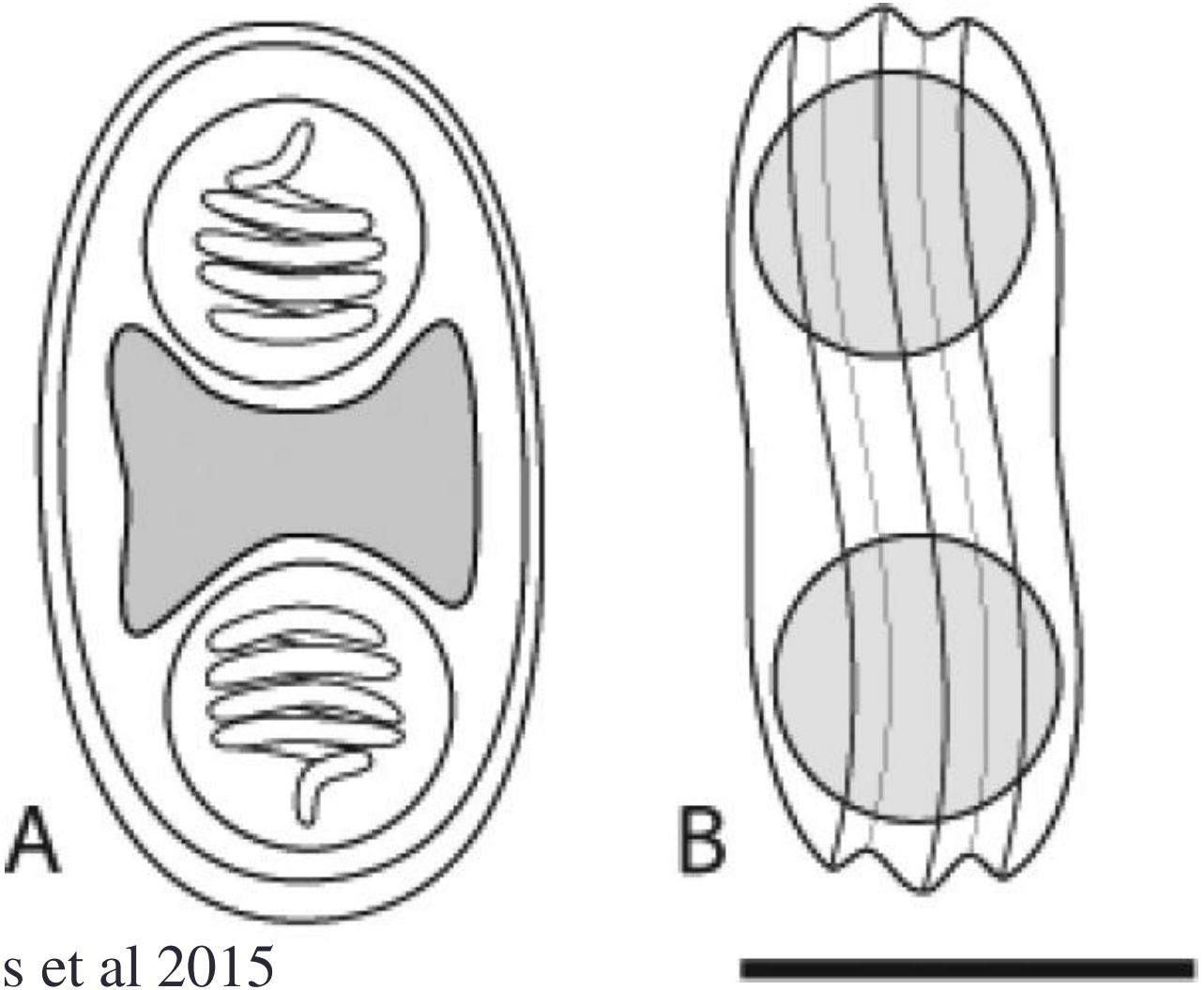
Parasitic Diseases: general pathology

- Space replacement/pressure atrophy
- Respiratory impediment – gill parasites
- Osmoregulatory – gill & skin infections
- Perforation of gut & 2nd bacterial infections
- Inflammatory changes

Common parasites of research zebrafish

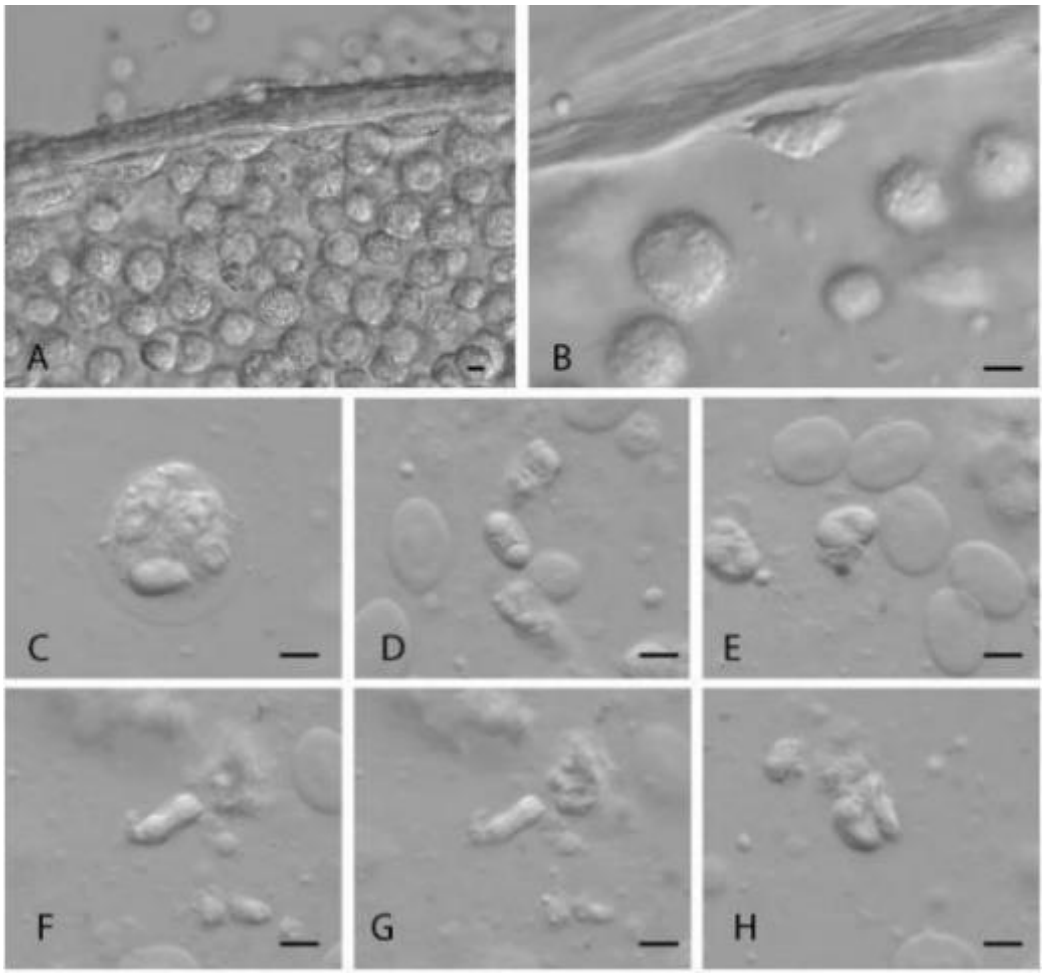
Parasite Group	Species	Reference
Dinoflagellate	*Piscinoodinium pillulare*	Westerfield 2000
Ciliate	*Ichthyophthirius multifiliis*	Matthews 2004
Nematode	*Pseudocapillaria tomentosa*	Kent et al. 2002
Myxozoan	*Myxidium streisingeri*	Whipps et al. 2015
Digenea	*Transversotrema patialense*	Womble et al. 2015
Microsporidia	*Pseudoloma neurophilia*	de Kinkelin 1980; Matthews et al. 2001
	Pleistophora hyphessobryconis	Sanders et al. 2010

Myxidium streisingeri



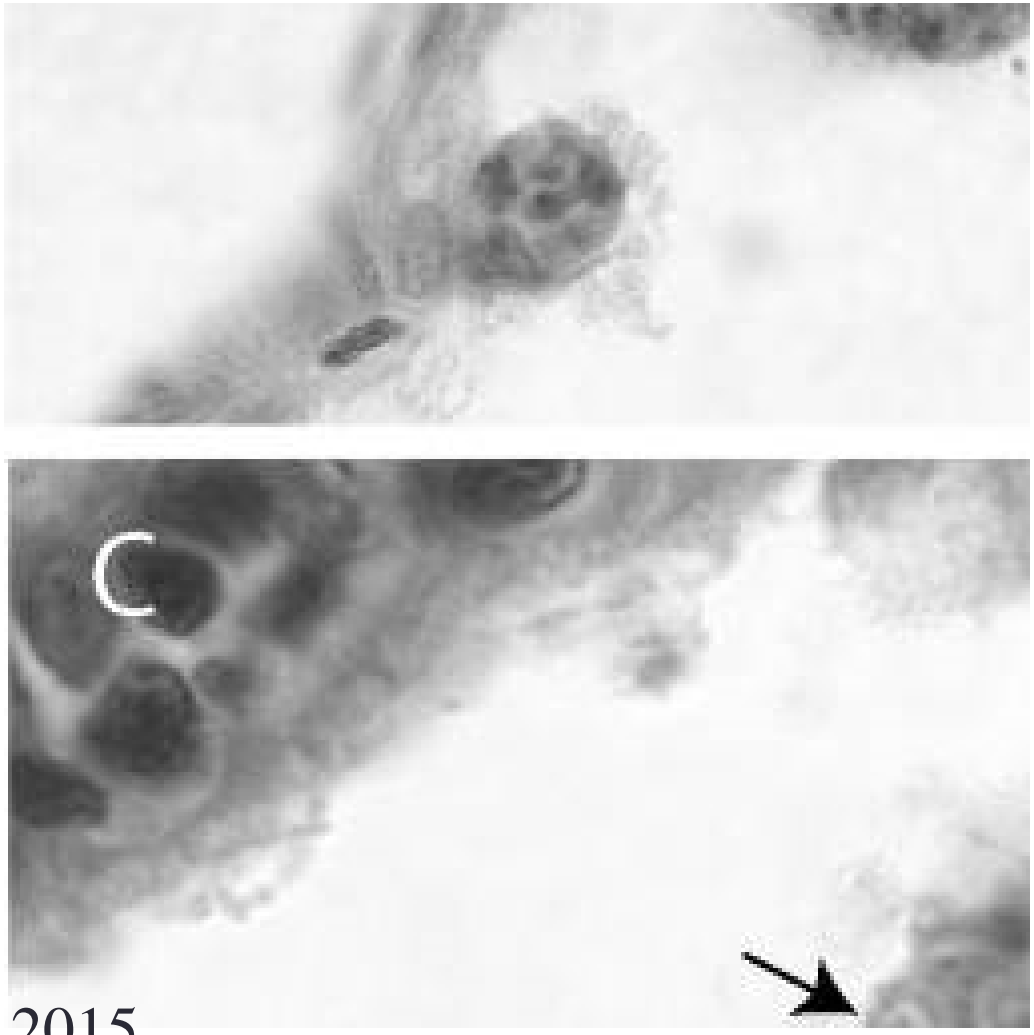
Whipps et al 2015

Myxidium streisingeri



Whipps et al 2015

Myxidium streisingeri

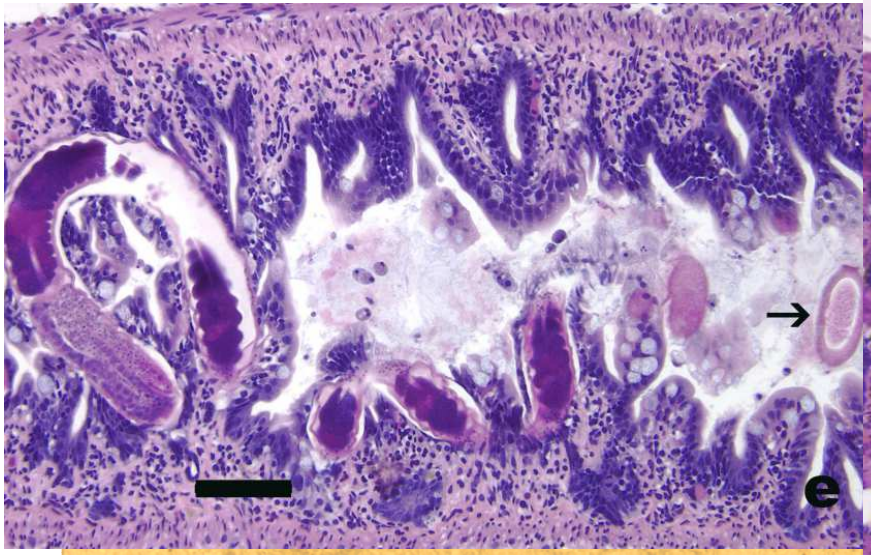


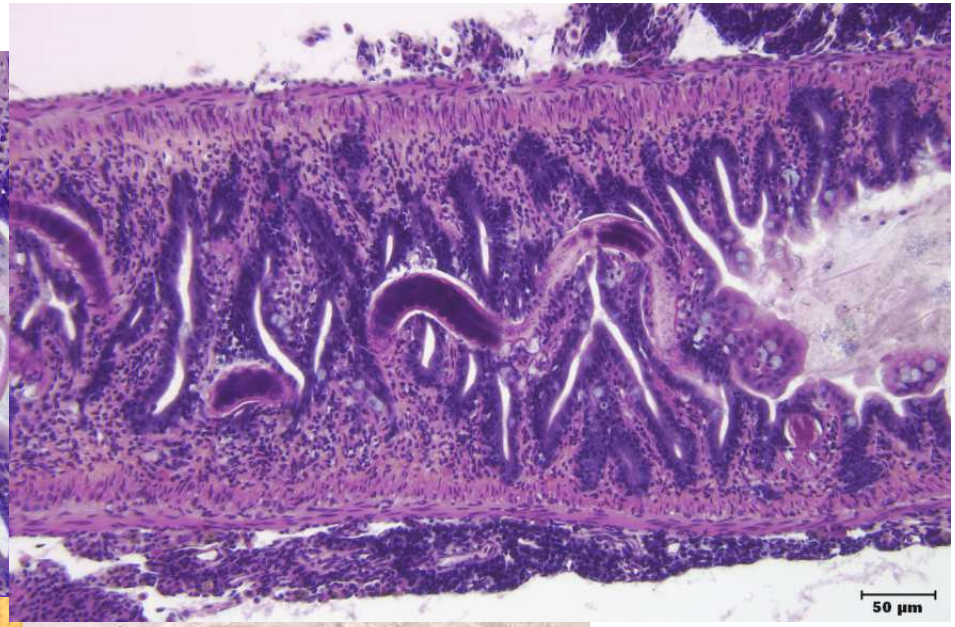
Whipps et al 2015

Myxidium streisingeri

Pathogenesis	Mode of transmission	Diagnosis	Control/Treament
Found in lumen of mesonephric and kidney ducts Associated with little to no observable pathology	Unknown: Direct? Requires oligochaete alternate host?	Wet mount: light microscopy; Histology PCR	Avoidance/quarantine

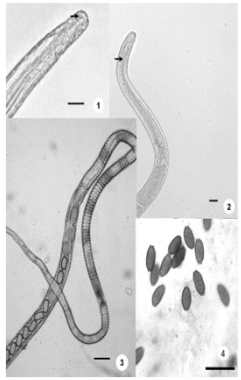
Pseudocapillaria tomentosa





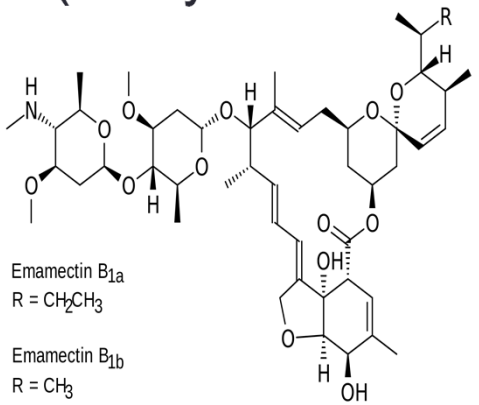
The zebrafish/*P. tomentosa* model: possible new treatments for nematodes

- Common antihelminthic (ivermectin) can have neurotoxic side effects
- Has been shown to have sublethal effects on embryonic zebrafish such as developmental malformations (Carlsson et al 2013)
- Treatment of adult zebrafish with ivermectin leads to behavioral changes, decreased fecundity, and higher mortality (Collymore et al 2014)
- Chereen Collymore, Virginia Watral, Julie R White, Michael E Colvin, Skye Rasmussen, Ravi J Tolwani, Michael L Kent. Tolerance and efficacy of emamectin benzoate and ivermectin for the treatment of *Pseudocapillaria tomentosa* in laboratory zebrafish (*Danio rerio*). Zebrafish. 11:490-497.

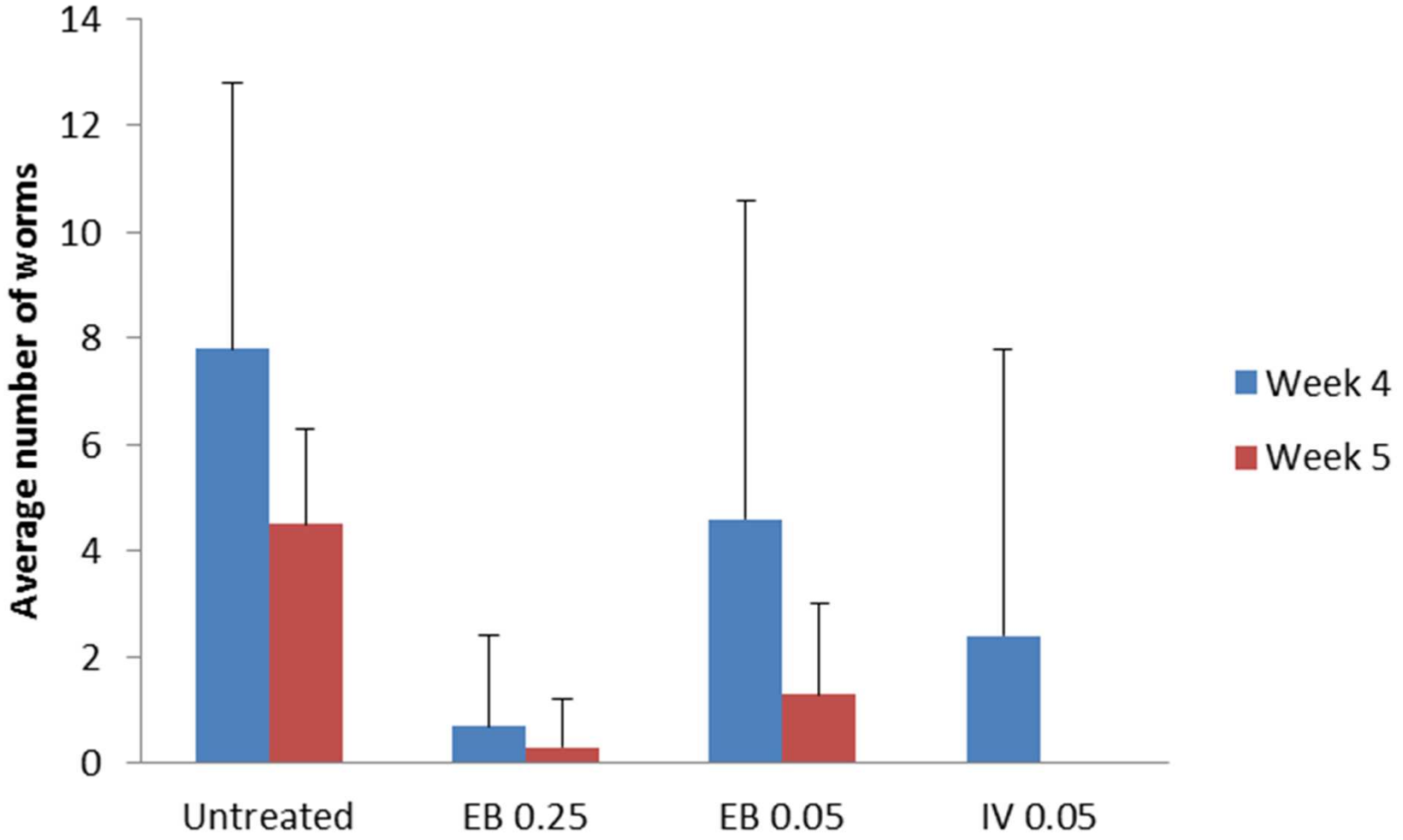


Emamectin benzoate

- Originally developed to control plant nematodes
- Extensively used in aquaculture to treat ectoparasites in trout and salmon (SLICE 0.2%; Merck Animal Health, Summit, NJ)
- Zebrafish have been shown to tolerate high doses of emamectin compared to ivermectin and has been demonstrated to reduce nematode burden (Collymore et al 2014)

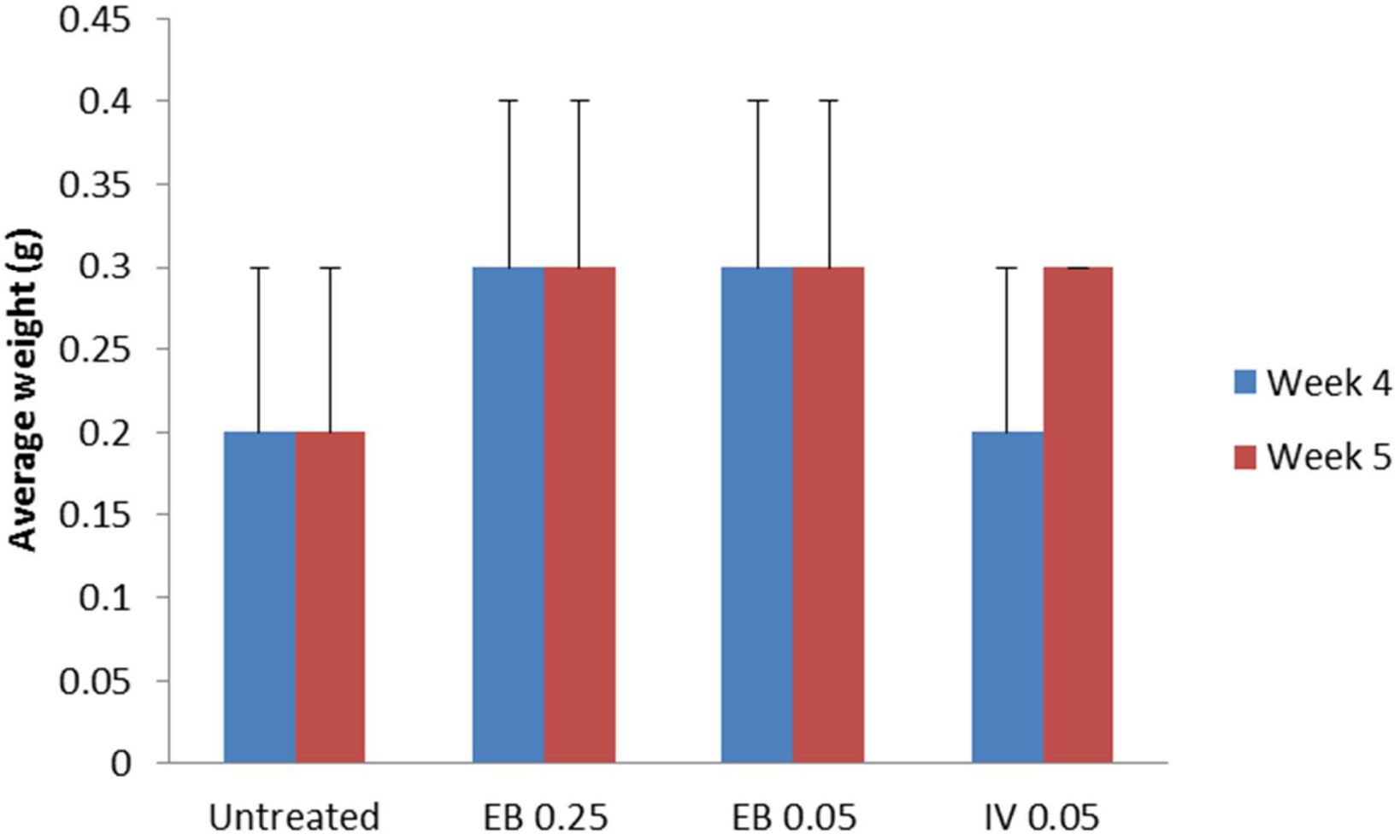


Total worm burden (Collymore et al)



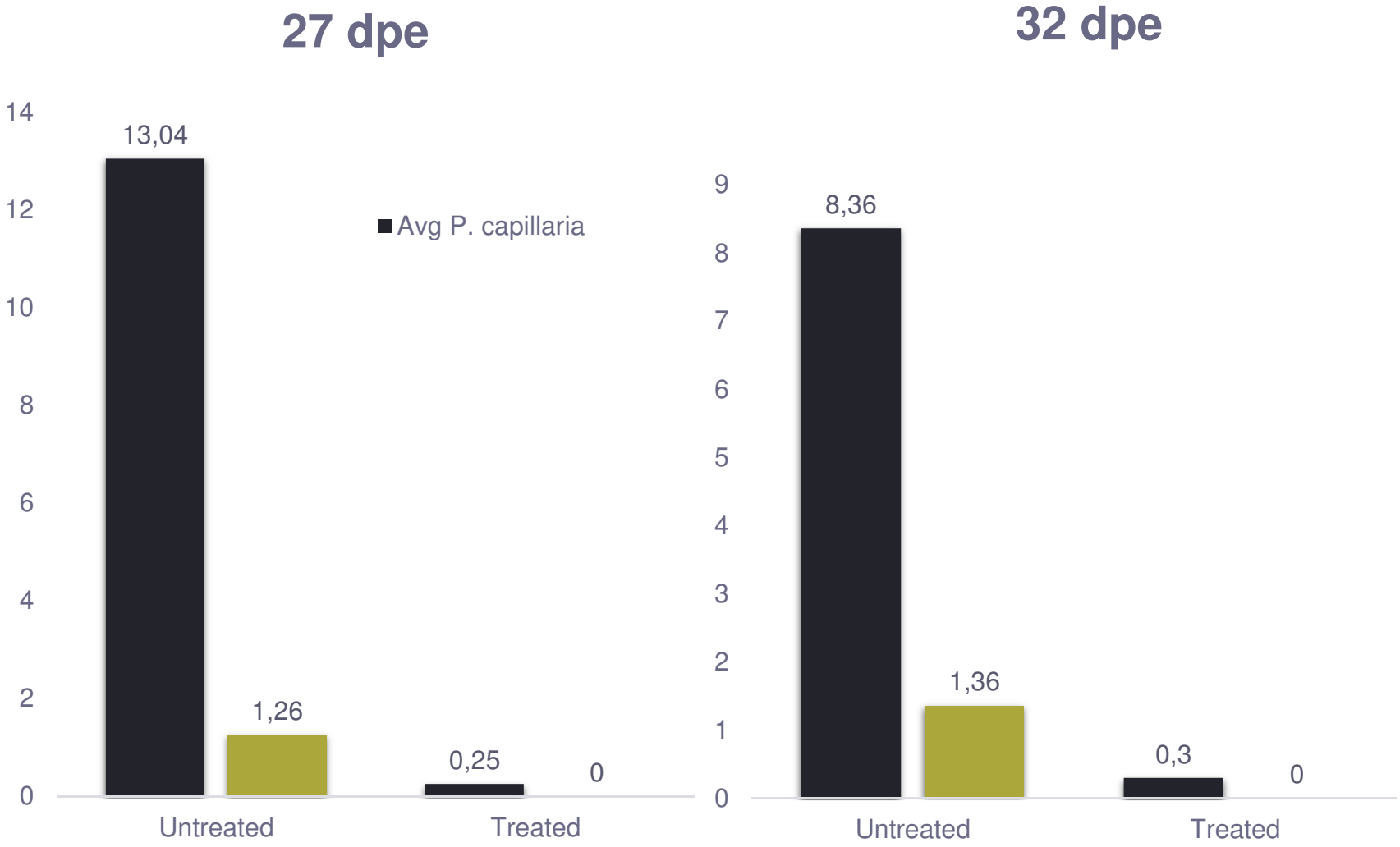
*$P \leq 0.05$; Tukey's pairwise comparison with Bonferroni correction

Average weight



*$P \leq 0.05$; Tukey's pairwise comparison with Bonferroni correction

Emamectin benzoate: Efficacy

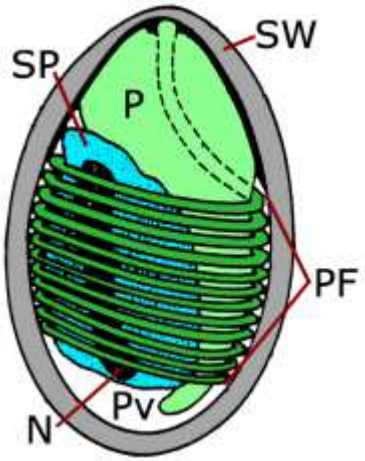


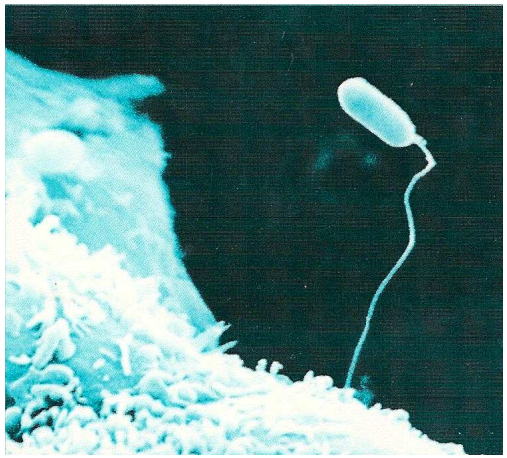
Pseudocapillaria tomentosa

Pathogenesis	Mode of transmission	Diagnosis	Control/Treament
Infects intestinal epithelium Severe chronic inflammation	Direct transmission through eggs	Wet mount: light microscopy, Worms **in fish** eggs in detritus PCR	Avoidance/quarantine Eggs resistant to chlorine Heat (50-60C) Emamectin

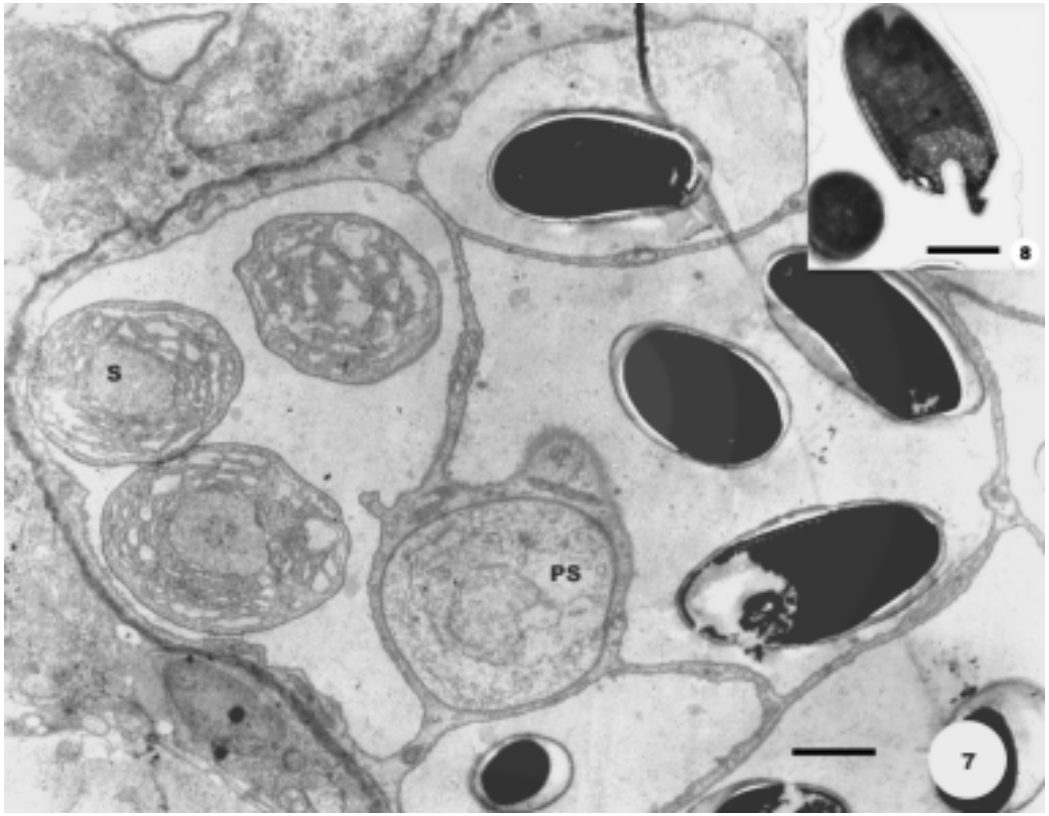
Microsporidia

- obligate intracellular parasite infecting a wide range of hosts
  - especially insects and fish
- defining characteristics:
  - small resistant spore (3-5 μm, some smaller)
  - 'polar filament' (=extrusion apparatus)





Life Cycle Phases



Infective Phase

- spore germination
- cell invasion

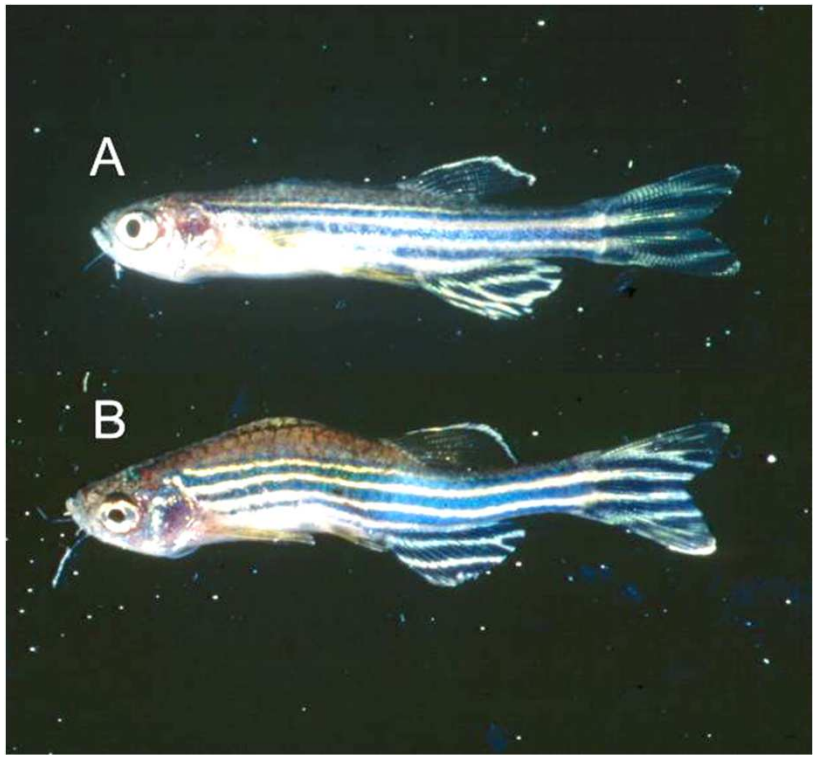
Proliferative Phase

- intracellular
- many modes of replication

Sporogonic Phase

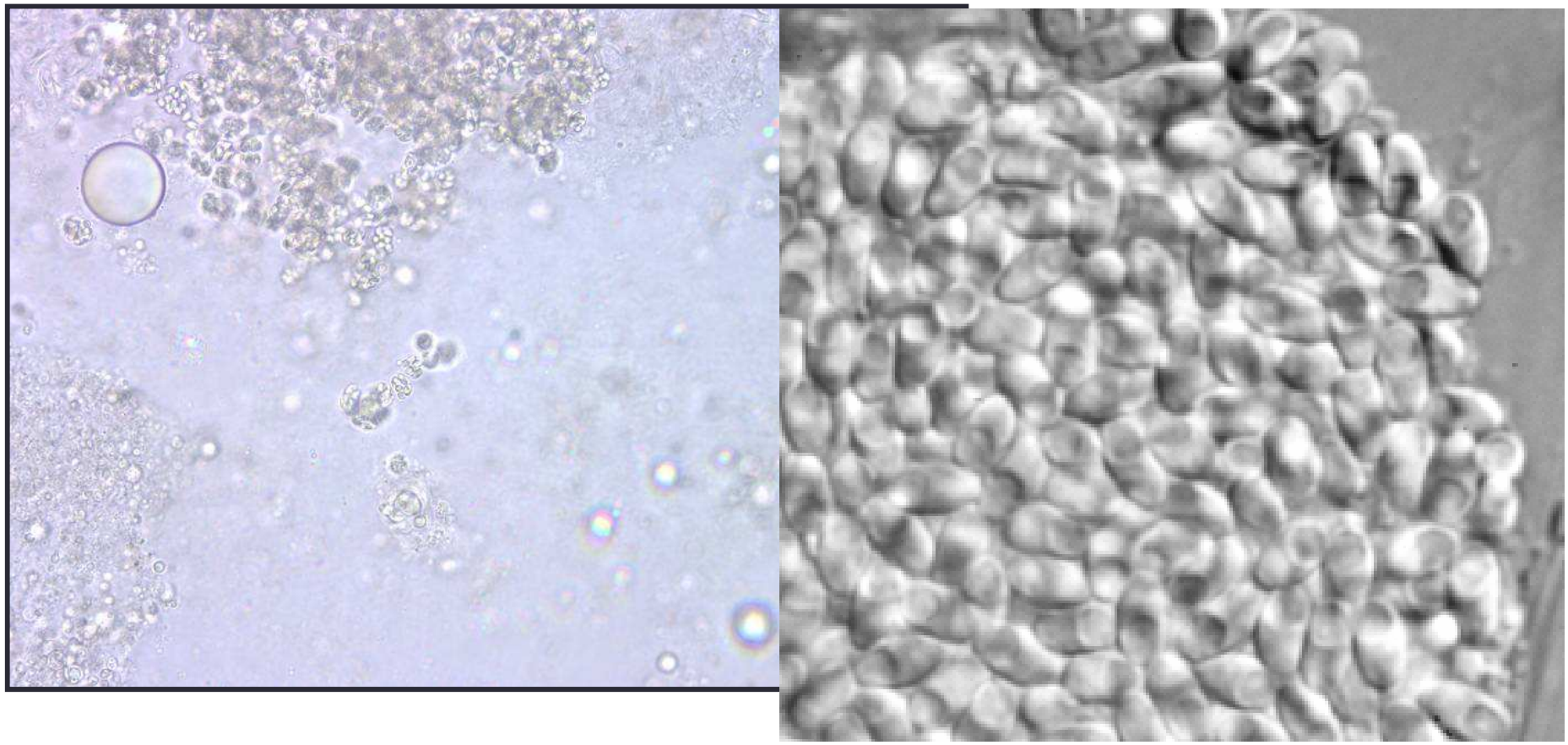
- terminal differentiation

Diagnosis of Microspordia - macroscopic

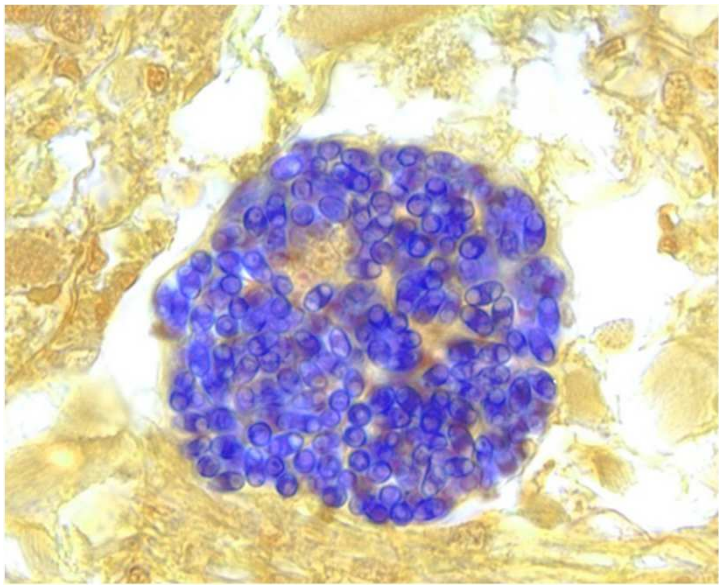


Neon tetra disease
Pleistophora hyphessobryconis
in muscle

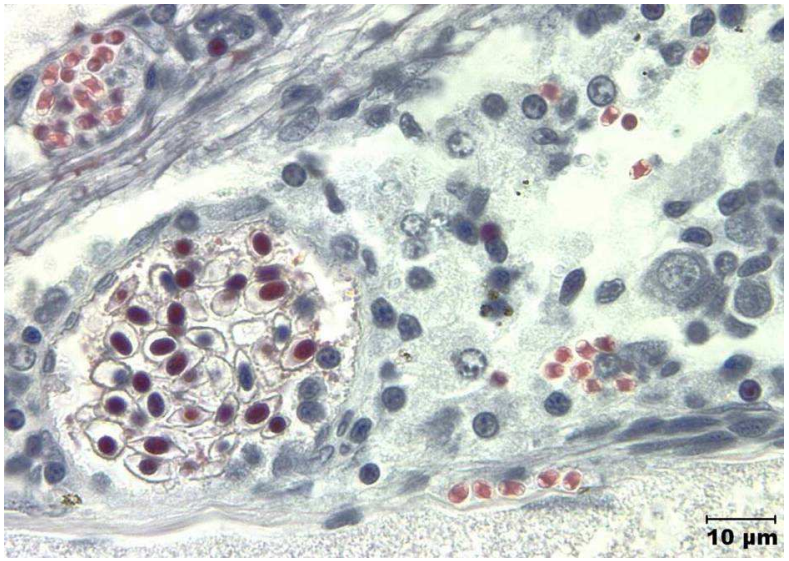
Wet mounts



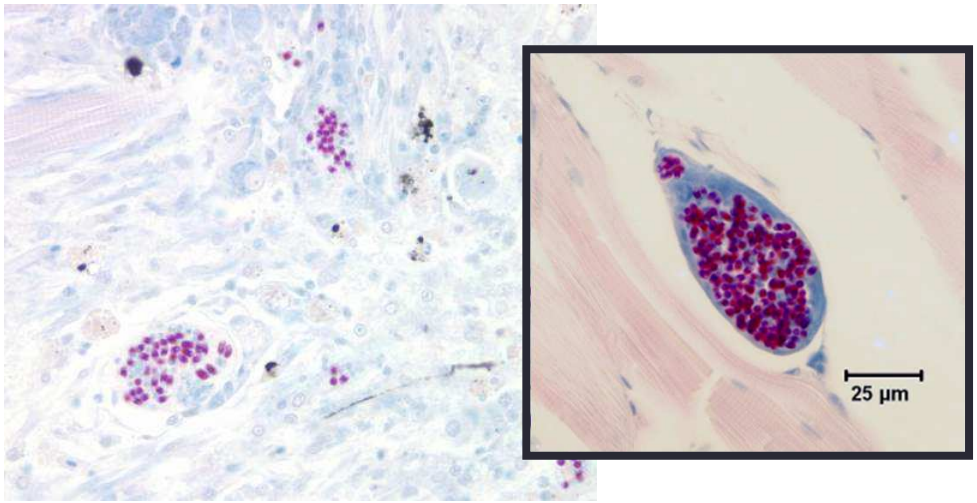
Gram Stain



Luna Stain

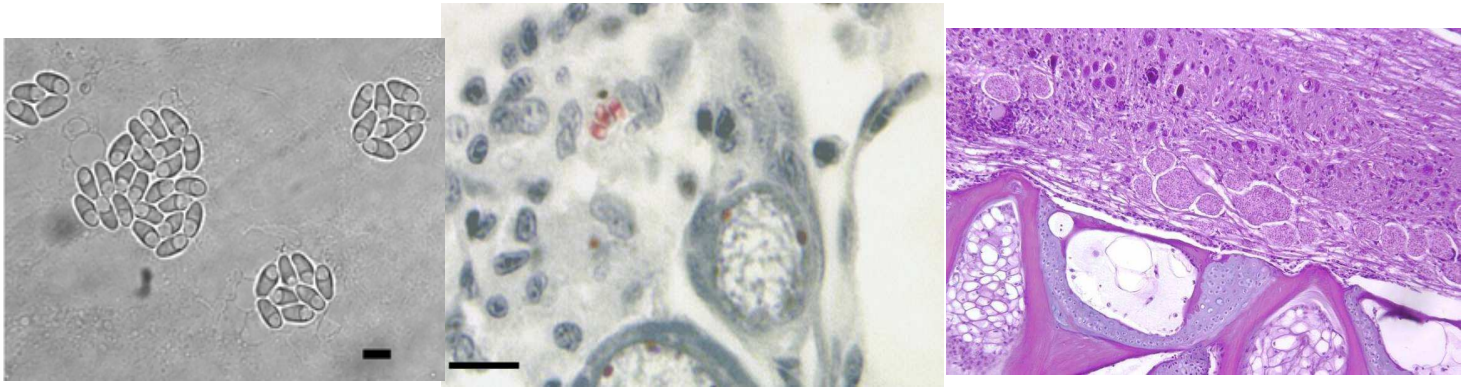


Acid-fast Stain

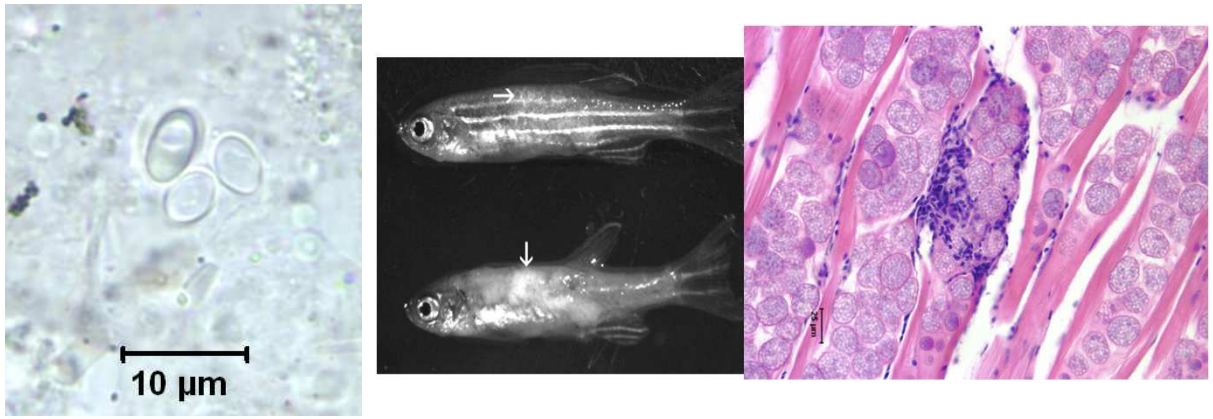


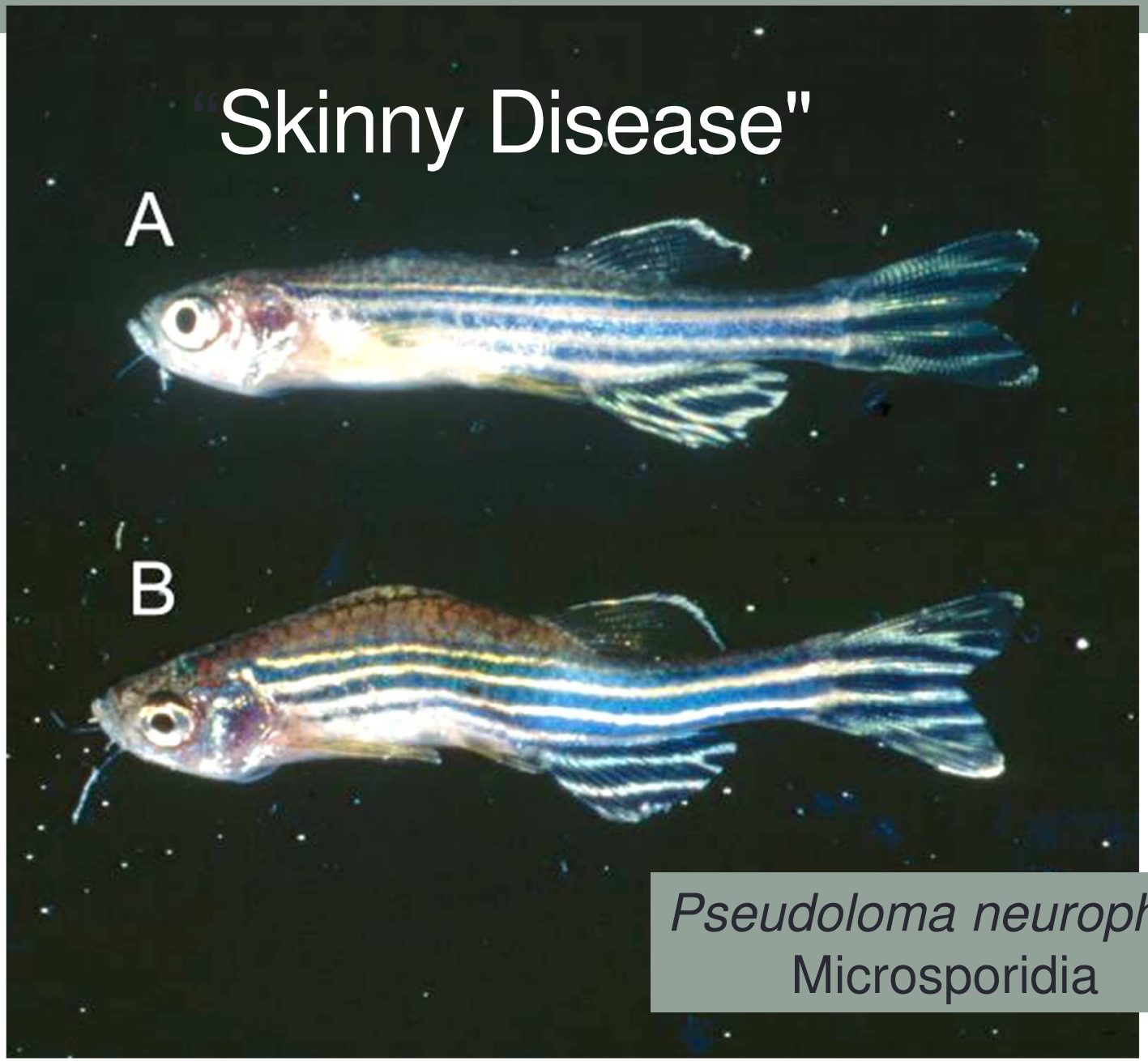
Common microsporidian parasites of zebrafish

- Microsporidia
  - *Pseudoloma neurophilia*

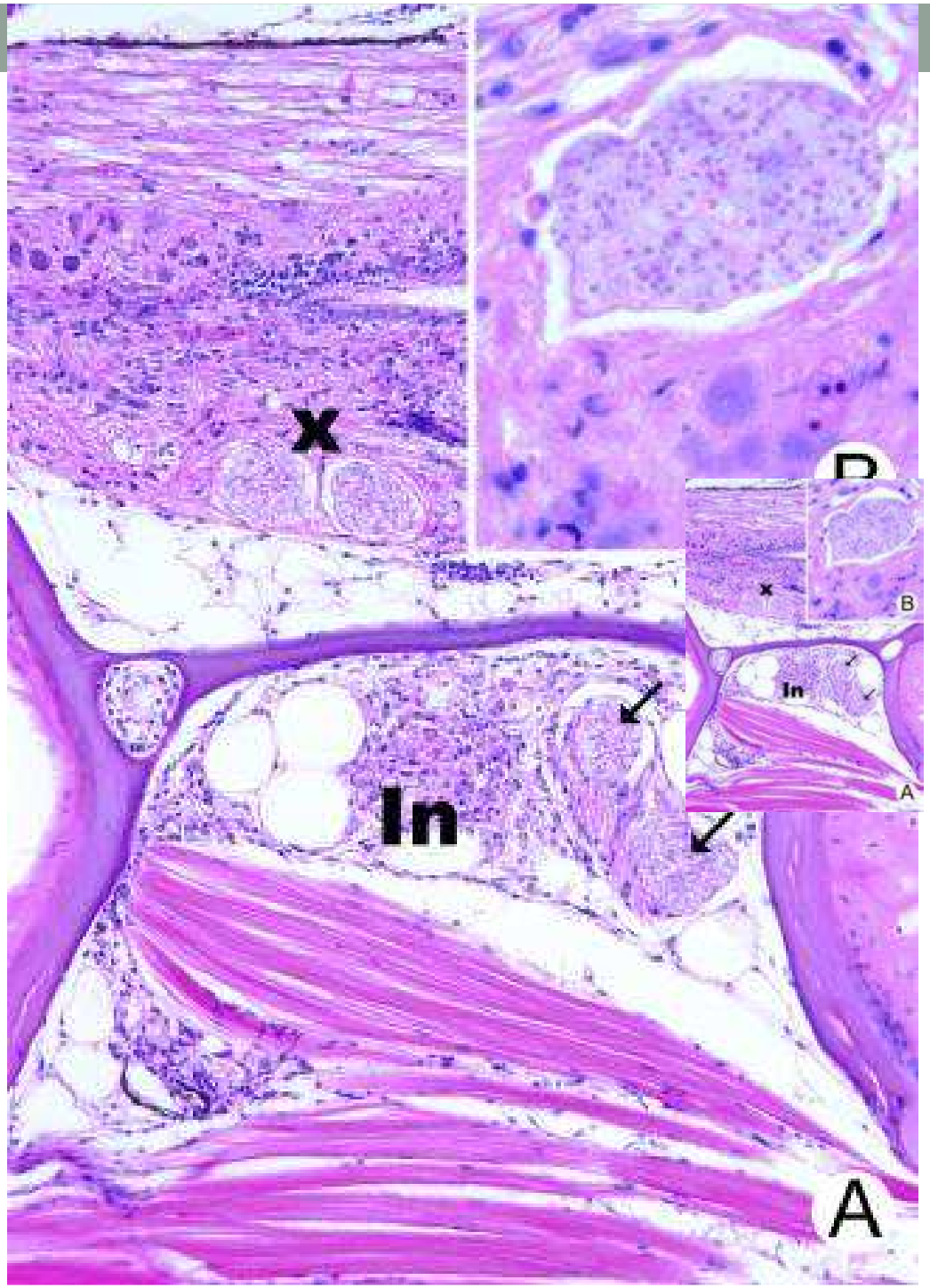


  - *Pleistophora hyphessobryconis*

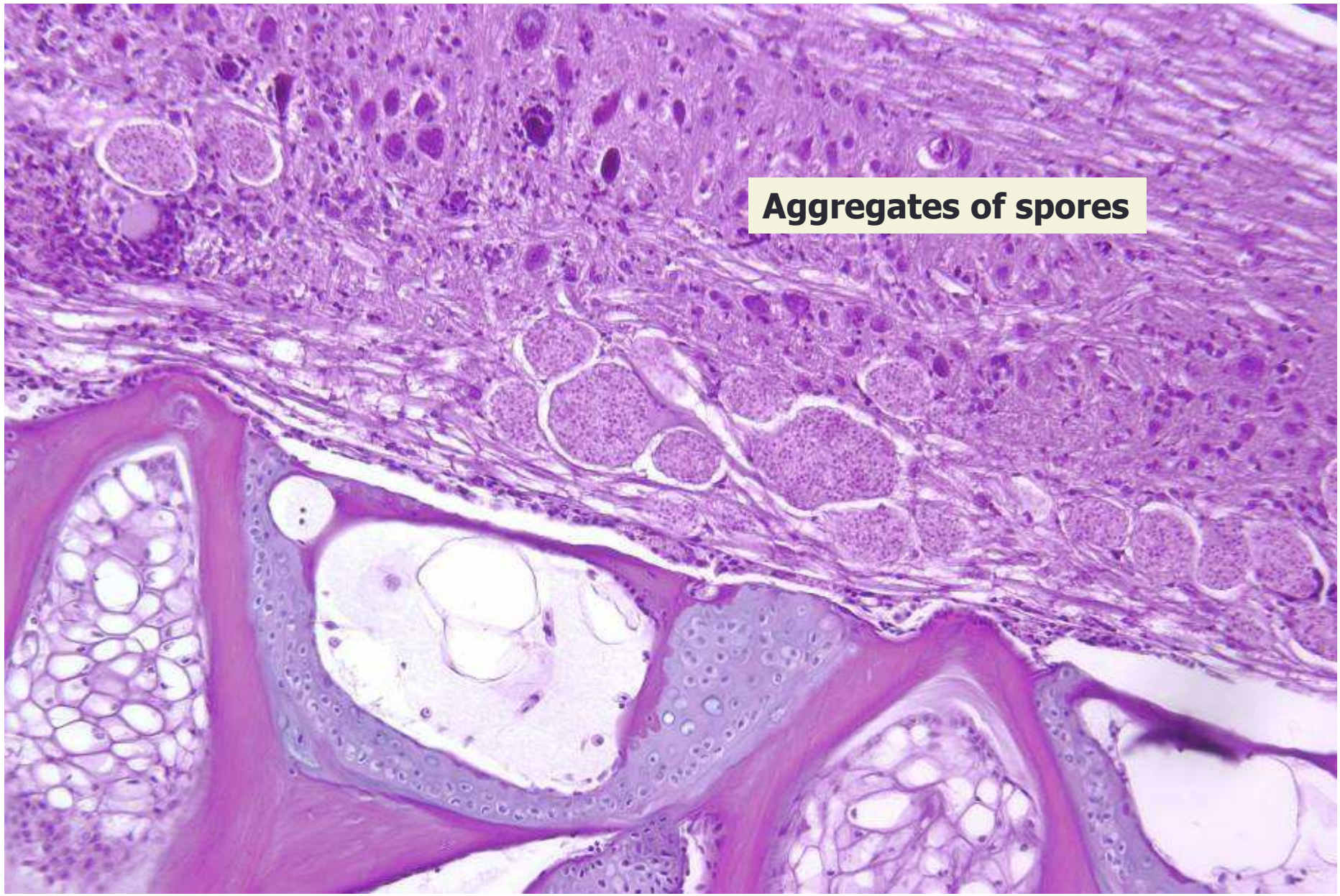




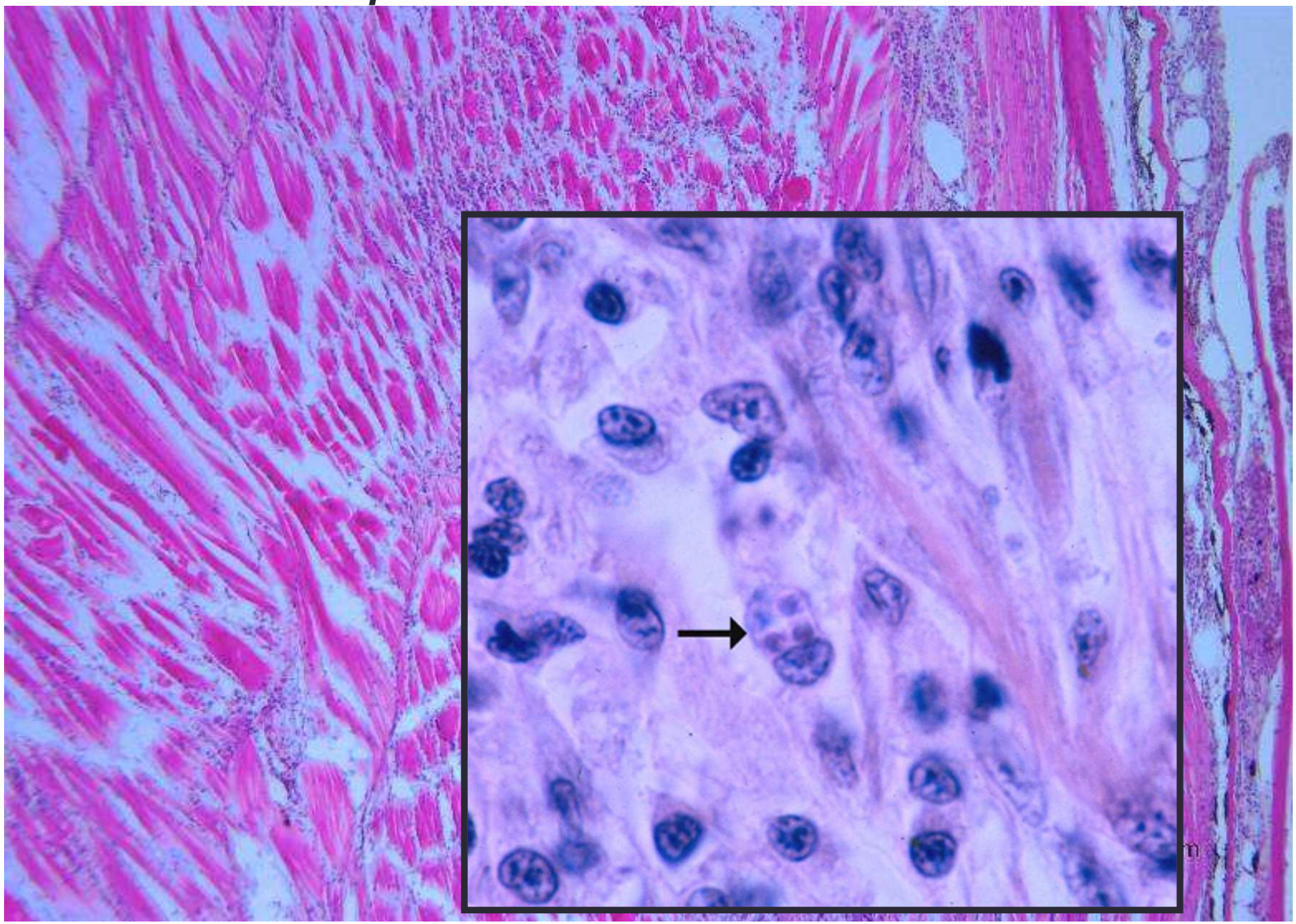
Pseudoloma neurophilia
Microsporidia

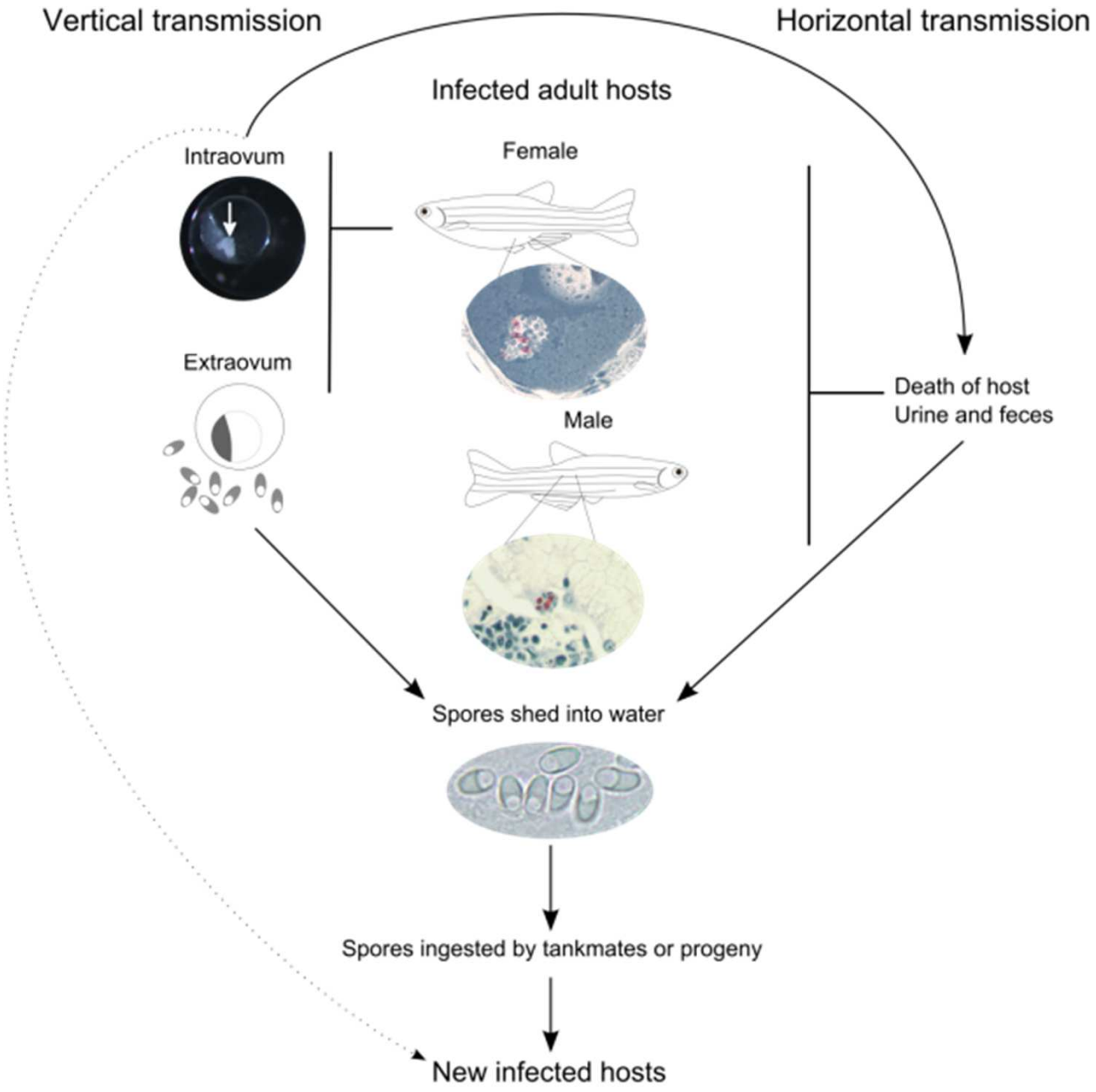


Pseudoloma neurophilia


Aggregates of spores

Diffuse, chronic myositis with *P. neurophilia*




Vertical transmission
Horizontal transmission
Infected adult hosts
Intraovum
Female
Extraovum
Male
Death of host
Urine and feces
Spores shed into water
Spores ingested by tankmates or progeny
New infected hosts

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Expansion of the Known Host Range of the Microsporidium, *Pseudoloma neurophilia*

Justin L. Sanders,[1] Virginia Watral,[1] Mark F. Stidworthy,[2] and Michael L. Kent[1,3]

- Betta, fathead minnow, giant danio, platys, and medaka are susceptible
- Goldfish – only very light infections
- Natural outbreak in fathead minnows

Control of Microsporidia
Disinfection

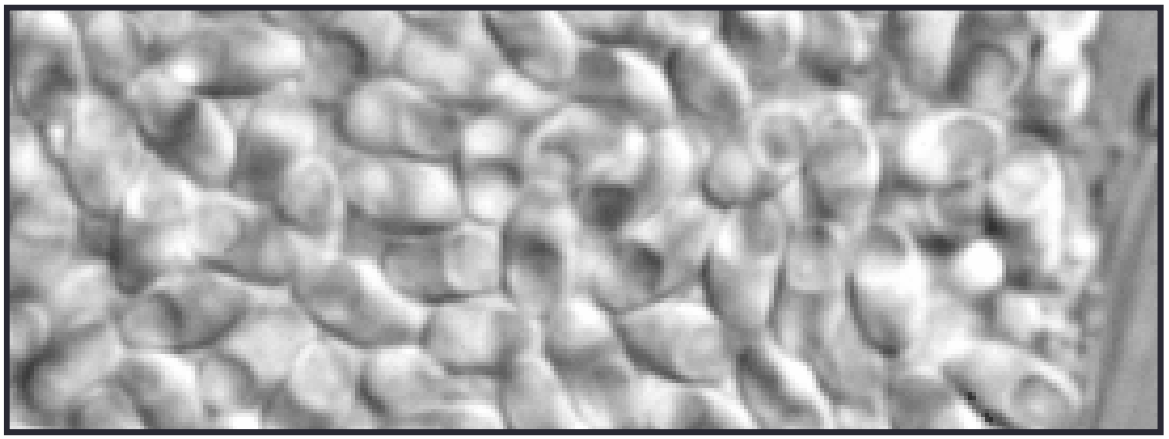
1. UV light sterilization of water
2. Chlorine for eggs, water, and equipment

UV is effective for Microspordia

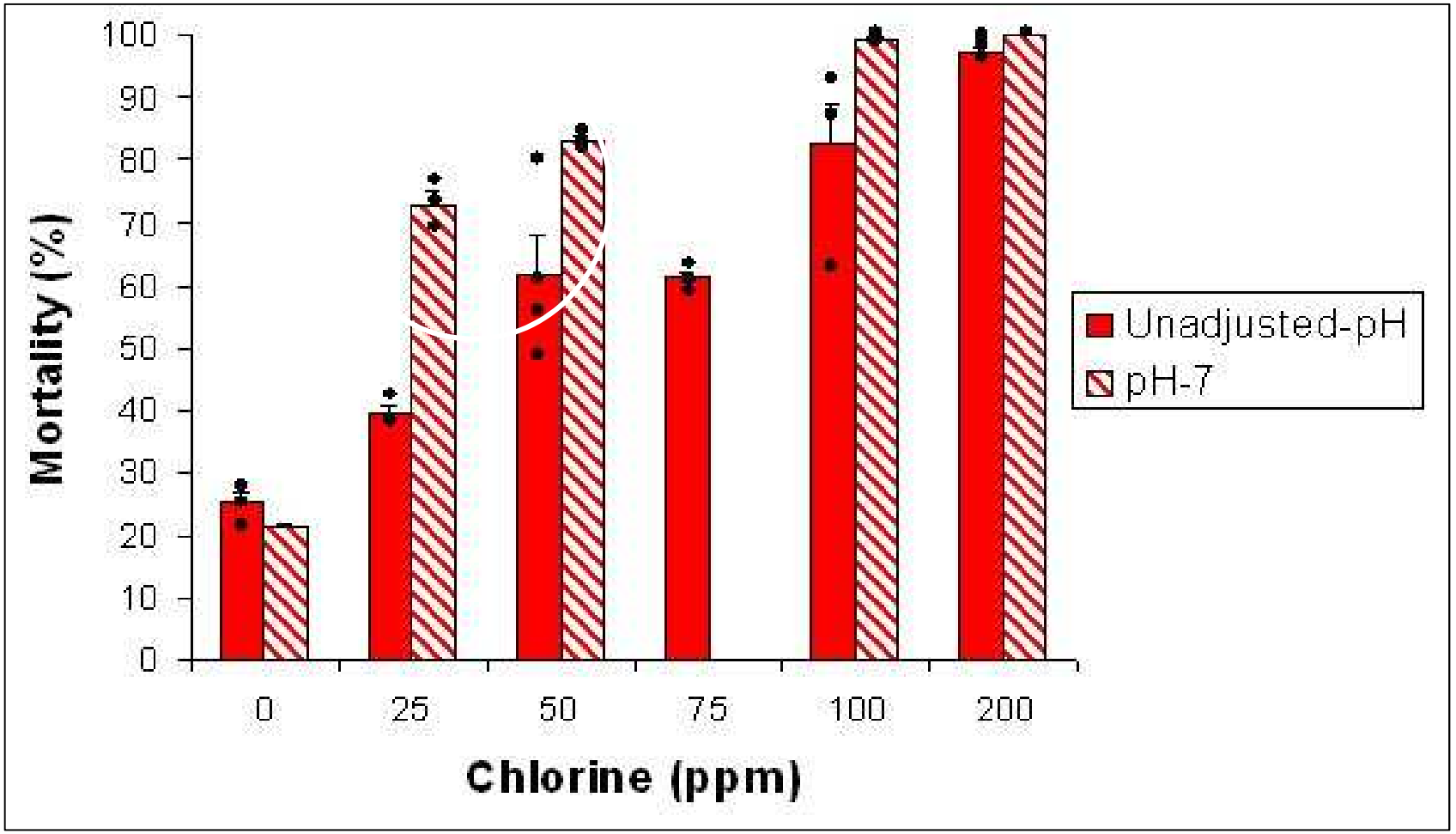
- *Pseudoloma*: 30-50,000 µWsec/cm2.
- Loma salmonae: 283,500 µWsec/cm2
  - Becker and Speare (2004)
- *Encephalitozoon* spp. 6-19,000 µWsec/cm2
  - Marshall et al. 2003

Efficacy of Chlorine

- 25 or 50 ppm/10 min: typical egg disinfection for zebrafish eggs
- Other studies (e.g., *Encephalitozoon spp.*
  - Ca 2 -5 ppm = 99% killing of spores



Pseudoloma



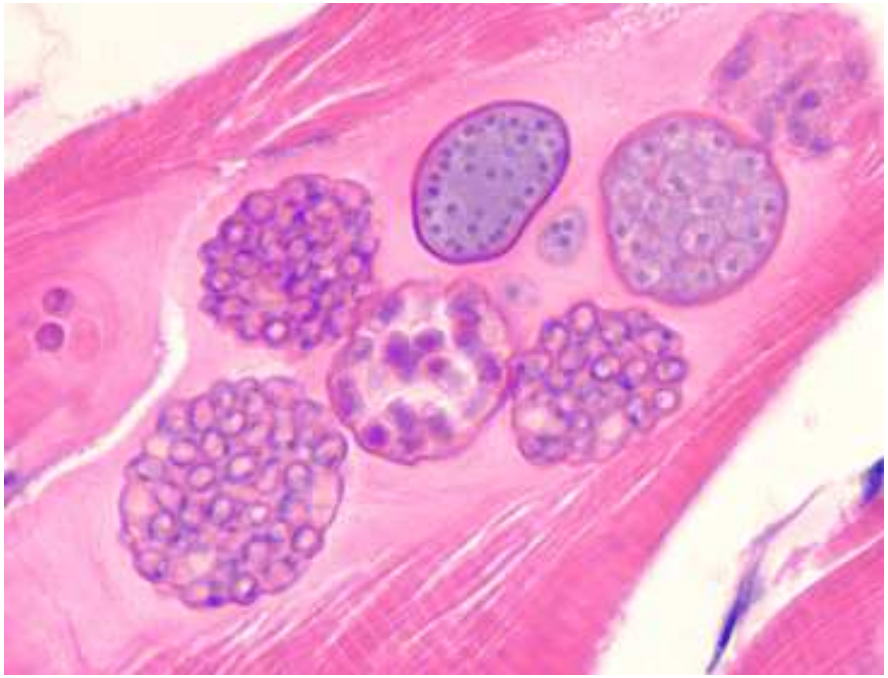
pH effect on chlorine efficacy on spores of *P. neurophilia* detected by SYTOX stain with standard error (black dots = data points). Unadjusted-pH trials (quadruplicate), ph-7 trials (triplicate). Note: 75 ppm @ ph-7 was not performed.

Pseudoloma neurophilia

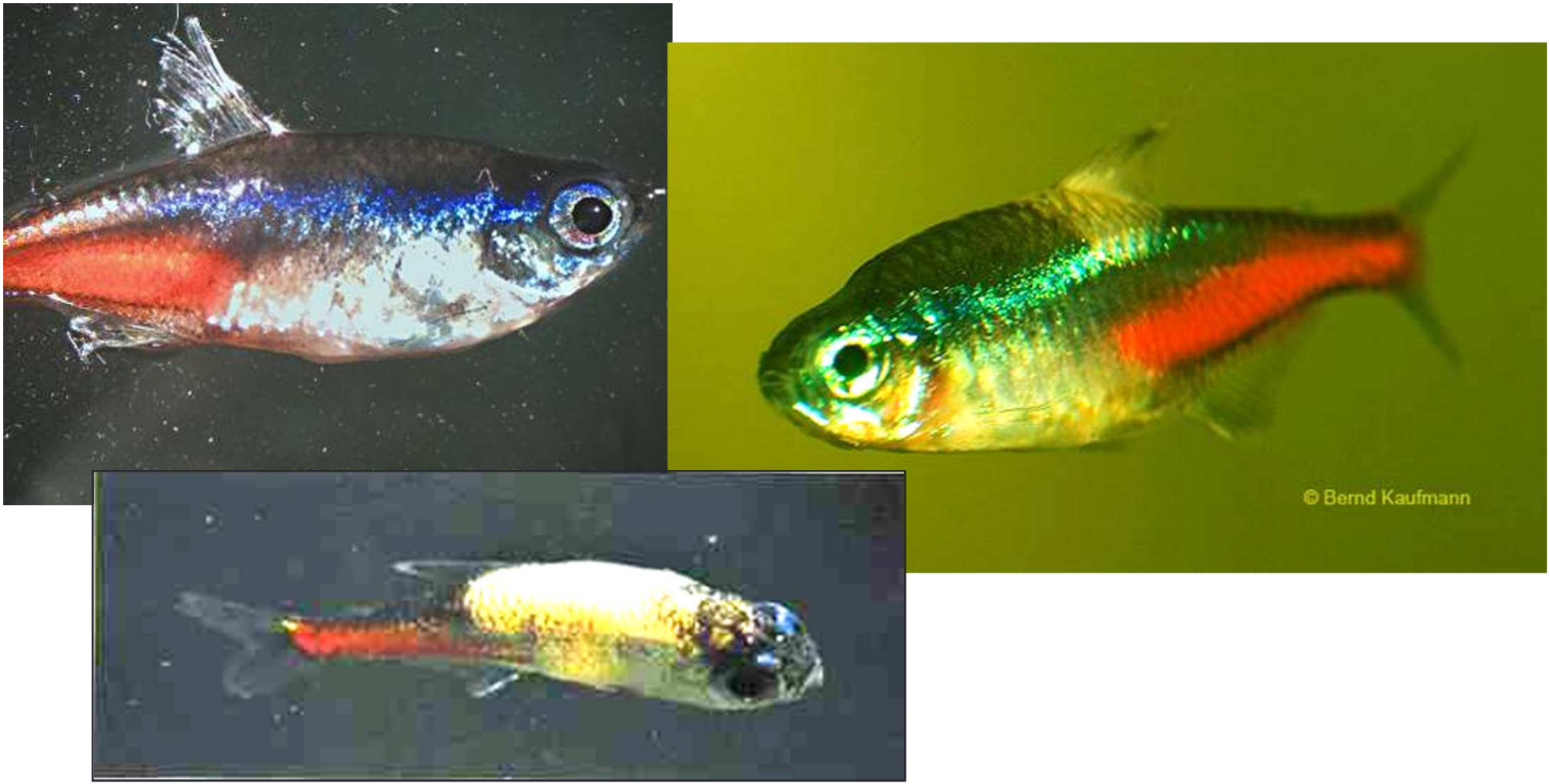
Pathogenesis	Mode of transmission	Diagnosis	Control/Treament
Emaciation, neural infections	Primarily horizontal through cannibalism, Vertical transmission directly to progeny or via exposure to spores during spawning	Wet mount: light microscopy, fluorescent fungal stains histology (Luna, acid-fast stains), PCR	Screening of broodstock; chlorination of equipment, UV of effluent

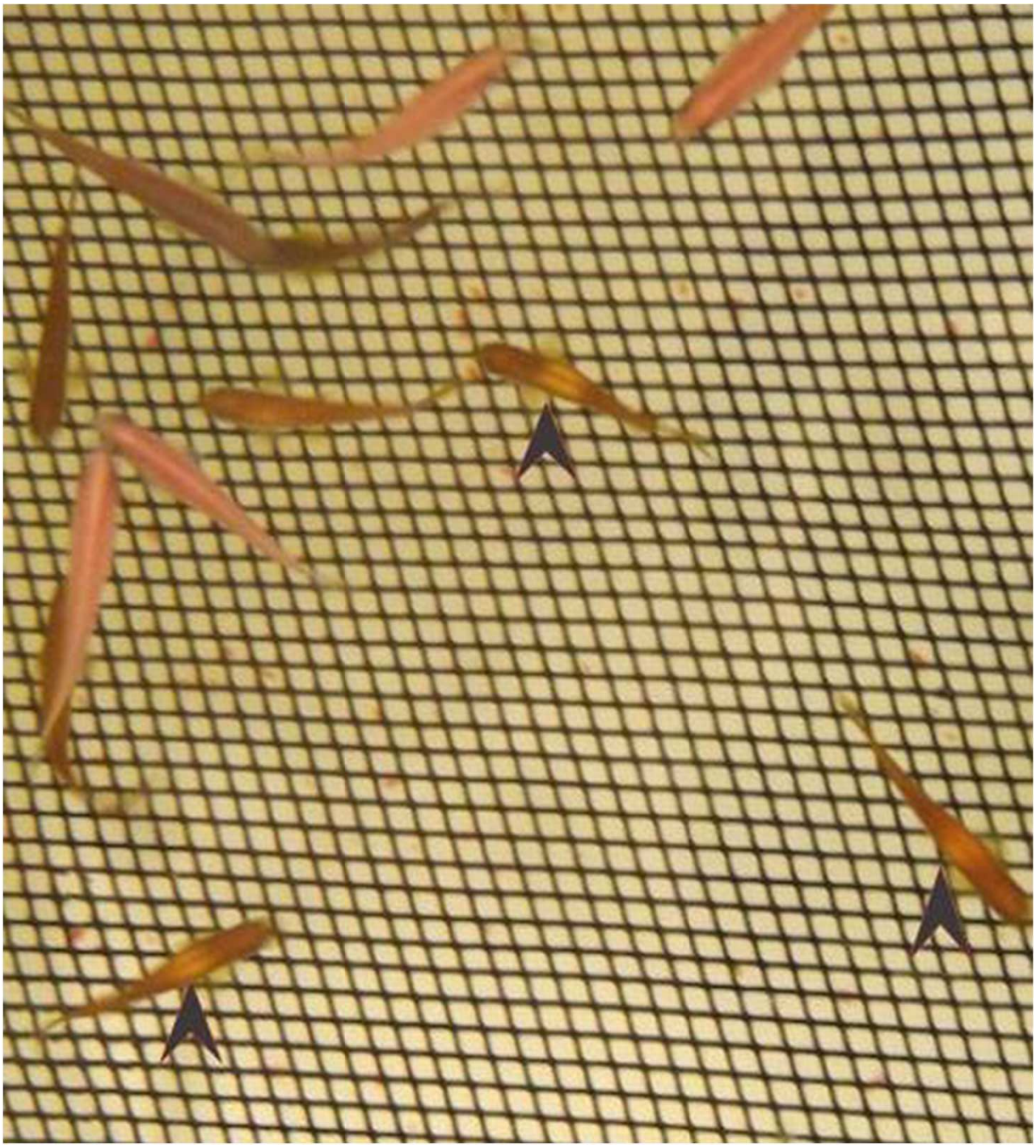
Pleistophora hyphessobryconis

- Commonly known as “Neon tetra disease”
- Infects many aquarium fishes
- Infects skeletal muscle
  - Massive involvement of myocytes
  - Necrosis and inflammation

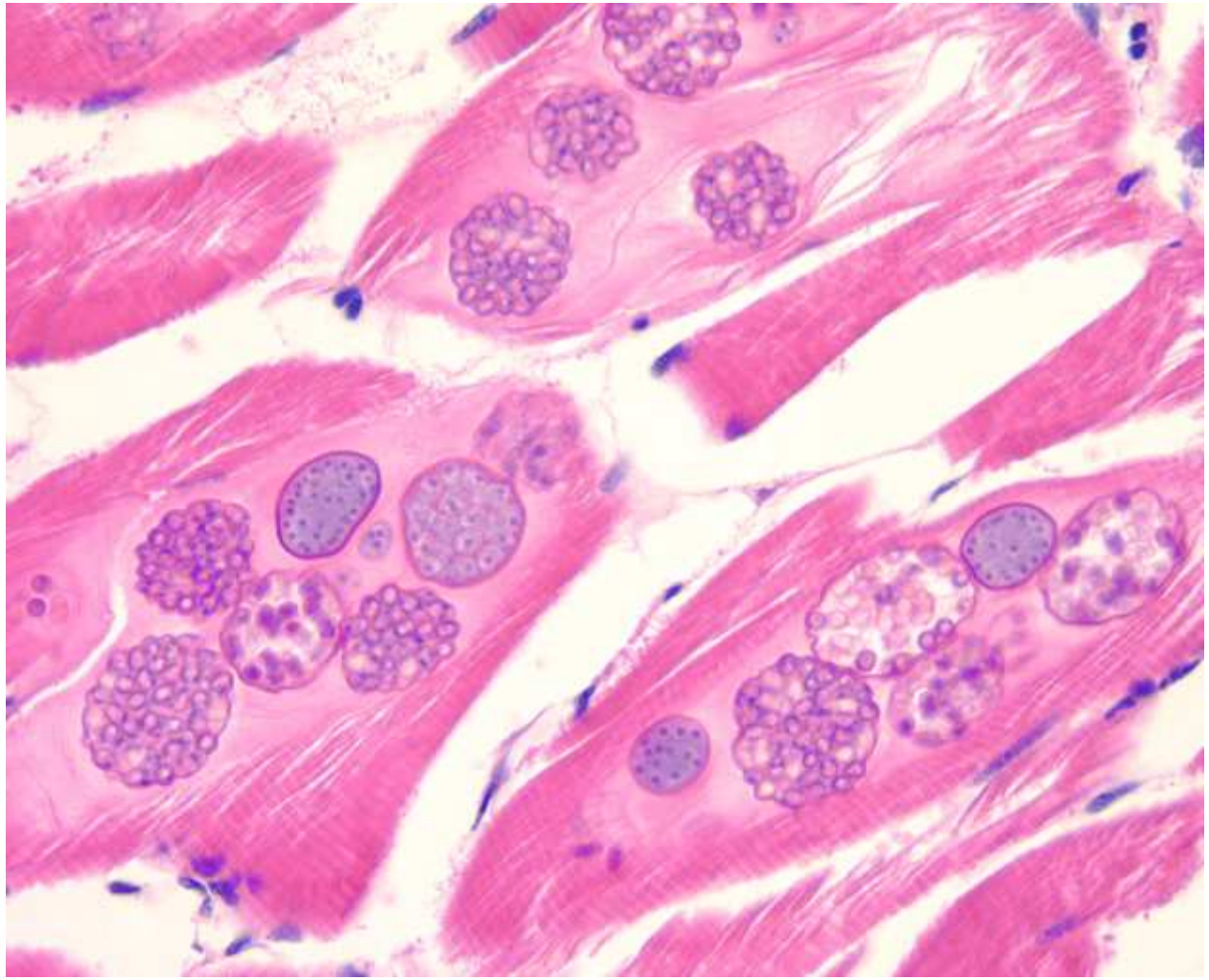


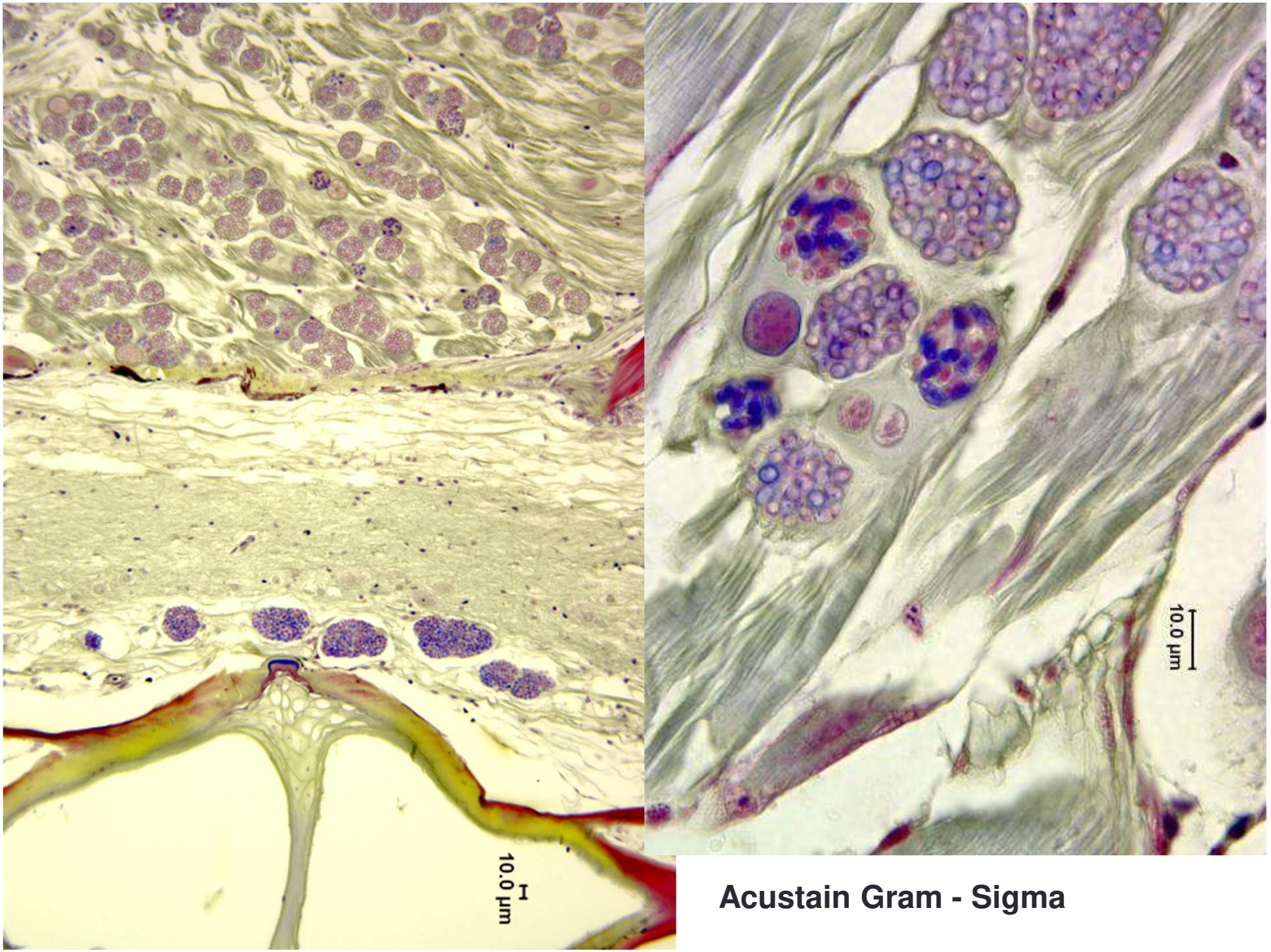
Neon tetra: *Paracheirodon innesi*



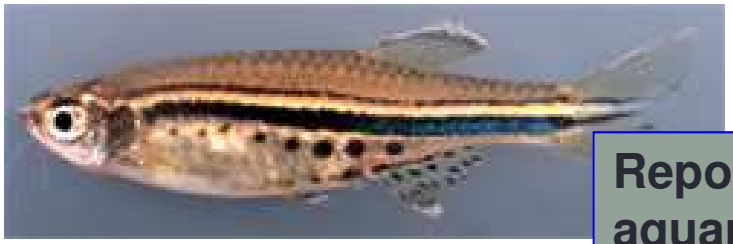


High magnification of *P.h.* in muscle with developmental stages





Acustain Gram - Sigma



Reported from 4 families and several species of aquarium fishes

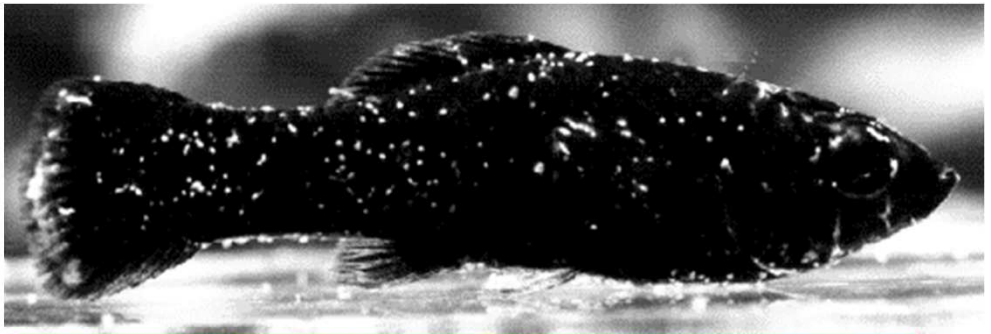
Pleistophora hyphessobryconis

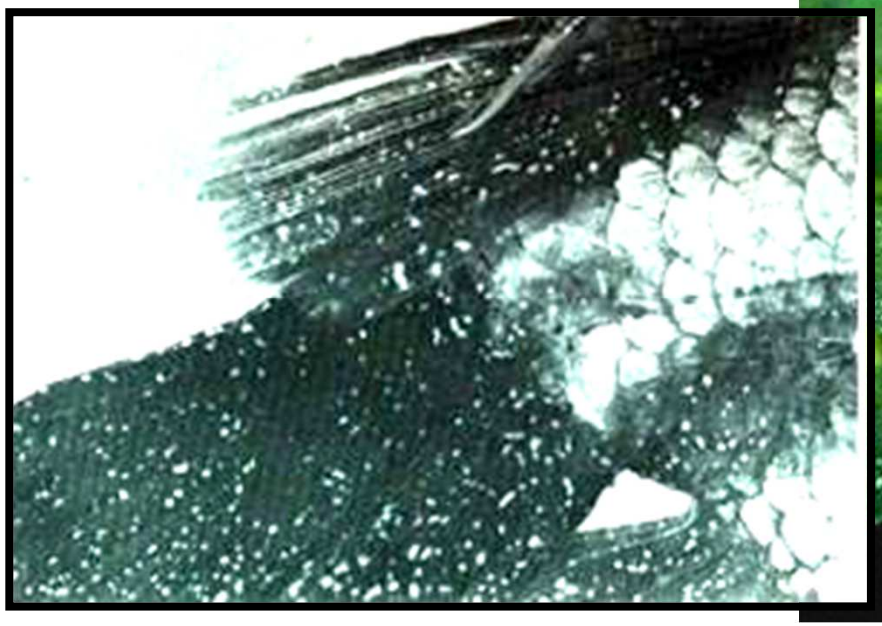
Pathogenesis	Mode of transmission	Diagnosis	Control/Treament
Infections of skeletal muscle Typically chronic becoming acute after stress/immunosuppression	Direct transmission through cannibalism	Wet mount: light microscopy, fluorescent fungal stains histology (Luna, acid-fast stains), PCR	Screening of broodstock; chlorination of equipment, UV of effluent

Ichthyophthirius multifiliis

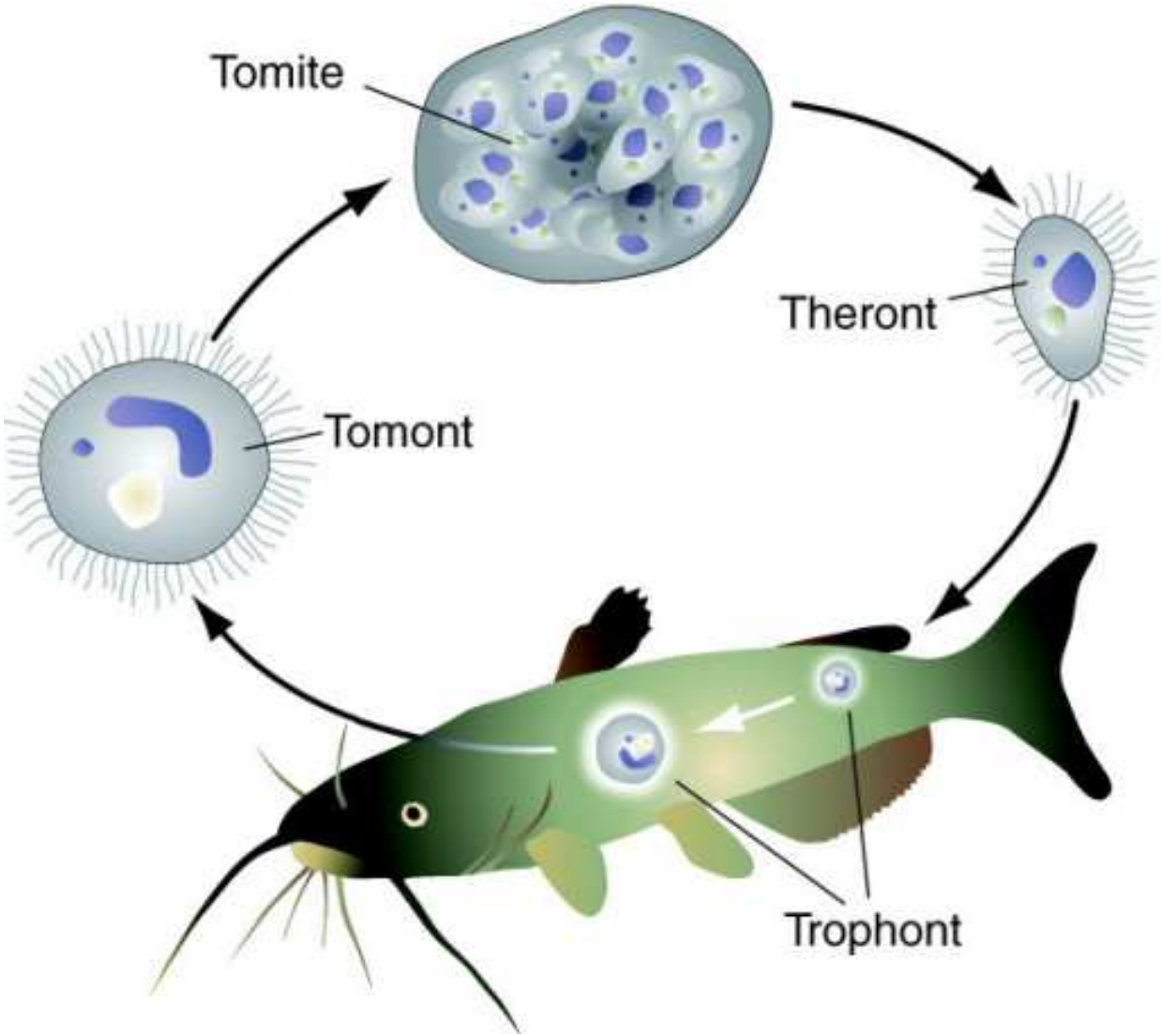
Ich" or white spot ciliate

- Direct Life Cycle – important in aquaculture and aquaria
- most species susceptible





“Ich”

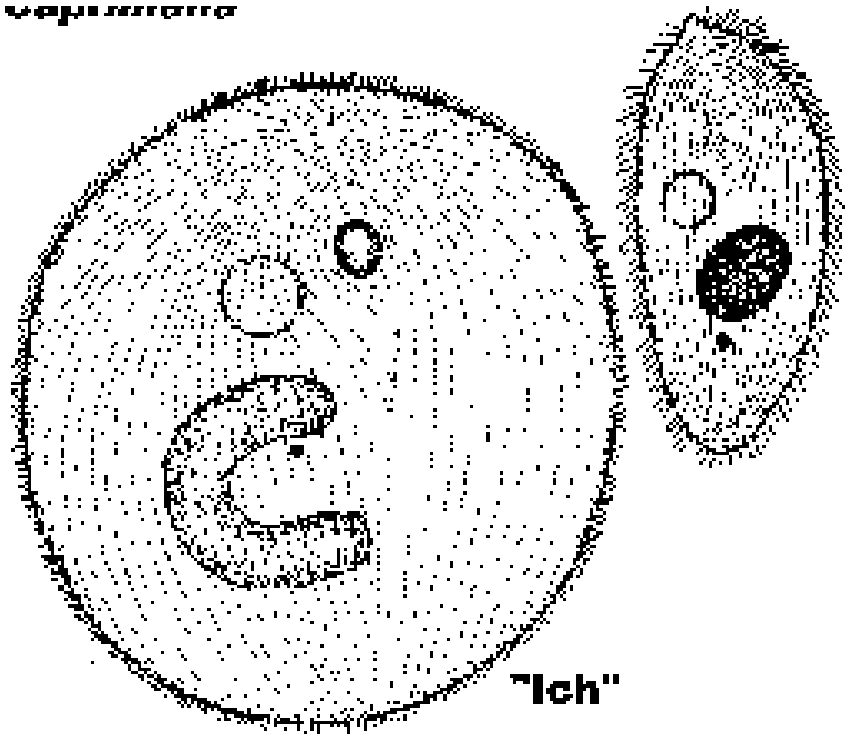


Ich

1. Penetrates epithelium of gills and skin
2. Causes severe epithelial hyperplasia
3. Death cause by osmoregulatory problems & asphyxiation (due to reduction in gill surface)

Diagnosis

Horseshoe shape macronucleus
Variable in size (some over 100 um)
Under epithelium



Formalin:
A common treatment for external parasites in fish

- **Bath:** 0.125 and 0.250 ml/L for 30 to 60 min
- (125 to 250 ppm).
- Remove fish from tank, then treat
  - But what about off-host stages
- **Long Term** : 15 – 25 ppm
  - Apply to system, kills off host stages
  - But may reduce bacteria in biological filters in recirculating systems
  - Aerate – formalin reduces O_2 levels

Control of Ich

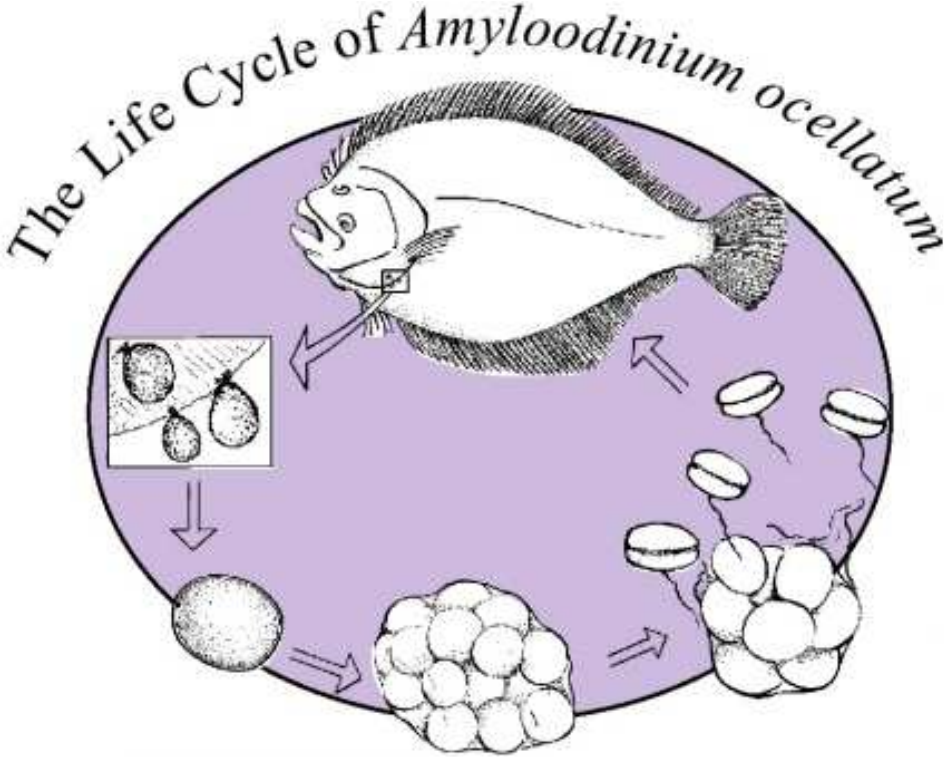
- Formalin: stages under epithelium are protected. So…must treat for many days
- Also several commercial products available
- High temperature > 85-90 F for fish that can tolerate (aquarium fishes)
- Acquired Immunity – Vaccines for catfish
  - (H. Dickerson, University of Georgia)

Ichthyophthirius multifiliis

Pathogenesis	Mode of transmission	Diagnosis	Control/Treament
Infections of gill and skin epithelium leading to hyperplasia Death due to osmoregulatory dysfunction/asphyxiation	Direct transmission through water exposure with off-host development	Wet mount: light microscopy; PCR	Associated with poor water quality (low temperature) Avoidance/quarantine Formalin treatment: several days

Velvet Disease
"Oodinium"

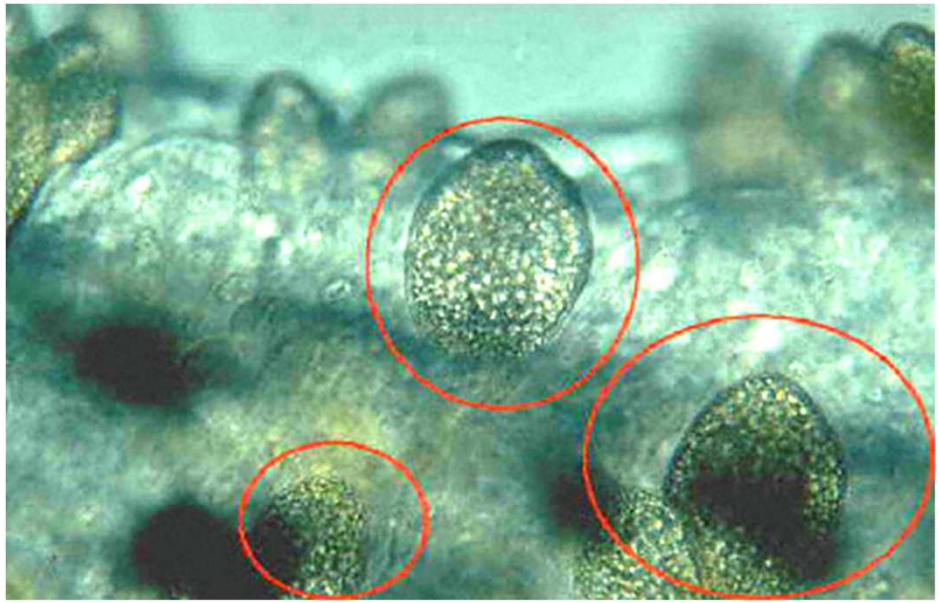
- Parasitic dinoflagellates
- ***Piscinoodinium pillulare – freshwater***
- ***Amyloodinium ocellatum – seawater***
- **Off host development**
  - **6 days at 25 C**

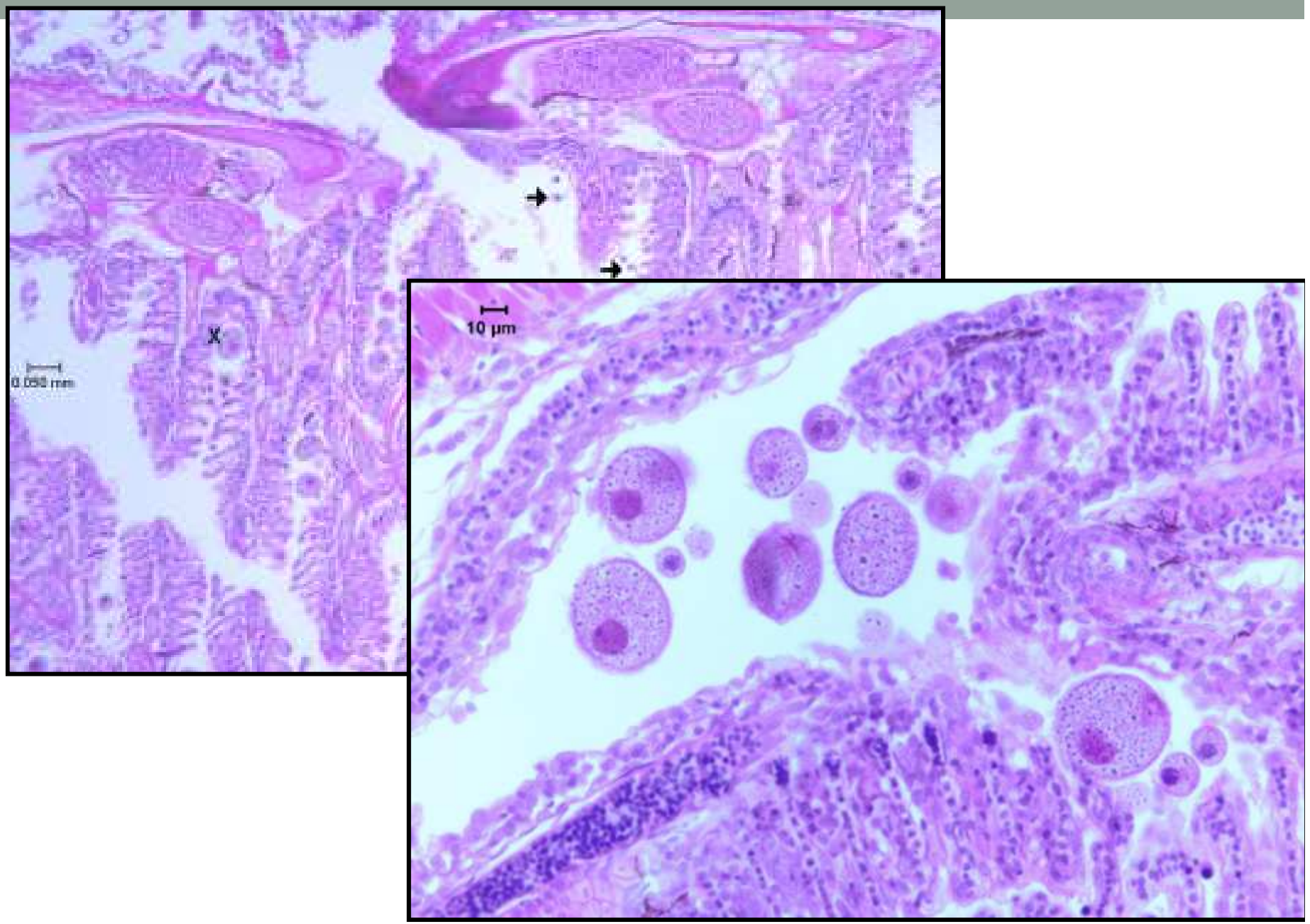



Lethargy, rapid respiration, flashing whitish “dust” on skin

“Oodinium” - Diagnosis

- 9-12 X 40-90 µm, - on gills and skin,
- Non-motile, large nucleus, starch granules




X
0.050 mm
10 µm

Treatment of “velvet disease”

1. **Salt + atabrine. Added over 3 day period. From “The Zebrafish Book” Westerfield, 2000, University of Oregon**
2. **Salt: Noga, E. “Fish Diseases, Diagnosis and Treatment. Mosby Press, St. Louis**

Control/Treatment

- Formalin Baths: 100-200 mg/l for 6-9 h
- Copper Sulfate - 0.15 - 0.20 ppm for 10-14 d
  - Amyloodinium – 0-10 ppt for 10-14 d
- Raise temperature to 29C/85 F?

Piscinoodinium pillulare

Pathogenesis	Mode of transmission	Diagnosis	Control/Treament
Infections of skin and gills	Direct transmission through water exposure with off-host development	Wet mount: light microscopy; PCR	Associated with poor water quality, esp in quarantine systems Avoidance/quarantine Salt treatment: several days Formalin bath Copper Sulfate Increased temperature

Clinical signs typical of mycobacteriosis

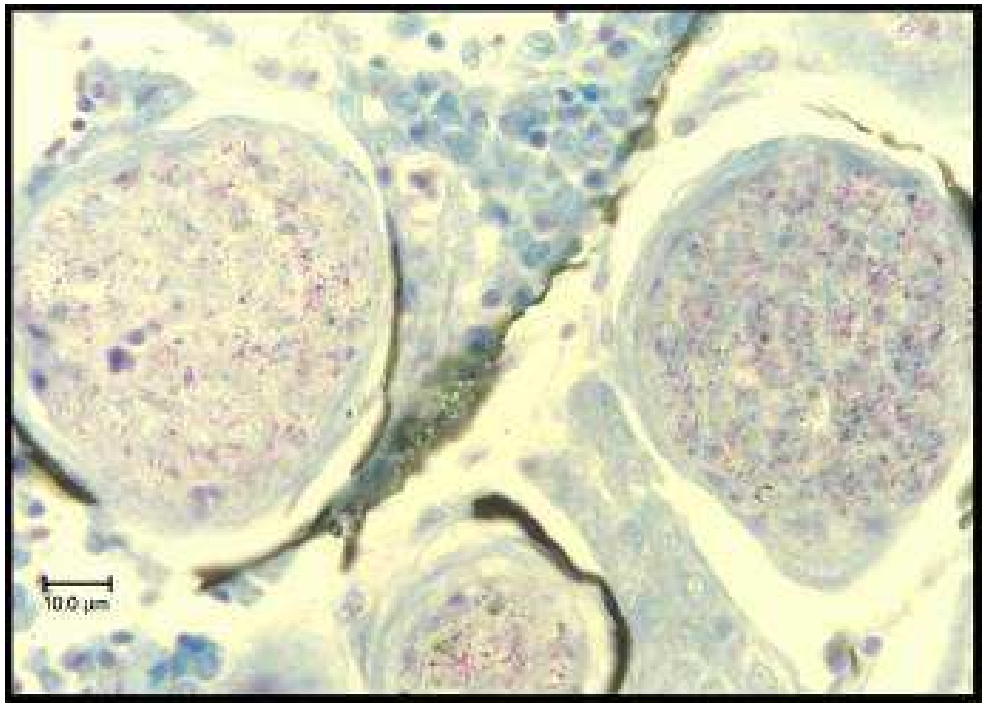
- Swollen belly
- Skin ulcers
- General malaise (e.g., lethargy, emaciation)

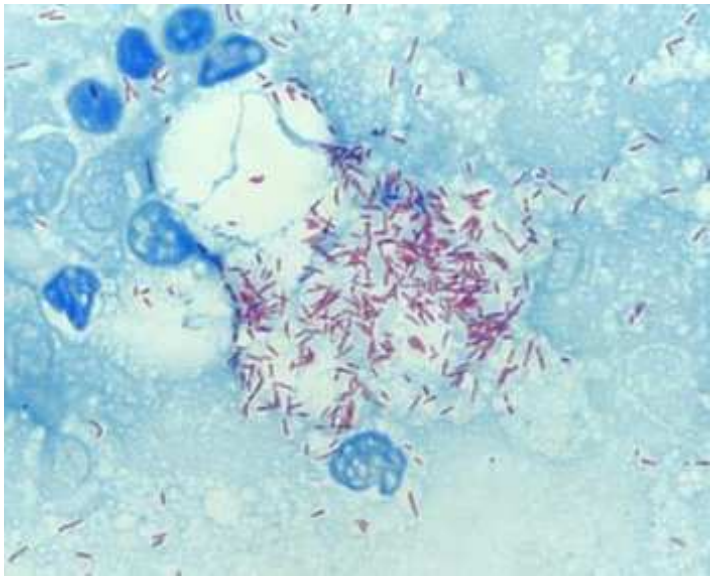
Internally

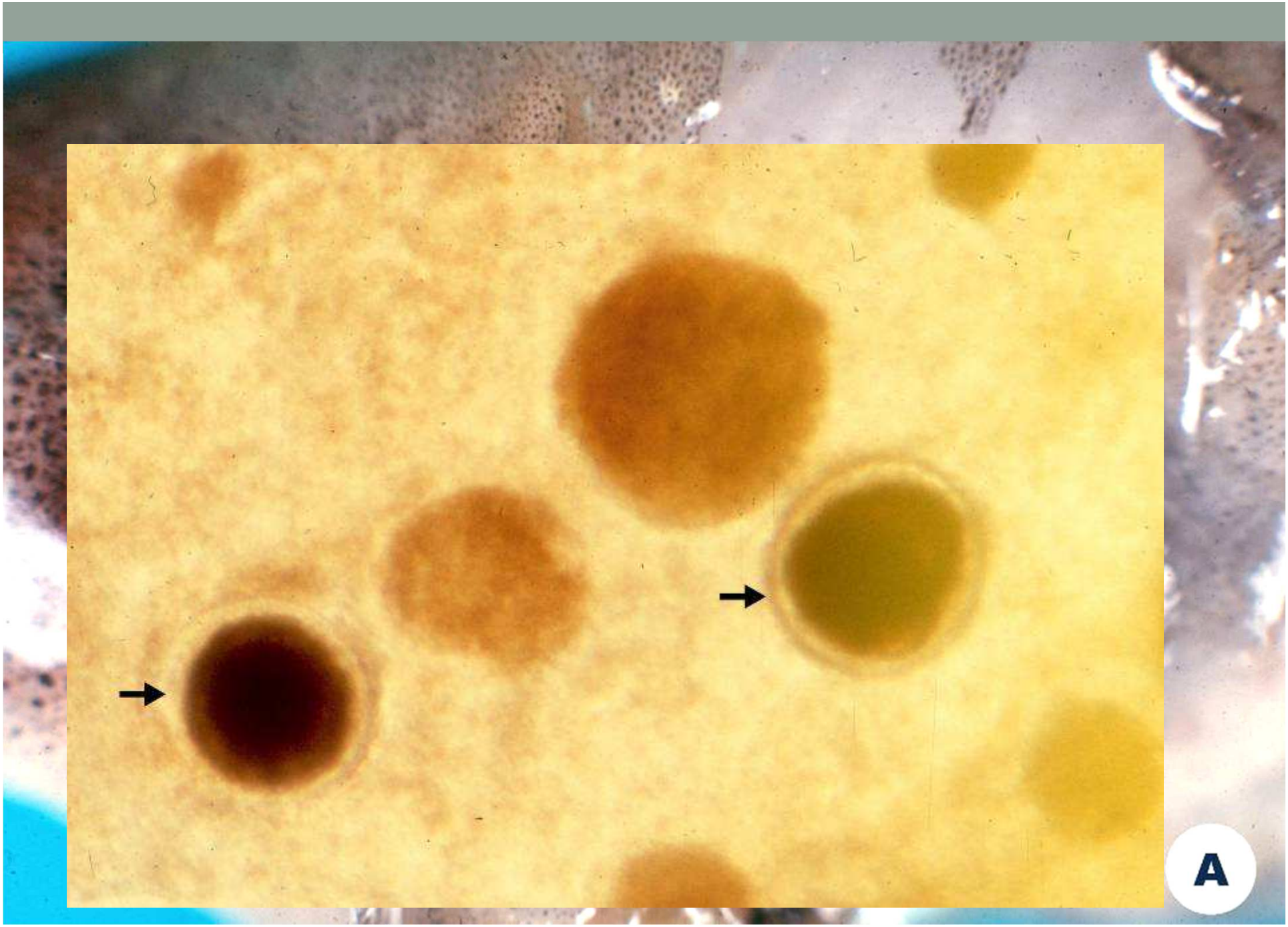
- Granulomas throughout viscera
- Swollen spleen, kidney, liver

Diagnosis

- Histology – granulomas and acid fast
- Tissue imprints – acid fast



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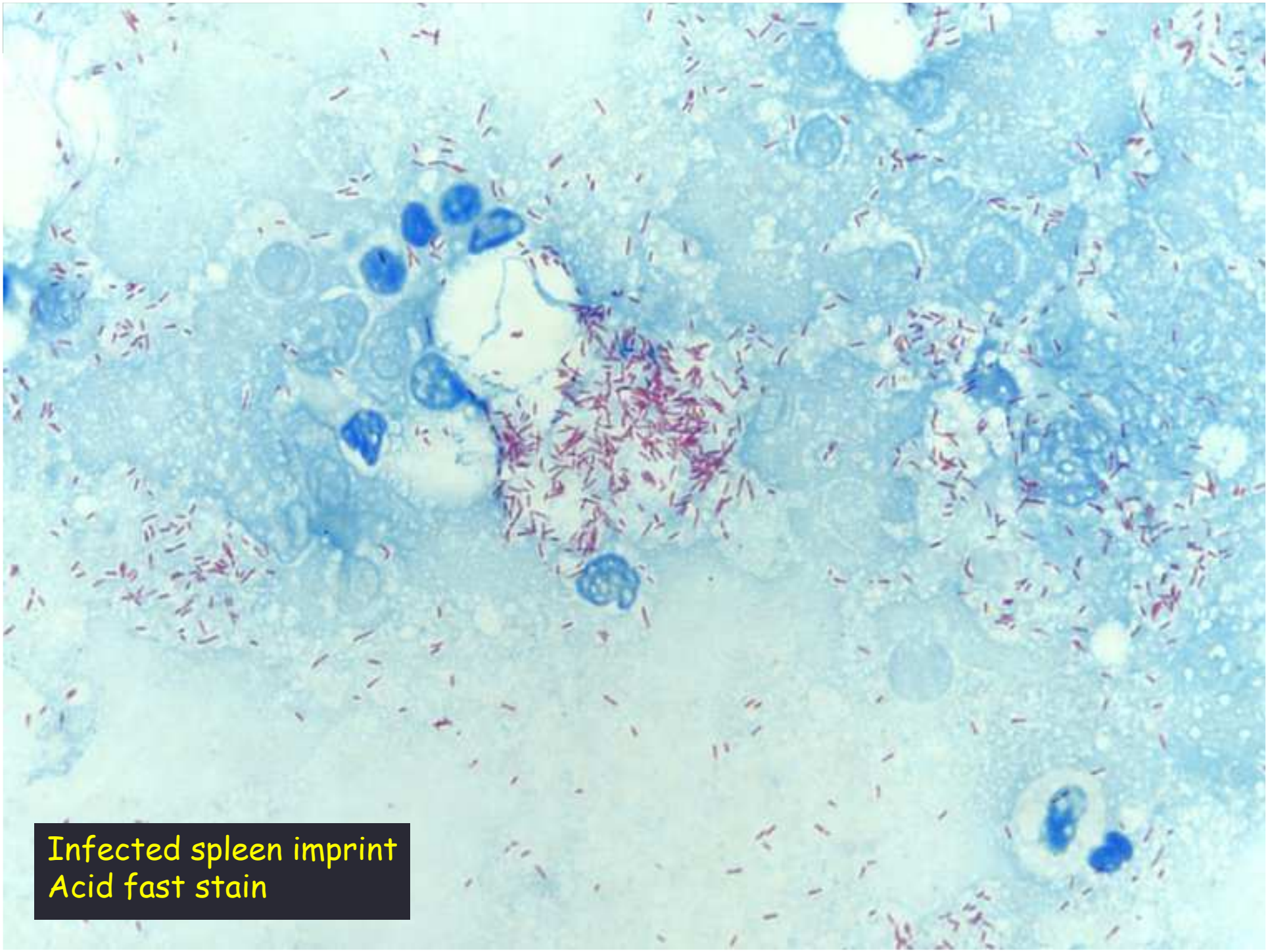
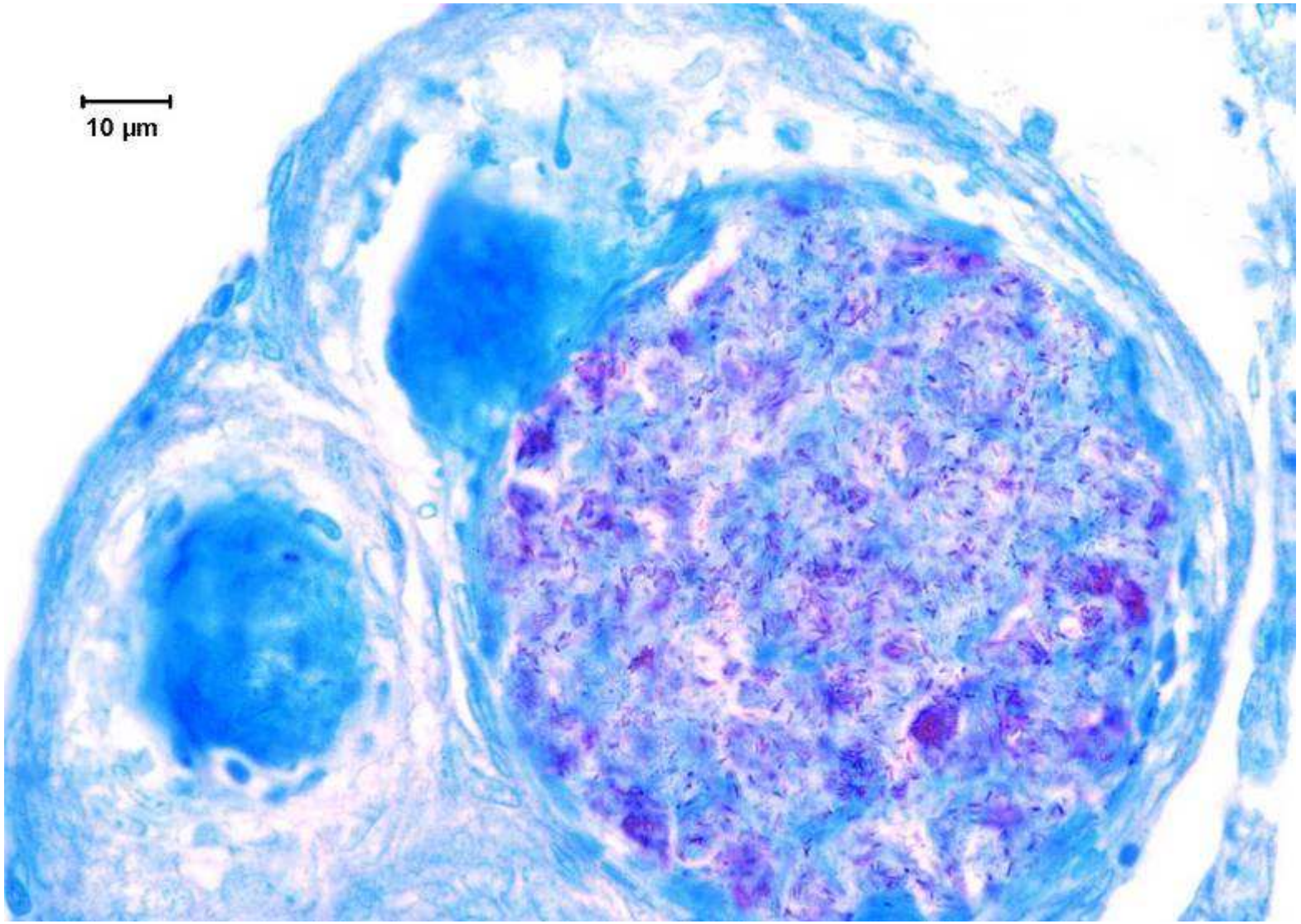

Infected spleen imprint
Acid fast stain

Acid Fast



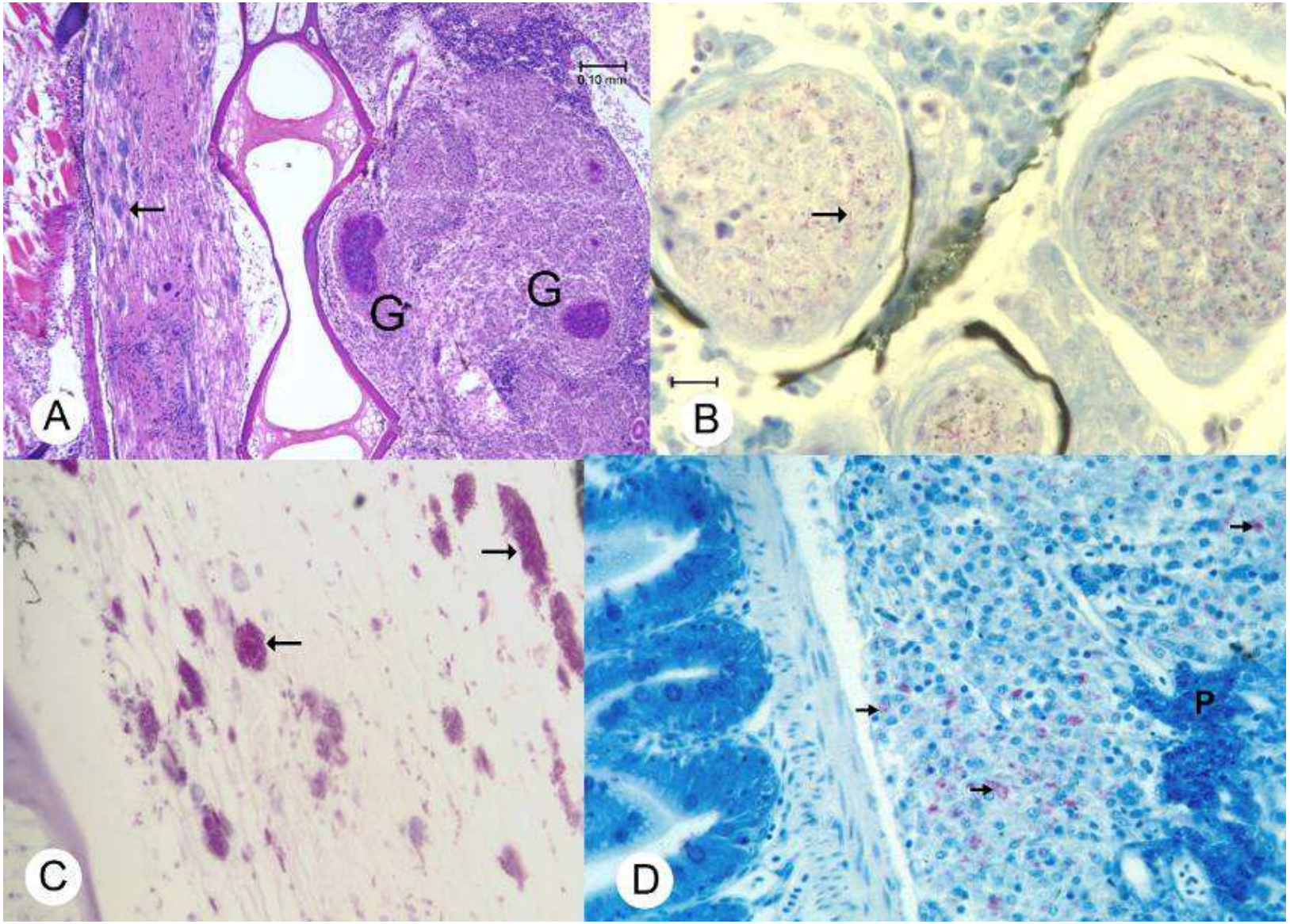
Molecular and PCR

- Directly from tissues (ethanol or frozen)

or

- Culture
- rDNA or hsp genes

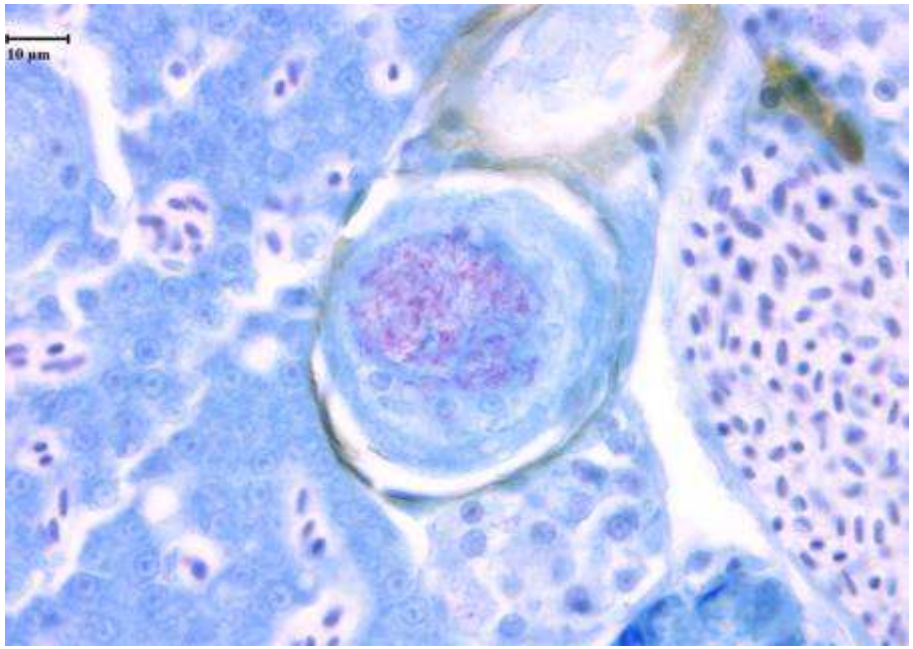
Mycobacterial infections by histology



-Kent, M. 1993

M. chelonae

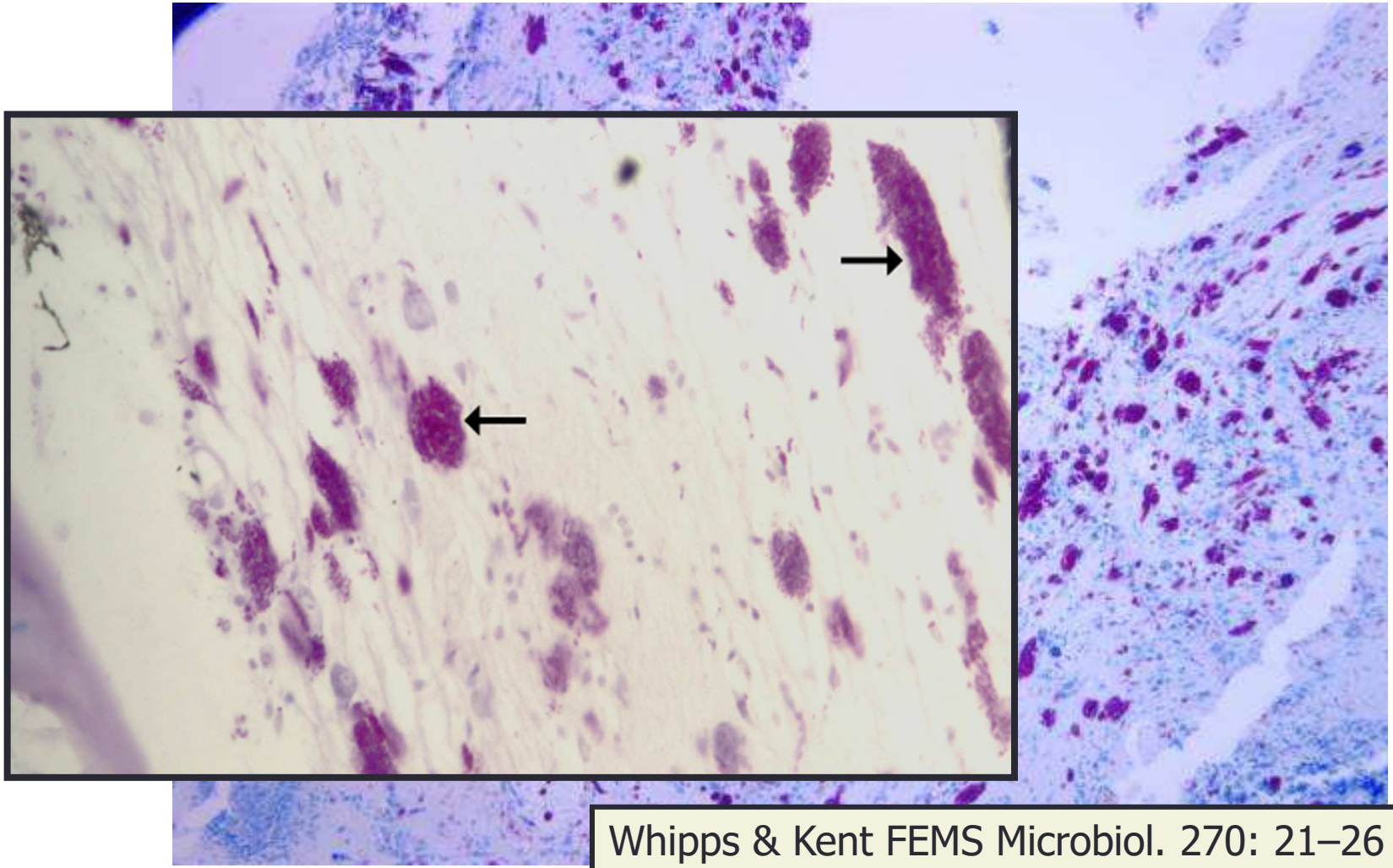
- Common in aquaria
- Most common mycobacterial infection
- Can be high prevalence
- Histology: typical fish myco, lots of granulomas
- Usually low mortality
- Non-protocol induced variation



Mycobacterium haemophilum

- See Whipps et al. 2007. FEMS Microbiol. Lett.
- About 7 outbreaks
- Massive infections, chronic mortalities
- Slow grower with special media needs
- Diagnosis – direct PCR

M. haemophilum neural infection



Whipps & Kent FEMS Microbiol. 270: 21–26

M. marinum

- Common in aquarium fish
- Highly pathogenic to zebrafish
- Zoonotic

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Strategies to Mitigate a *Mycobacterium marinum* Outbreak in a Zebrafish Research Facility

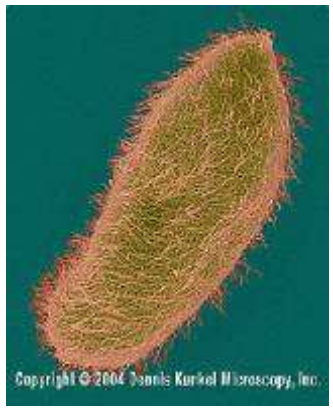
Timothy Mason,[1] Kathy Snell,[1] Erika Mittge,[2] Ellie Melancon,[3] Rebecca Montgomery,[1] Marcie McFadden,[1] Javier Camoriano,[1] Michael L. Kent,[4] Christopher M. Whipps,[5] and Judy Peirce[3]

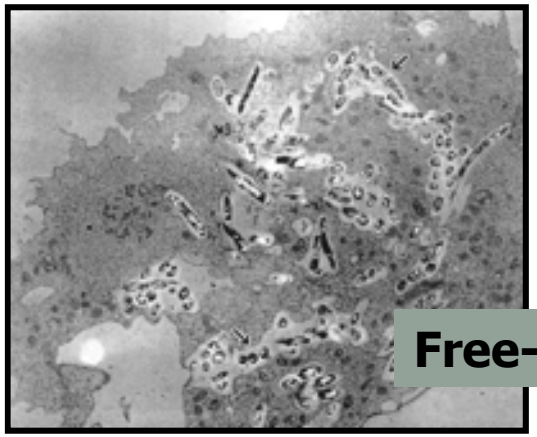
Transmission

- Myco is common in water and biofilms,
- But fish often remain uninfected
- Lab studies – rely on IP injections
- Harriff et al. (2007) J. Fish Dis.
  - Oral route
  - Hard to transmit by water borne exposure
- Biological vectors
- Feeding on infected tissues

Invertebrates common in zebrafish tanks that may be vectors for mycobacteria

Paramecium – used for larvae food





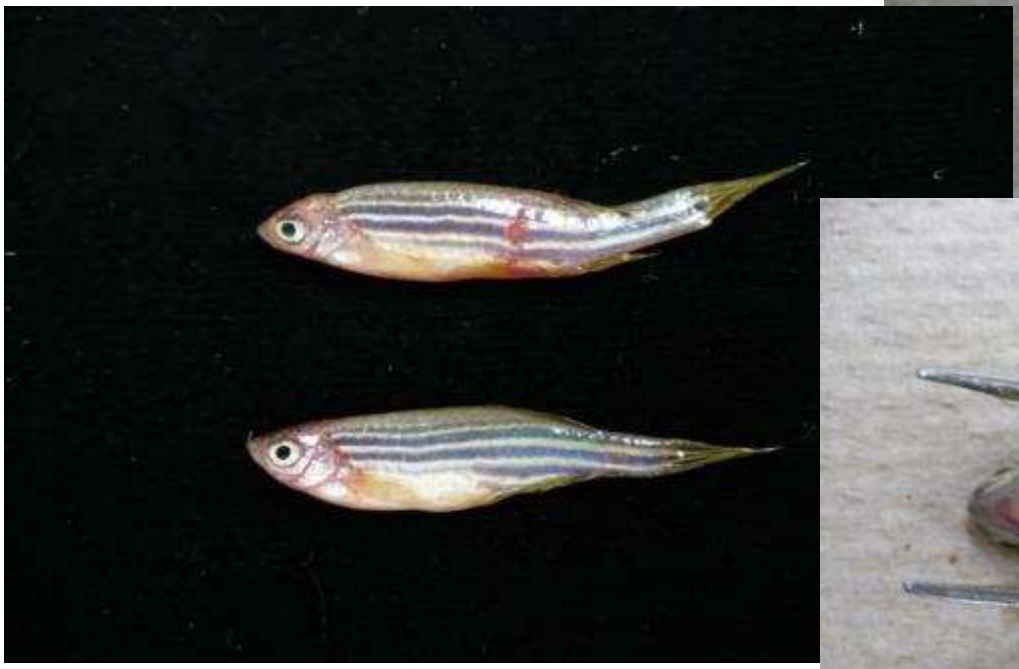
Free-living amoeba with mycobacterium

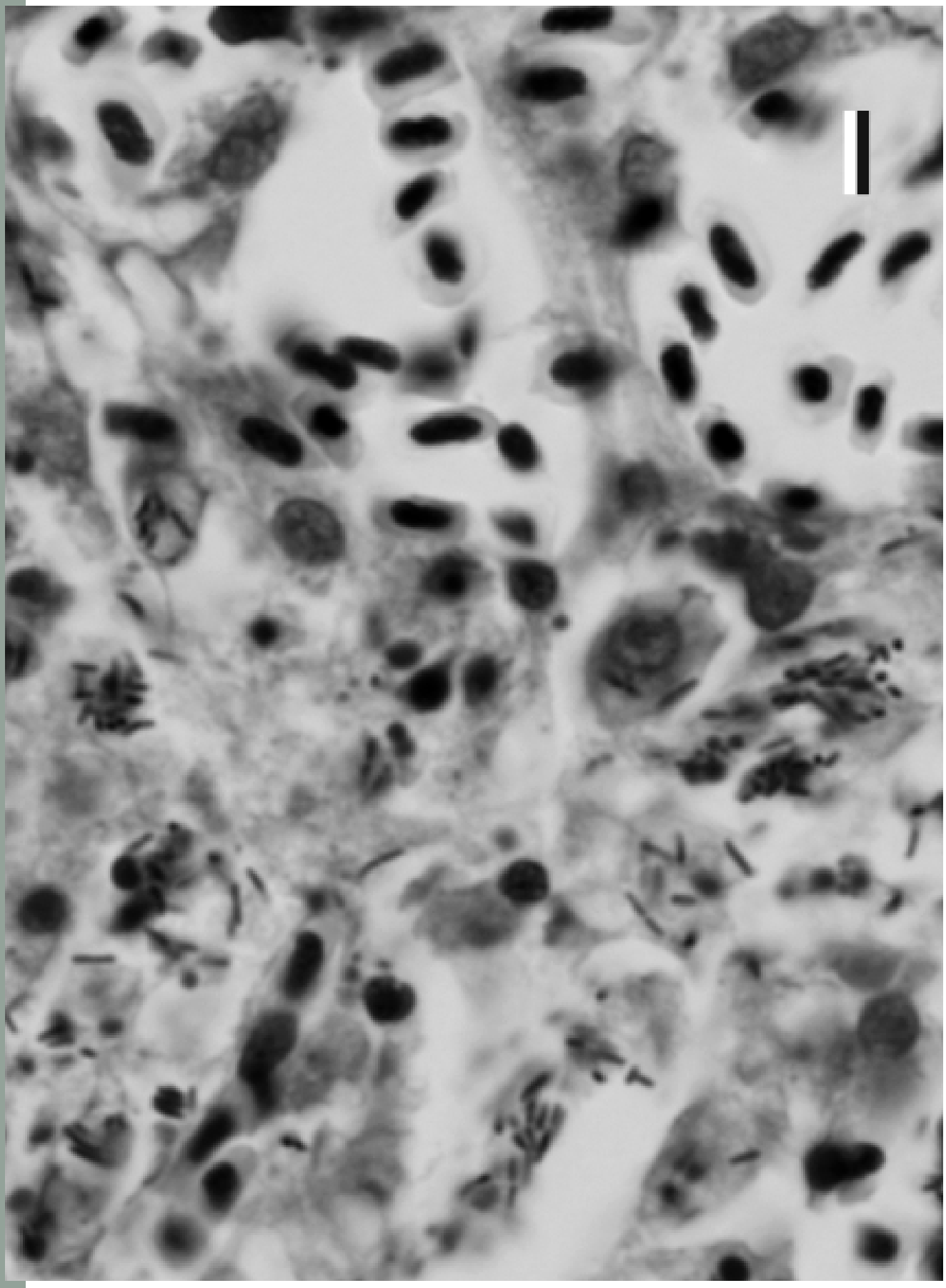
Edwardsiella ictaluri

Collaboration with J. Hawke, LSU

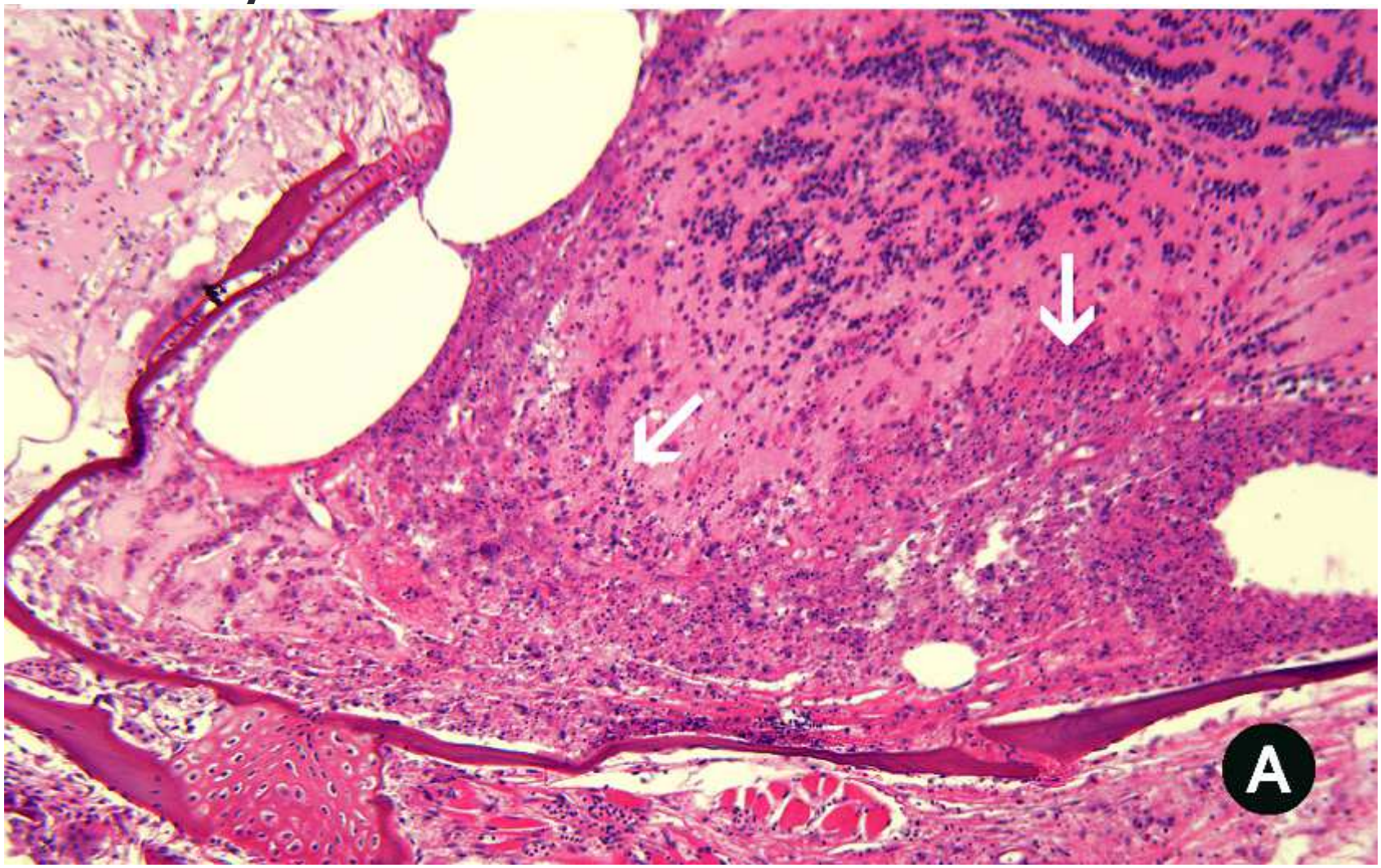
- Serious disease of channel catfish
- More pathogenic than opportunistic aeromonds
- Reports in aquarium fish, including danios
- Research zebrafish
  - 3 cases detected in quarantine
  - 1 outbreak in main facility

Courtesy of J. Hawke, LSU





Infection in Forebrain/ olfactory bulbs

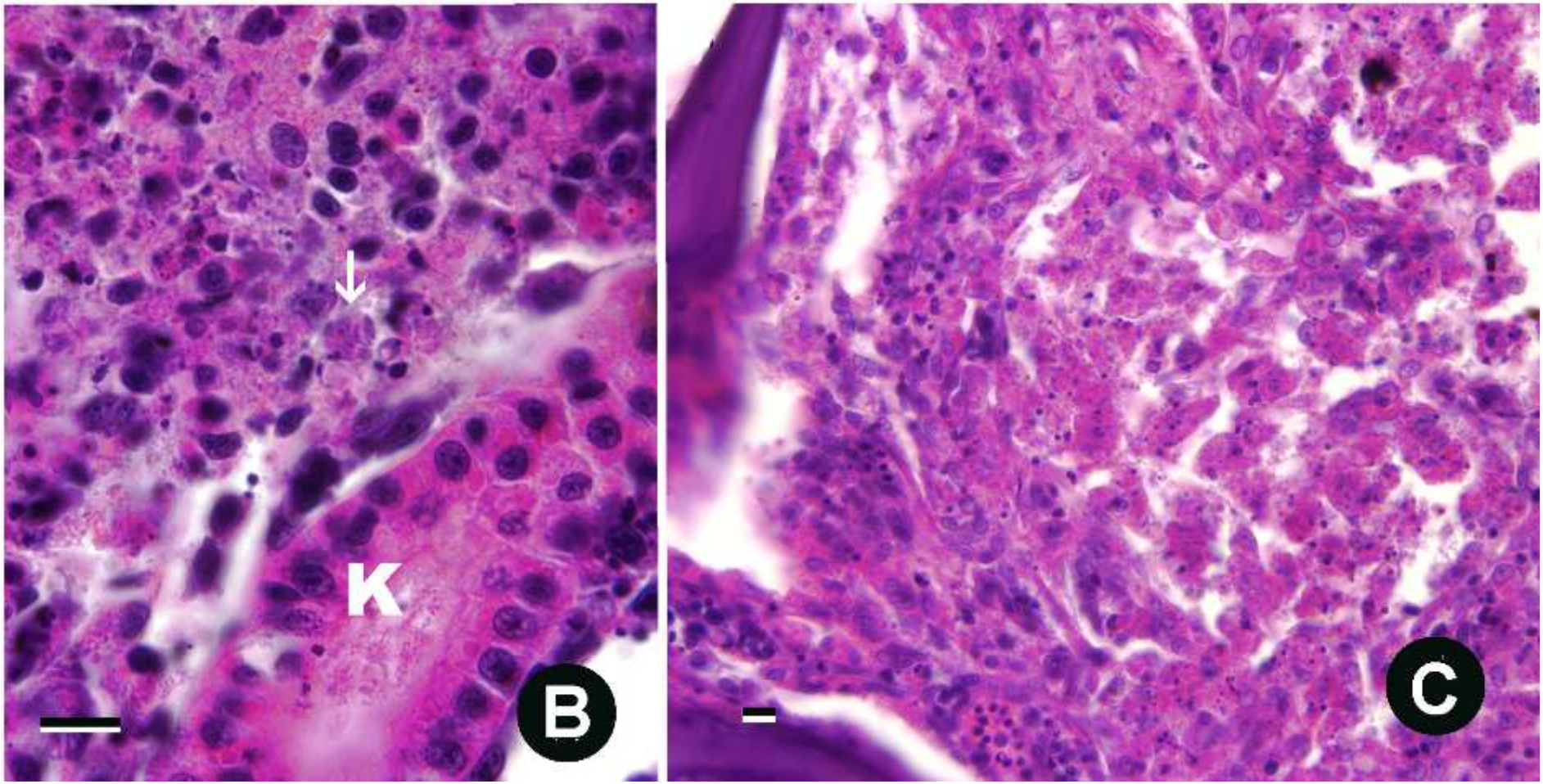


Diagnosis

- Distinctive pathology
  - Bacteria in phagocytes in nares, forebrain, kidney and spleen
  - Culture – blood agar
    - Slow grower, often overgrown by Aeromonds
    - Co-infections with aeromonads do occur
    - In contrast to catfish strains, zebrafish strains are “non-motile”

E. ictaluri

Kidney (B), Nares (C)



In vitro chlorine

- Sytox – dead spores
- FungiFluor Polar Tube Extrusion:
  - Live Spores