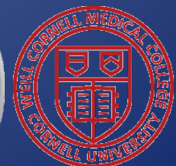


Infectious Diseases of Laboratory Zebrafish



Dr. Christine Lieggi, DVM, DACLAM
Memorial Sloan-Kettering Cancer Center and
Weill Cornell Medical College
5th Annual International Zebrafish Husbandry Course



HOSPITAL
FOR
**SPECIAL
SURGERY**

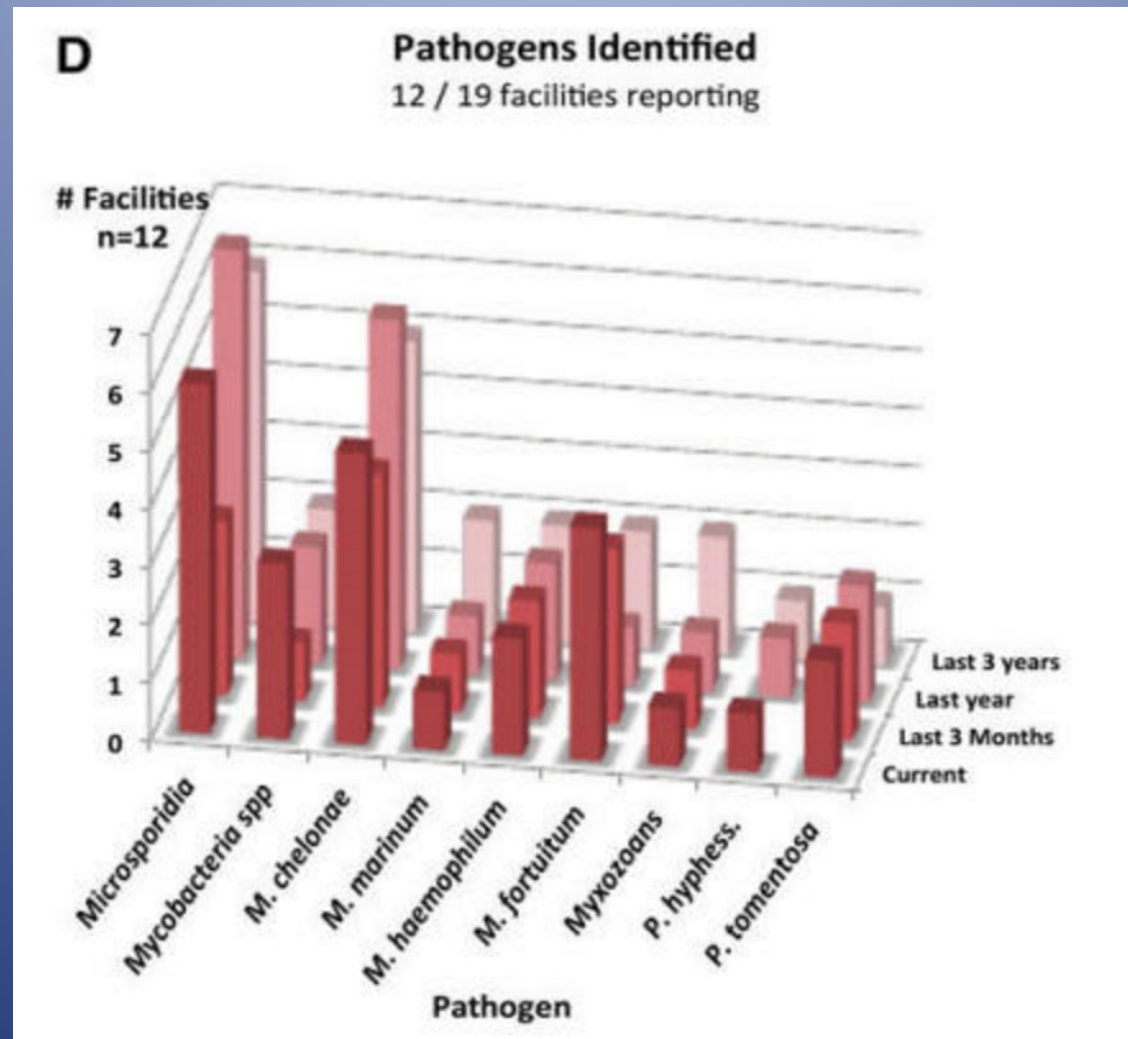
Expression of Disease

- Stress (Poor water quality, handling, overcrowding, tank aggression)
 - More susceptible to infection
 - Earlier onset of disease
 - Increased severity of infection
- Health status
 - Nutrition
 - Other diseases

Expression of Disease

- Experimental Treatments
 - Irradiation
 - Medications
 - Handling
- Genetic background
 - Modulate response to disease
 - Immune status

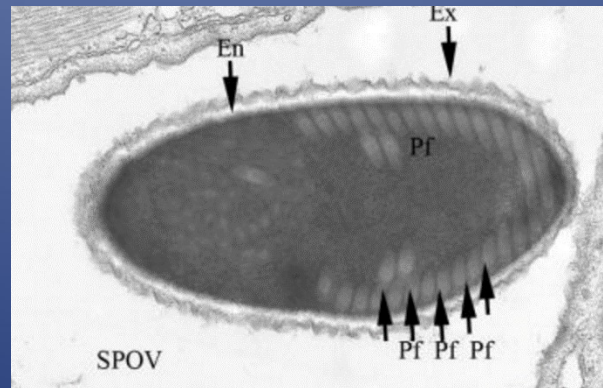
What's Common?



Lawrence, 2016

Microsporidiosis

- Microsporidium
 - Spore forming, eukaryotic, obligate intracellular parasitic fungi
 - Infect insects, crustaceans, fish, and mammals
 - Over 50% of microsporidia genera infect aquatic organisms

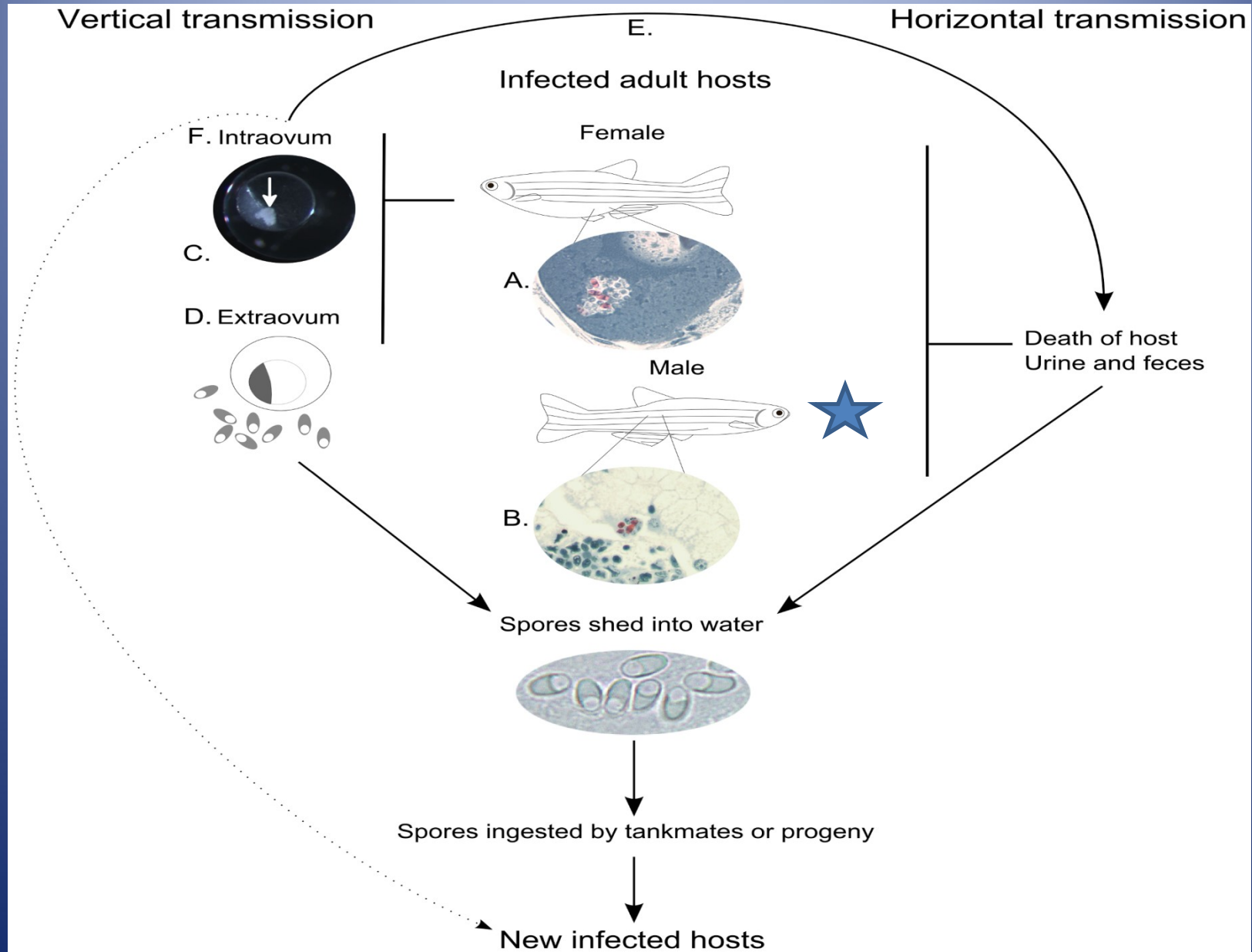


Cali, A. *et al.*, 2012

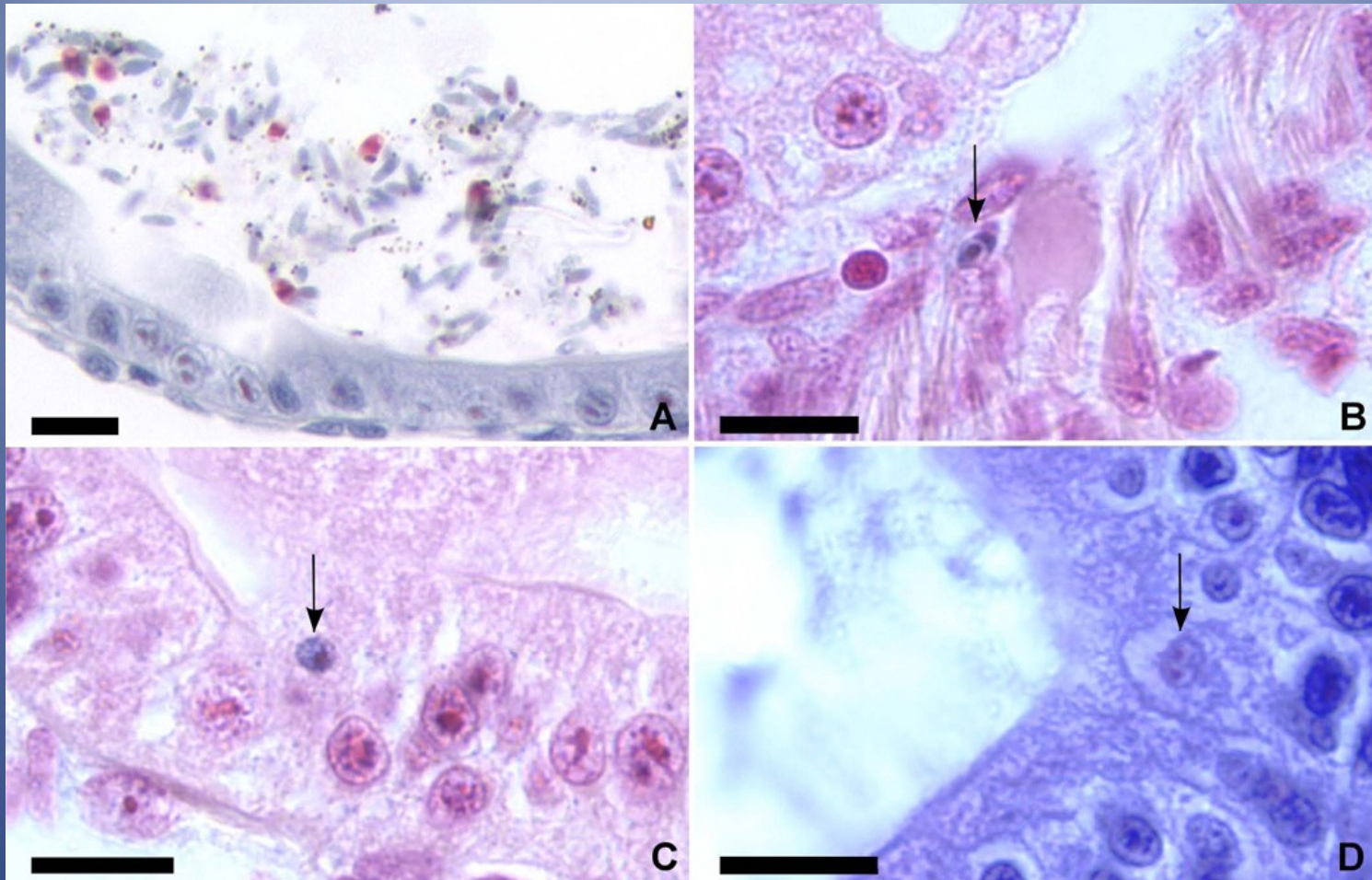


- *Pseudoloma neurophilia*
 - 1st reports from petstore zebrafish (1980s)
 - At least 7 susceptible fish species
- *Pleistophora hyphessobryconis*
 - Neon-tetra disease
 - 1st reported in laboratory zebrafish 2010
 - At least 20 susceptible fish species

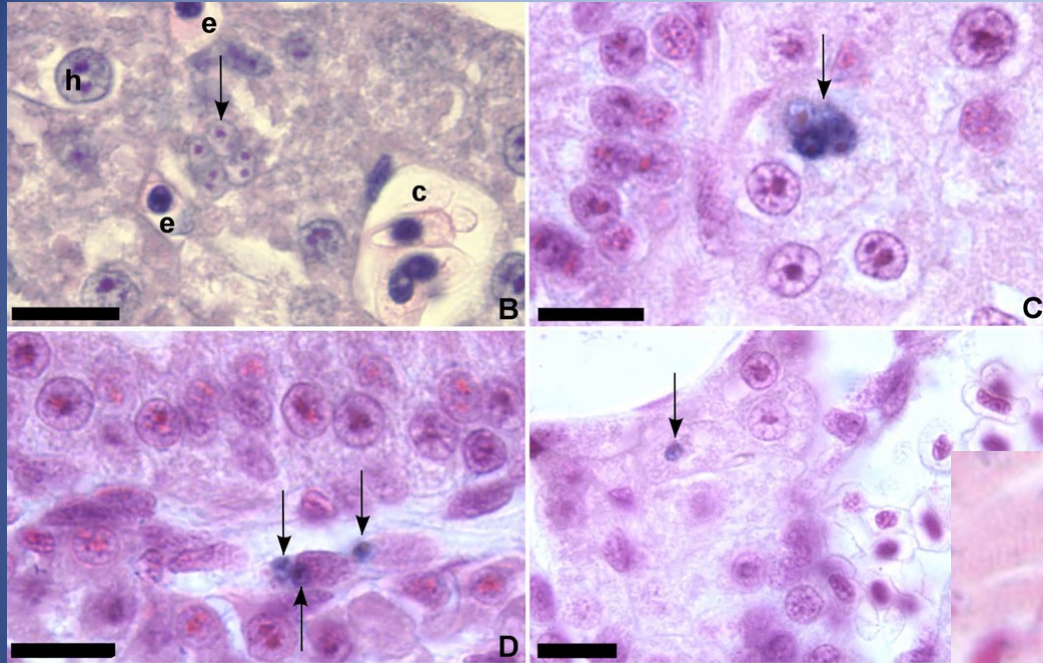
Pseudoloma neurophilia*



From: Verification of Intraovum Transmission of a Microsporidium of Vertebrates: *Pseudoloma neurophilia* Infecting the Zebrafish, *Danio rerio*; Justin L. Sanders, Virginia Watral, Keri Clarkson, Michael L. Kent

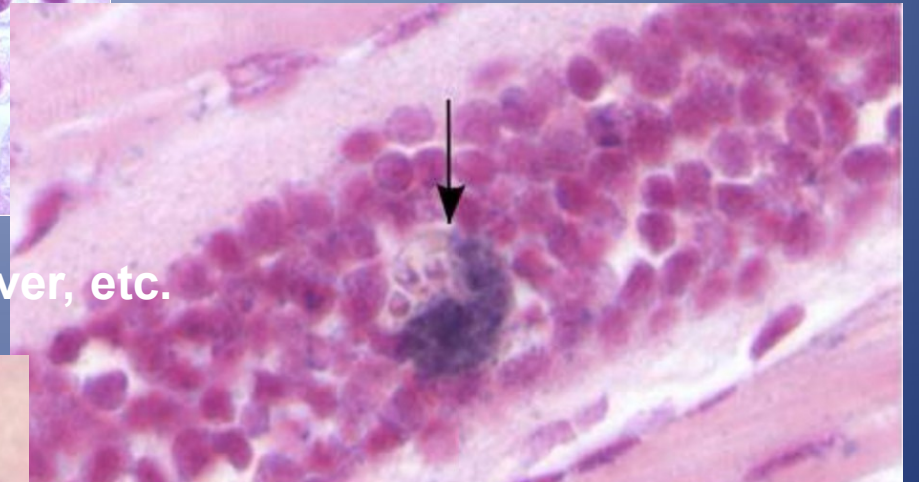


- A. 12 h: Spores in intestinal lumen
- B. 36 h: Proliferative stages in pharyngeal epithelium
- C. 48 h: Single proliferative stage in intestinal epithelium
- D. 72 h: Multiple proliferative stages in intestinal epithelium

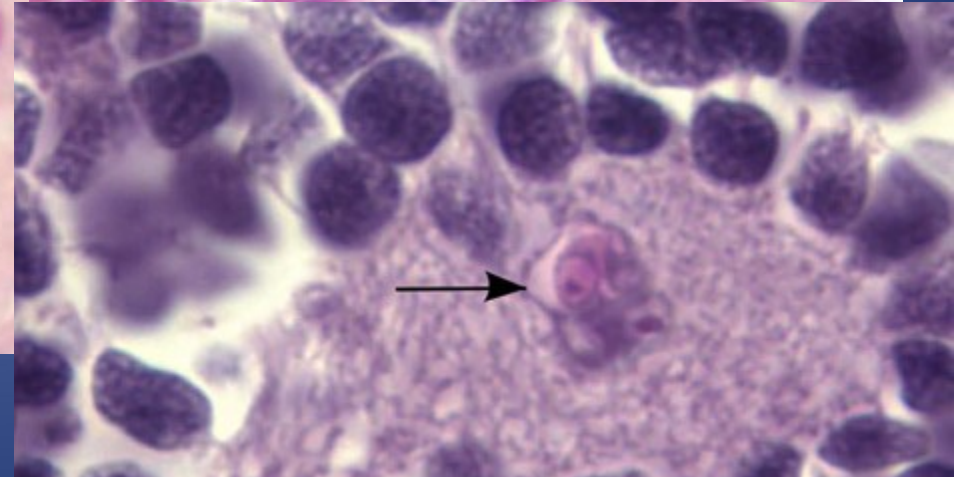


72 Hours

96 Hours: Mature spores in epithelium, liver, etc.

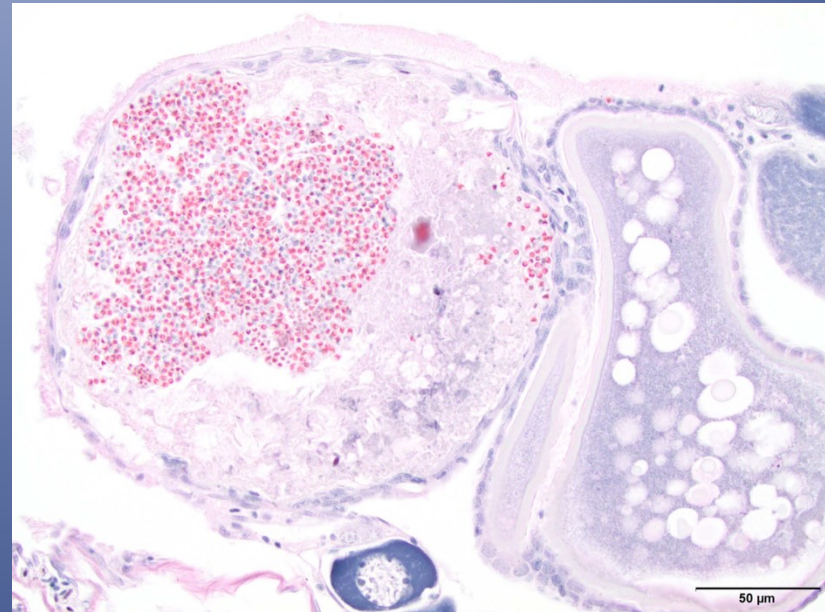


120 Hours



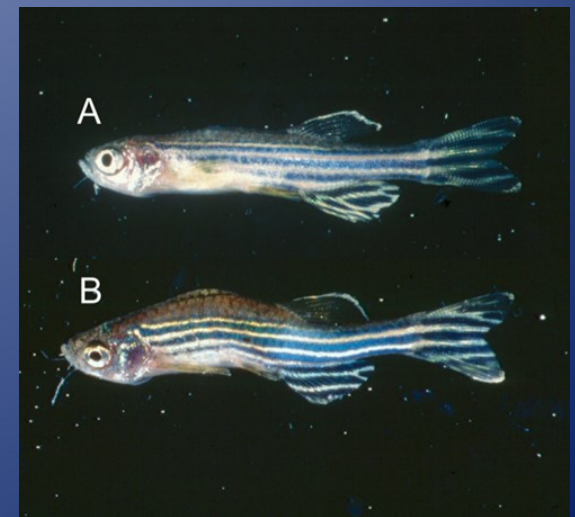
Results of Infection

- Foci of inflammation associated with the spores
 - Ovarian tissue
 - Hindbrain
 - Spinal cord
- Extensive infection
 - Other tissues



Clinical Signs

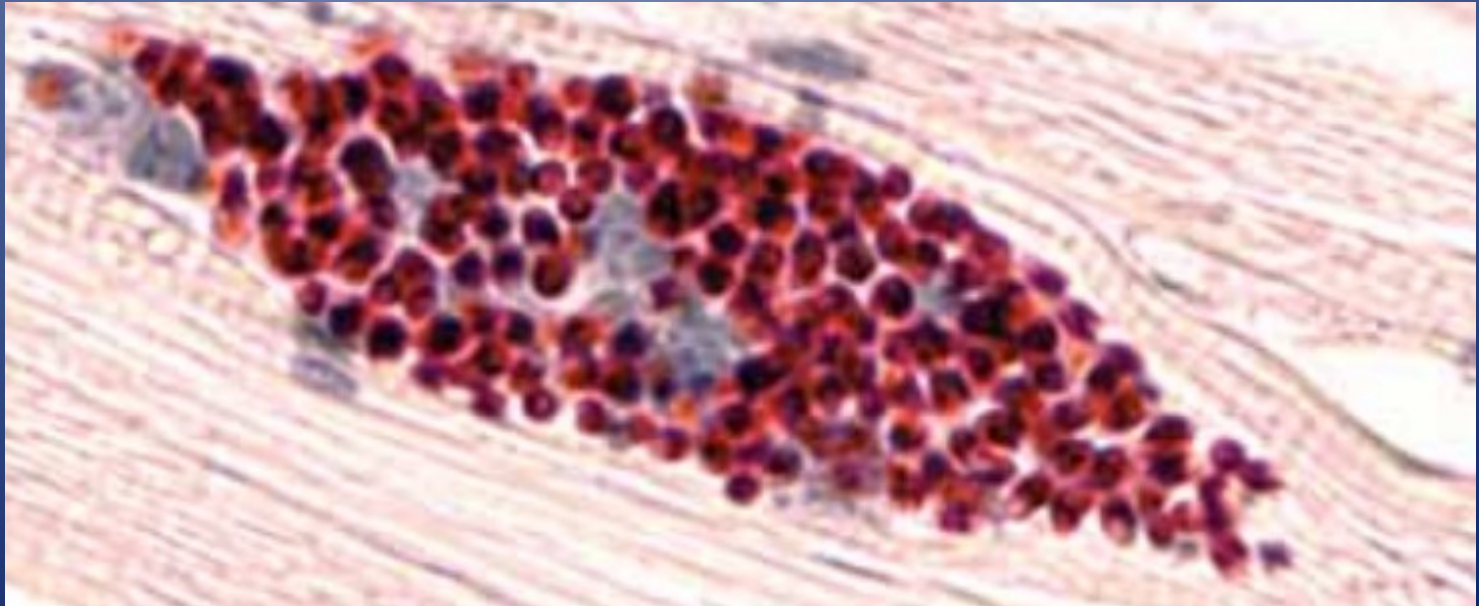
- **None**
 - Variable Emaciation
 - Spinal deformity
 - Death
-
- TL strains more likely to show clinical signs
 - Male more commonly infected



Courtesy of ZIRC

Subclinical Signs

- **Decreased fertility**
- **Behavioral changes**
- Experimental variables



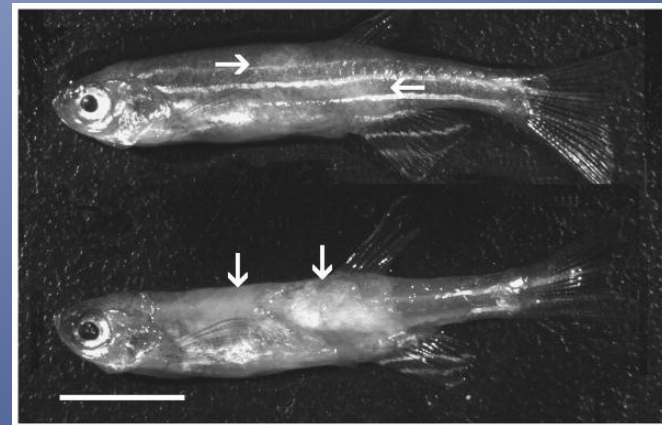
Pleistophora hypnessobryconis

- Primary host is... neon tetra (*Paracheirodon innesi*)
 - Broad host specificity
 - Caution with shared facilities
- Ingestion
 - Cannibalism
 - Exposure to spores
- Vertical?
 - Not yet proven



Results of Infection and Clinical Signs

- Large numbers of spores in skeletal muscle
 - Inflammation from released spores
- Clinical Signs
 - None
 - Sluggish, bloated
 - Spinal curvatures
 - Multifocal to coalescing white-grey, slightly raised regions
 - Morbidity/Mortality



Sanders, JL, 2010

Treatment of Microsporidian Infections

None!!!

Management Strategies

- Quarantine system with embryo sanitization
 - Not 100% effective
 - Doesn't capture vertical transmission
- Remove sick and old fish
- Practice safe spawning
 - Screen brood fish (replace +)
 - Embryo disinfection for brood stock
 - Dump spawn water down drain
- Minimize stress

Management Strategies

- One net/tank
- Effective UV sanitization
 - 45-50,000 $\mu\text{Wsec}/\text{cm}^2$
- Disinfect equipment
 - 100 ppm bleach x 10 min kills 99% spores
 - Wescodyne Soak (75 ppm) x 10 min
 - Wescodyne Spray (175 ppm) x 10 min

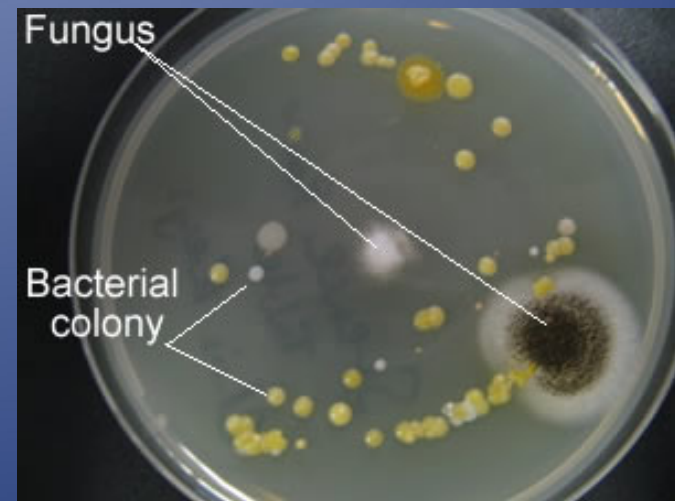


Bacteria

Opportunistic: Cause disease in compromised host

Pathogenic: Cause disease in healthy individuals

Many are zoonotic!



Opportunistic Bacteria

- Normal bacteria species that may cause disease
 - *Aeromonas hydrophila*
 - *Staph spp.*
- Typically secondary to water quality problems
- Stress
 - Stocking density
 - Handling
 - Shipping



Clinical signs that may be associated
with bacterial infections



Other Bacteria

- Pathogenicity may be dose dependent
- *Flavobacterium columnare*
- *Vibrio spp.*
- *Staphylococcus spp.*



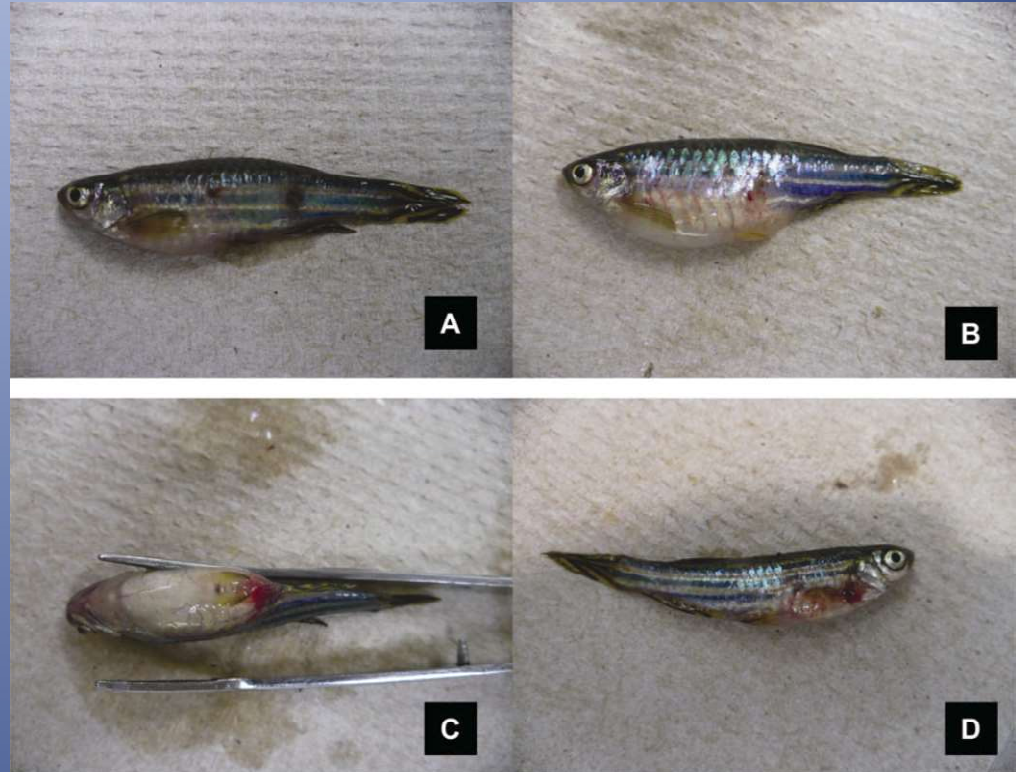
Picture courtesy of J. Cockington

Edwardsiella ictaluri

- Primary, obligate pathogen
- Causative agent of Enteric Septicemia of Catfish (ESC)
- Transmitted from fish to fish by close contact, via the water, by fecal shedding, or orally

Edwardsiella ictaluri

- Acute mortality over 1-2 weeks
 - 280 deaths within 10 days
- First signs within 24-hours of arrival possible
- Treatment
 - Not proven curative



HAWKE ET AL. 2014

Quarantine!

General Treatment Recommendations

- Experimental history?
- Check water quality
 - Best growth < 7.2; alkalinity???
- Embryo treatment
 - 25-50 ppm bleach for most gram (-)



Picture courtesy of J. Cockington

Treat or Cull?

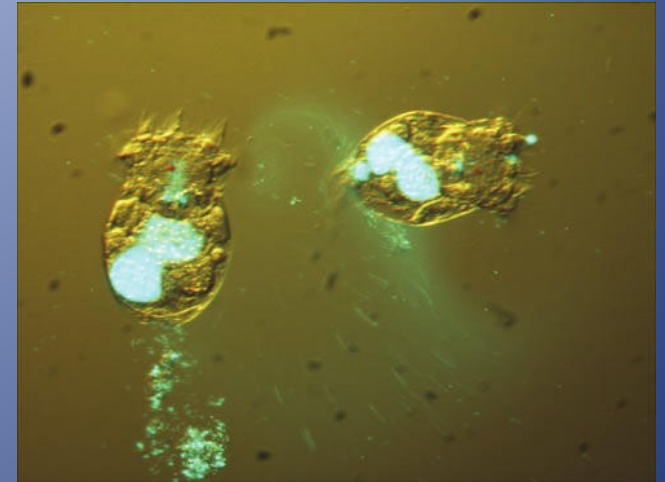
- Case by case basis
 - Depopulate
- Antibiotic treatment generally not recommended *
 - Culture/sensitivity
 - Possible adverse effects of medications

Treatment Considerations

- Treat off-system or flow- through
 - Consider impact on biofilter
- Before treatment consider.....
- Pilot studies
- Potential impact on research
- Potential impact on reproduction

Mycobacteriosis

- Primarily through intestinal tract
 - Shedding from infected fish
 - Fish tissue, debris, food
- Live feed reservoirs
- Found in biofilms
- Contaminated equipment/personnel



Paramecium caudatum* enhances transmission and infectivity of *Mycobacterium marinum* and *M. chelonae* in zebrafish *Danio rerio

Tracy S. Peterson^{1,*}, Jayde A. Ferguson², Virginia G. Watral¹, K. Nadine Mutoji³, Don G. Ennis⁴, Michael L. Kent^{1,5}

Does Species Matter?

TU may be more susceptible

- Variable Pathogenicity
 - *M. haemophilum* and *M. marinum*
 - *M. fortuitum*
 - *M. abscessus*, *M. chelonae*, *M. saopaulense*, *M. peregrinum*



Clinical Signs

- None (subclinical)
- Myositis, ulcerations
- Granulomatous lesions
 - EAI
- Exophthalmia
- Anorexia, lethargy
- Dropsy/Edema
- Death



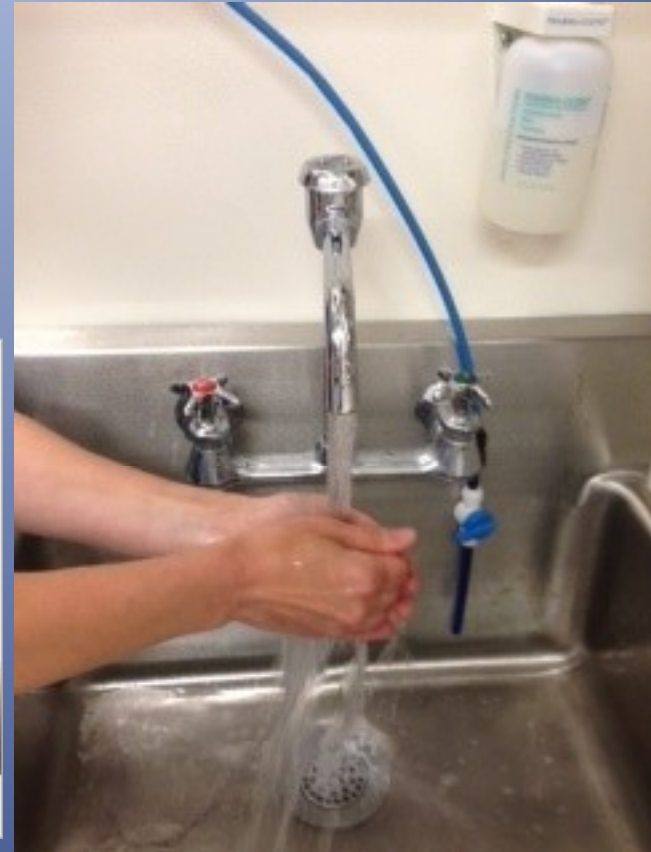
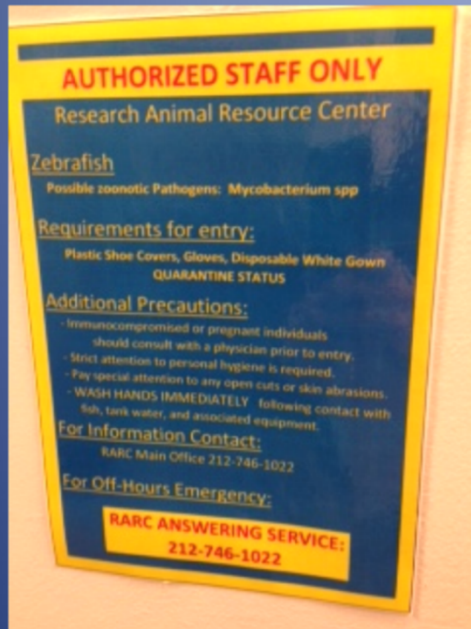
Prevention

- Ubiquitous in the aquatic environment
- Exclusion of all species likely cost prohibitive
- Surface sanitization of embryos
 - Variable pathogen susceptibility
 - 30 ppm x 10 min for *M. marinum*
 - No *M. marinum* in 6 dpf larvae
- Disinfect facility surfaces and hands
 - 70% ethanol or other validated method

Prevention

- One net/tank
- Enforce workflow order
 - Food first
 - Nursery
 - Main colony
 - Quarantine
- Staff education and appropriate PPE

Mycobacteriosis and Human Safety



Unknown source



Babamahmoodi et al.

Human Treatment

- *M. marinum*
 - Non-healing wound and exposure to aquaria
- 3 months- 2 years of antibiotics
 - Multi-drug resistance
 - Combination therapy common
 - Hepatotoxicity
- Surgical drainage

Control of Infection

- *Remove sick and dead fish*
- *Follow prevention procedures*
- Cull elderly fish
 - Keep them lower
- Track sick/dead fish
 - System/Rack/Room
 - Lab/ User specific
 - Stock specific
- Conduct environmental testing
 - Where is it coming from and/or how being spread?

Treatment

- Pharmaceutical tx not recommended
 - Development of resistance
 - No definitive elimination of infection
- Implement Control Procedures
- *Depopulate and Disinfect*

Control of Infection

TABLE 3. STRATEGIES TO MITIGATE ISSUES FROM A MYCOBACTERIAL OUTBREAK

<i>Strategy</i>	<i>Rationale</i>	<i>Practical effect</i>
Provide personnel training on mycobacteria	<i>Guide for the Care and Use of Laboratory Animals</i> , 2011	Reduces risk of zoonotic infection; Reduces pathogen vectors
Wear personal protective equipment (gloves)	<i>Guide for the Care and Use of Laboratory Animals</i> , 2011	Reduces risk of zoonotic infection
Use 70% ethanol to disinfect facility surfaces and hands	Mainous 2005	Eliminates bacteria on facility surfaces and hands
Use embryo surface disinfection	Our experimental results	Reduces bacterial counts on embryo chorion
Track diseased fish with tank labels	<i>Guide for the Care and Use of Laboratory Animals</i> , 2011, and observations at UO	Provides surveillance data
Perform environmental sampling	Adapted from rodent health monitoring. Pritchett-Corning 2014.	Provides surveillance data
Plan and direct personnel movements	<i>Guide for the Care and Use of Laboratory Animals</i> , 2011	Reduces pathogen spread through personnel movements
Remove elderly fish	Keller 2004 and Sasaki 2013	Removes potential disease carriers
Remove dead and moribund fish	Kent 2009	Removes potential disease carriers
Place young fish highest on housing racks	“Because water is an excellent vehicle for pathogens.” Kent 2009	Reduces risk of pathogen spread through water spill
Dedicate wild-type fish for outcrosses	Noga 2010 and Murray 2012	Reduces pathogen spread through shared fish for outcrosses
Remove spawn water and water from tank changes from RAS	Adapted from Murray 2012	Eliminates potentially pathogenic bacteria from RAS
Change tanks every 3 weeks	Observations in our facility	Reduces biofilm and algae
Evaluate and validate sanitation	<i>Guide for the Care and Use of Laboratory Animals</i> , 2011	Reduces pathogen spread through soiled equipment

Parasites



Nematodiasis: *Pseudocapillaria tomentosa*

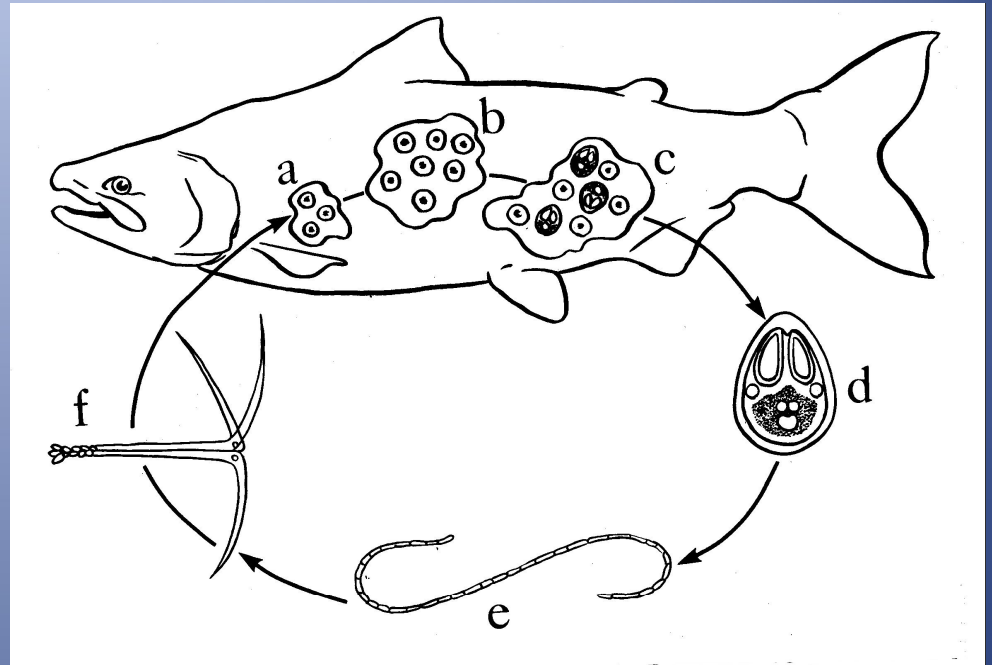
- Capillarid nematode
- Thin, transparent worms in lumen of the intestine
- Import of infected fish



David Maley, et al.

Transmission

- Direct Transmission
 - Co-habitation/spawning with infected fish
 - Ingestion of eggs
 - Clinical signs within 2 weeks
- Indirect Transmission
 - Ingestion of infected hosts
 - oligochaetes



Clinical Signs

- None
- “Bulge” in posterior abdomen
- Darker in color
- Lethargy
- Death

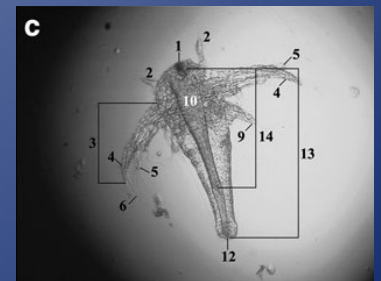
Seyed-Mohammadreza Samaee. Zebrafish. 2015 June



Murray and Peterson, JAVMA 2015

Treatment Options

- Fenbendazole soaked *artemia* +/-algae
 - Daily or twice daily treatment x 3 d, **repeated in 2 weeks**
 - Effective on nematode adults only
- Mebendazole or Praziquantel soaked *artemia* + algae
 - Twice daily treatment x 3 days
 - Eliminates parasite eggs + adults
 - Anorexia & lethargy x 3 days in sick with Praz
 - Anorexia x 3 days/lethargy

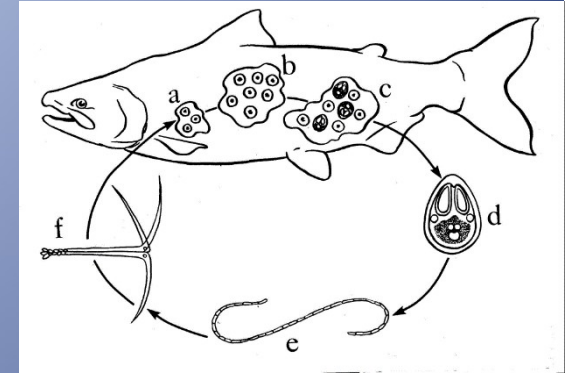


Treatment Options

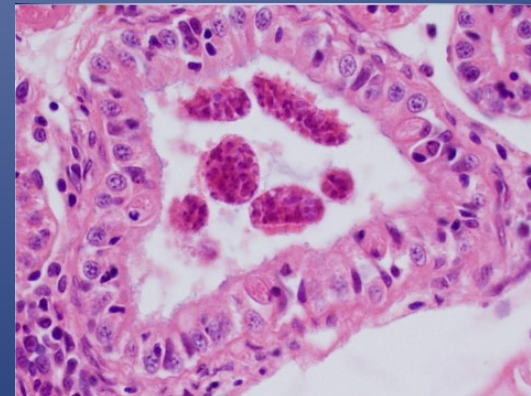
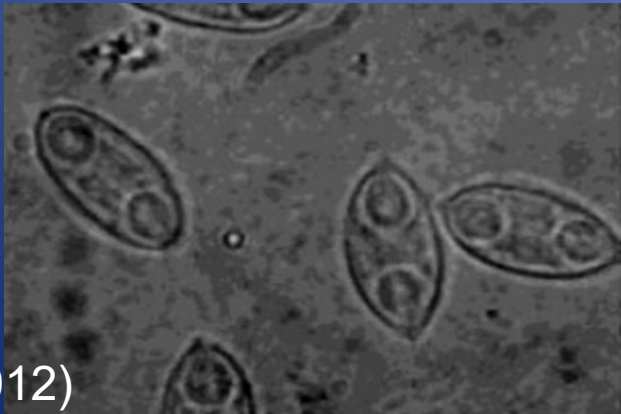
- Ivermectin
 - Soaked *artemia + algae*
 - Twice daily x 1 d
 - Eliminates infection eggs + adults
 - Coated pelleted feed*
 - Twice daily, twice/week x 4 weeks
 - Safety concerns- narrow margin of safety
- See earlier slide re: treatment considerations

Myxidium streisingeri

- Coelozoic parasite
 - Typically required intermediate host
 - Life cycle currently unknown
- No associated clinical signs
 - Mesonephric ducts and the lumen of the kidney tubules
 - No appreciable histopathologic changes



Courtesy of ZIRC



Protozoan Parasites

- Many species may infect zebrafish
 - Pet stores and ornamental fish

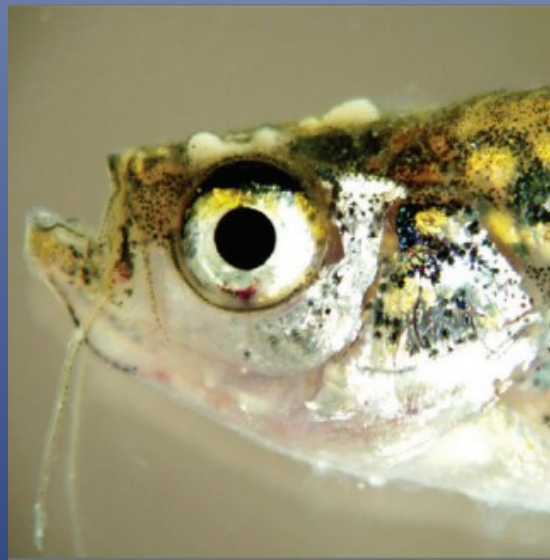
Piscinoodinium pillulare

“velvet disease”

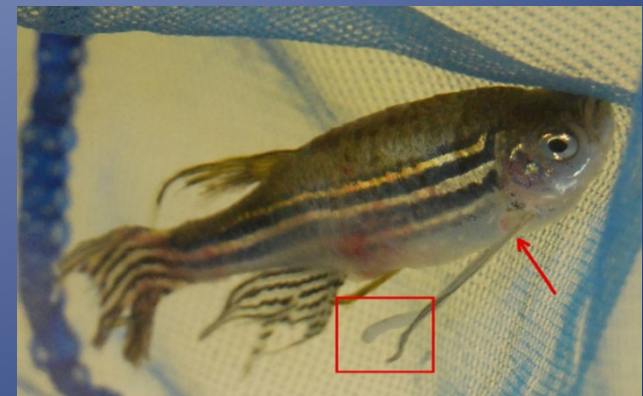


<http://goldfish2care4.com/goldfish-diseases/velvet.html>

Ichthyophthirius



IDEXX



https://c1.staticflickr.com/5/4043/4324457486_558b2384ac_b.jpg

Treatment

- Check water quality!
- Temperature Increase
 - Standard housing temps= 4-day life cycle
 - 30° can kill parasite (decreased O₂)
- Salt bath: 2-3ppm
- Formalin bath, Malachite Green, Copper sulfite
- Treatment considerations...

Parasite Prevention

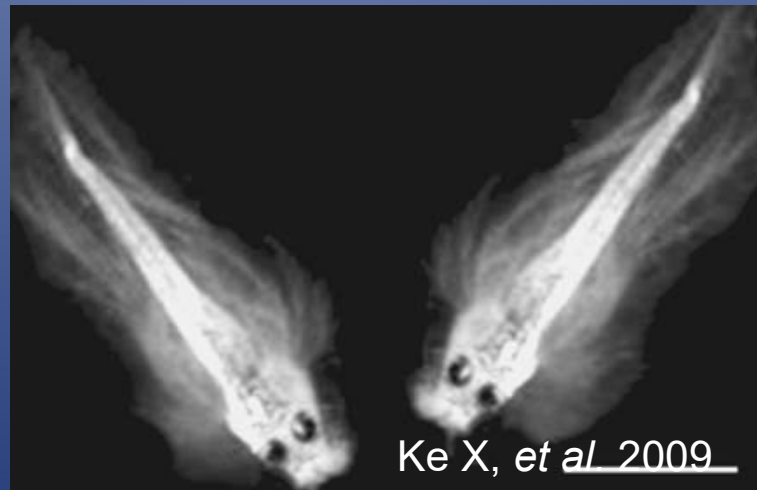
- Effectiveness of egg surface sanitization is not known for all parasites
 - 3000 ppm chlorine for *P. tomentosa*
 - Ensure adequate agitation
- Treat incoming fish of unknown health status
- **Be careful with your “import” water!**
- Adequate disinfection of equipment
 - 50 or 60 °C for 30 min or 1 h or *P. tomentosa*
- Maintain good water quality

Fungal Infections

- Opportunistic pathogens
 - *Saprolegnia brachydanis*
 - *Exophiala sp.*
- Direct transmission
 - Fungus on inanimate objects or other fish contacts damaged skin



Courtesy of J. Cockington



Treatment

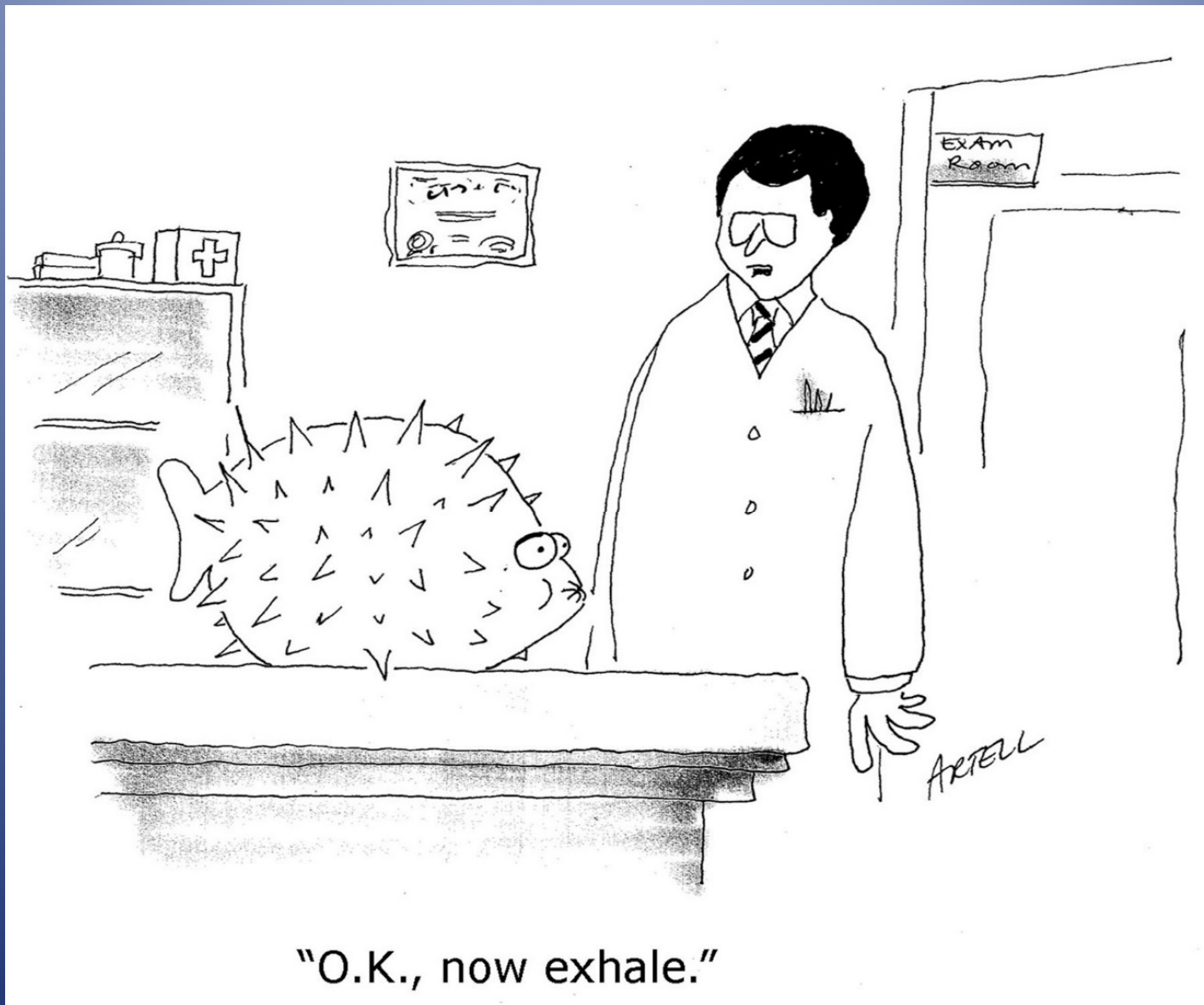
- Check water quality and minimize stress
- Check for “hiding” debris
 - Adequate sanitization essential
- Rinse/sanitize embryos
- Salt bath
- Methylene blue

Viral Infections

- Endogenous retroviruses described
- One report of infection by Red-spotted grouper nervous necrosis virus
- No naturally transmissible viruses that cause disease have been identified at this time*
- Susceptible to other viruses
 - Spring Viremia of Carp
 - IPNV
 - ISKNV
 - IHNV

Spring Viremia of Carp Virus

- Rhabdovirus
- Hemorrhagic viremia in cyprinids
 - *Cyprinus carpio* primary host
- Wild or commercial fish
- $< 17^{\circ}\text{C}$
- Intraceoelomic injection can result in infection in zebrafish
- No natural infections known



"O.K., now exhale."

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