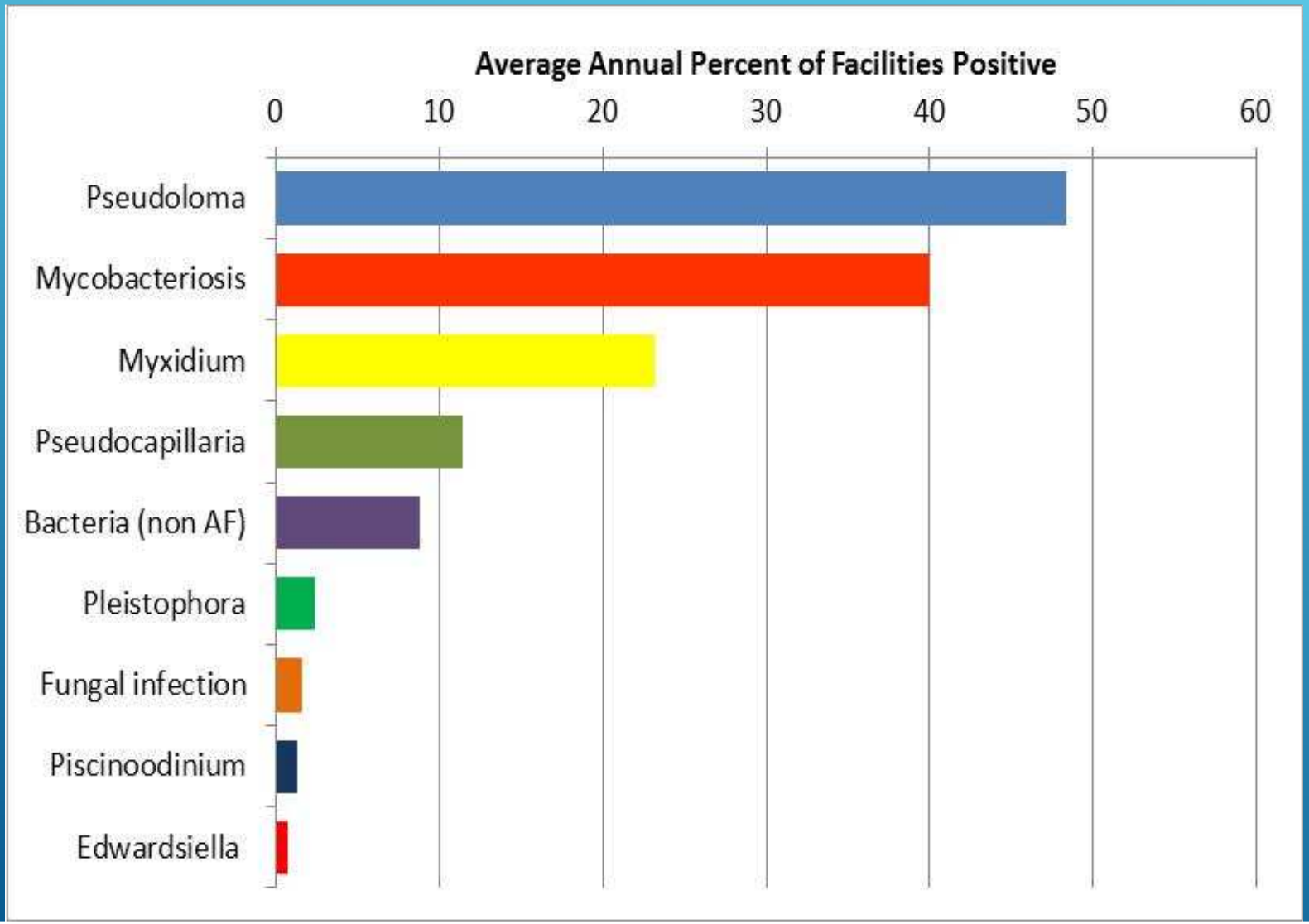
ZEBRAFISH CHRONIC DISEASES AND RESEARCH OUTCOMES

Justin L. Sanders

Department of Biomedical Sciences

7th Annual International Zebrafish Husbandry Course

PATHOGENS IN ZEBRAFISH FACILITIES 2006-2015: – CA 10,000 FISH, 100 LABORATORIES



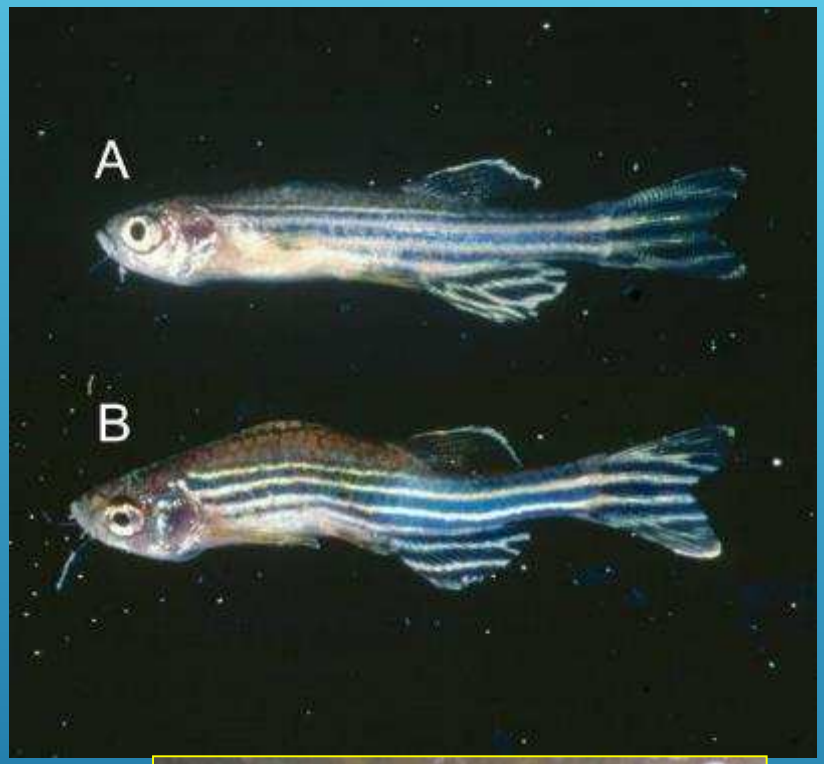
NEGATIVE IMPACTS OF CHRONIC INFECTIONS IN ZEBRAFISH

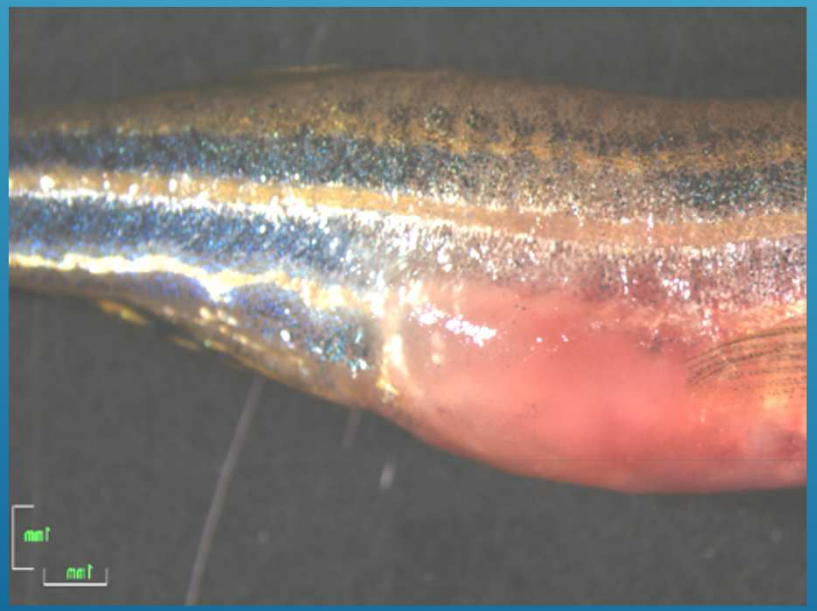
- Wide range of external indicators of infection

- Husbandry:
  - Reduced fecundity
  - Increased mortality
- Experimental
  - Non-protocol induced variation

Kent ML, Harper C, Wolf JC (2012) Documented and potential research impacts of subclinical diseases in zebrafish. ILAR J 53: 126–134.

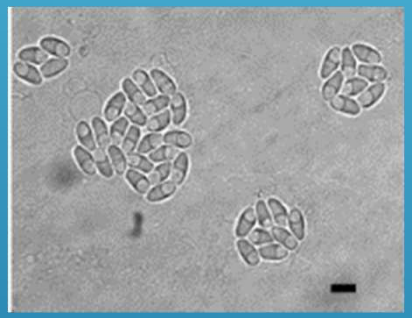
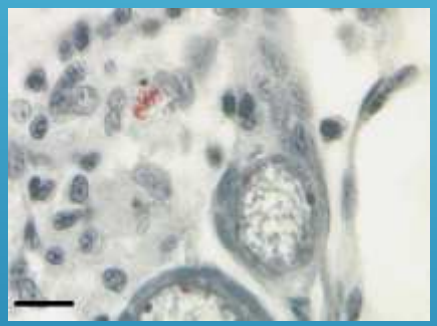
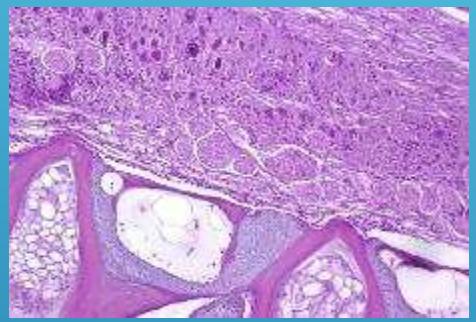
CLINICAL SIGNS OF DISEASE



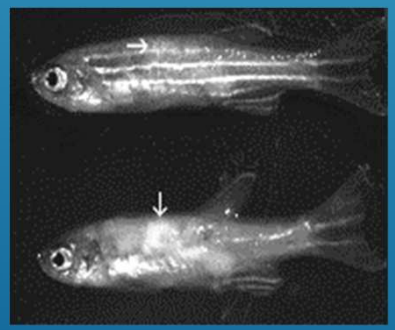
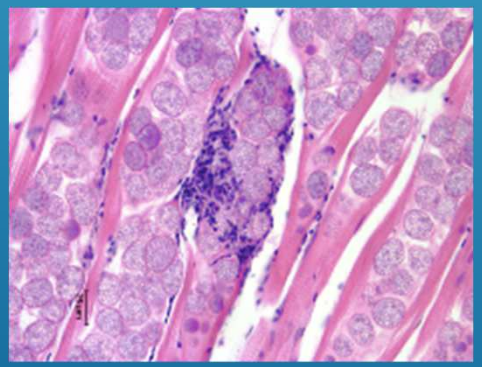


COMMON MICROSPORIDIAN PARASITES OF ZEBRAFISH

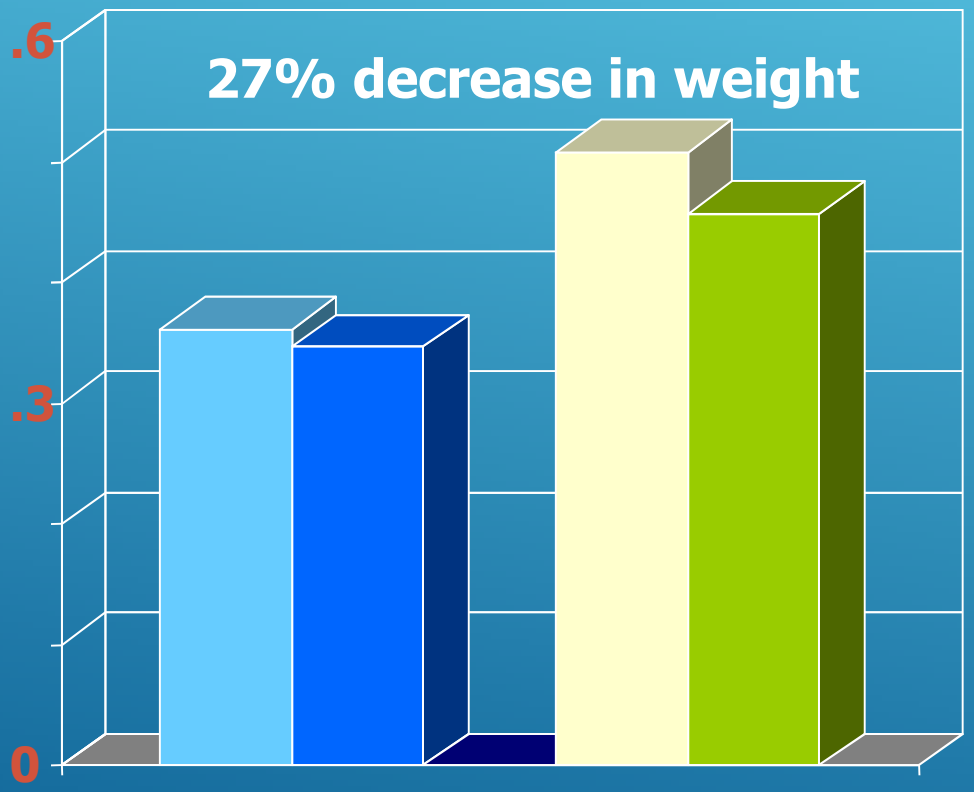
- Microsporidia
  - *Pseudoloma neurophilia*

  - *Pleistophora hyphessobryconis*

IMPACT ON GROWTH: WEIGHT

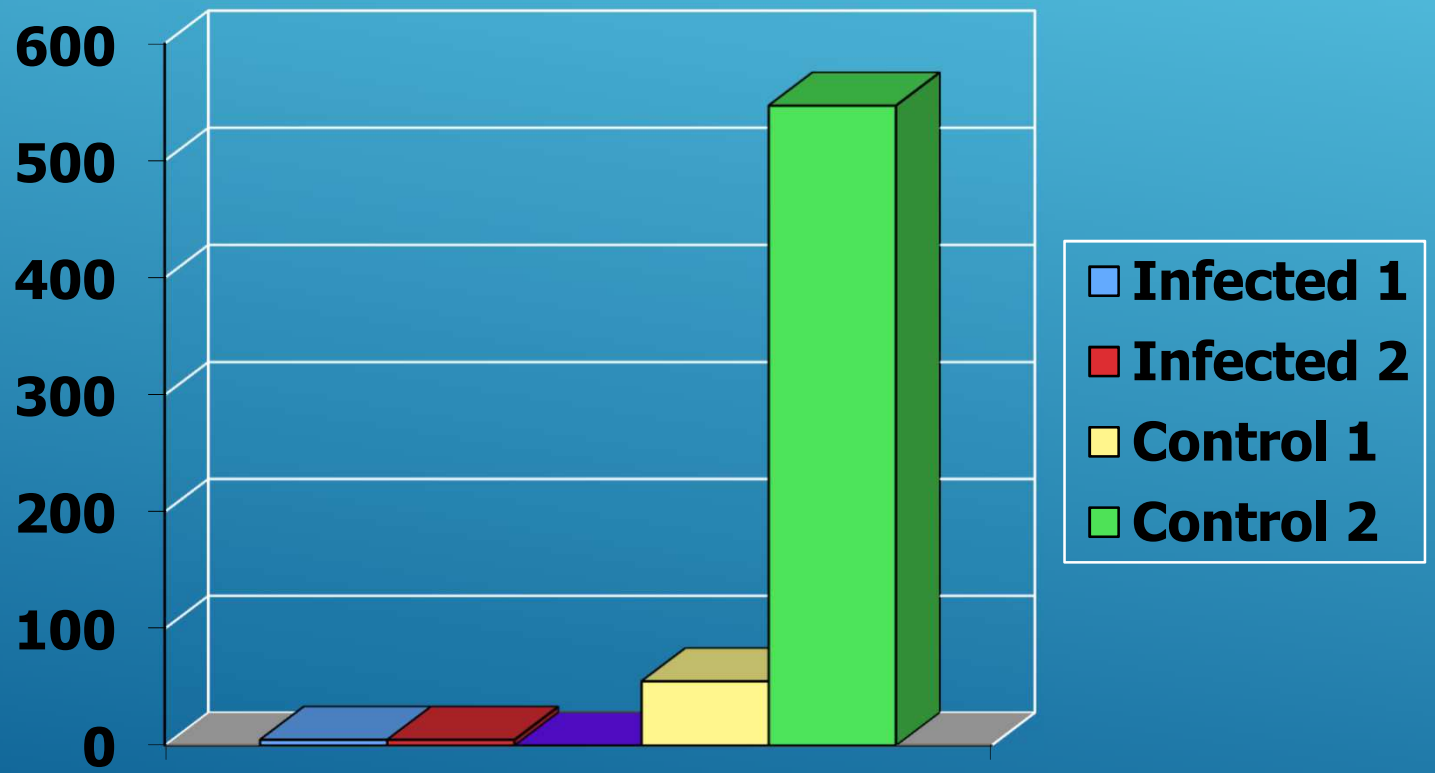


Infected 1
Infected 2
Control 1
Control 2

Ramsay, J. M., V. Watral, C. B. Schreck, and M. L. Kent. 2009. *Pseudoloma neurophilia* infections in zebrafish *Danio rerio*: effects of stress on survival, growth, and reproduction. Diseases of Aquatic Organisms 88: 69–84.

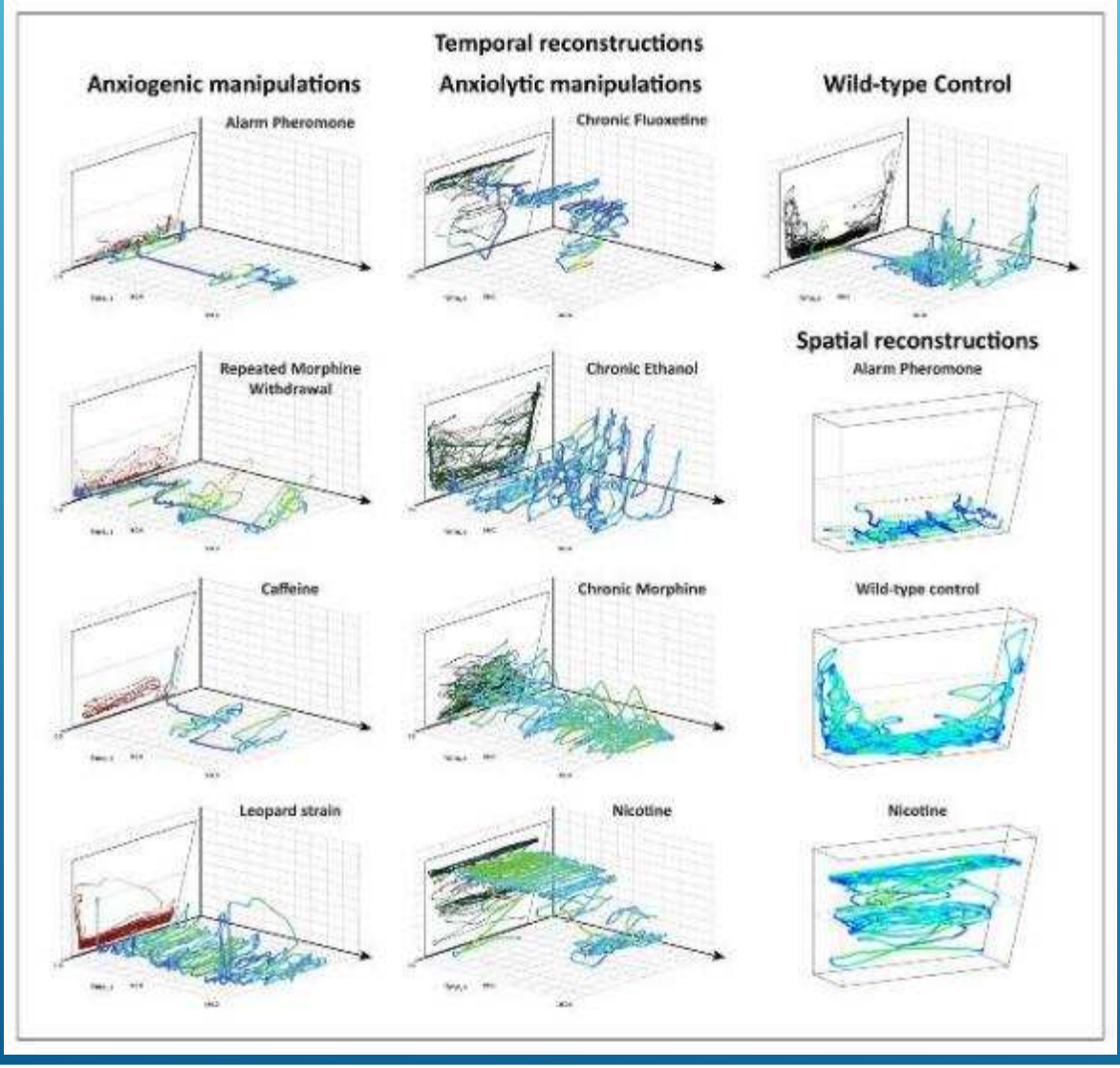
IMPACT ON REPRODUCTION: EGG PRODUCTION

Tank 1: 69% (11/16)
Tank 2: 82% (14/17)

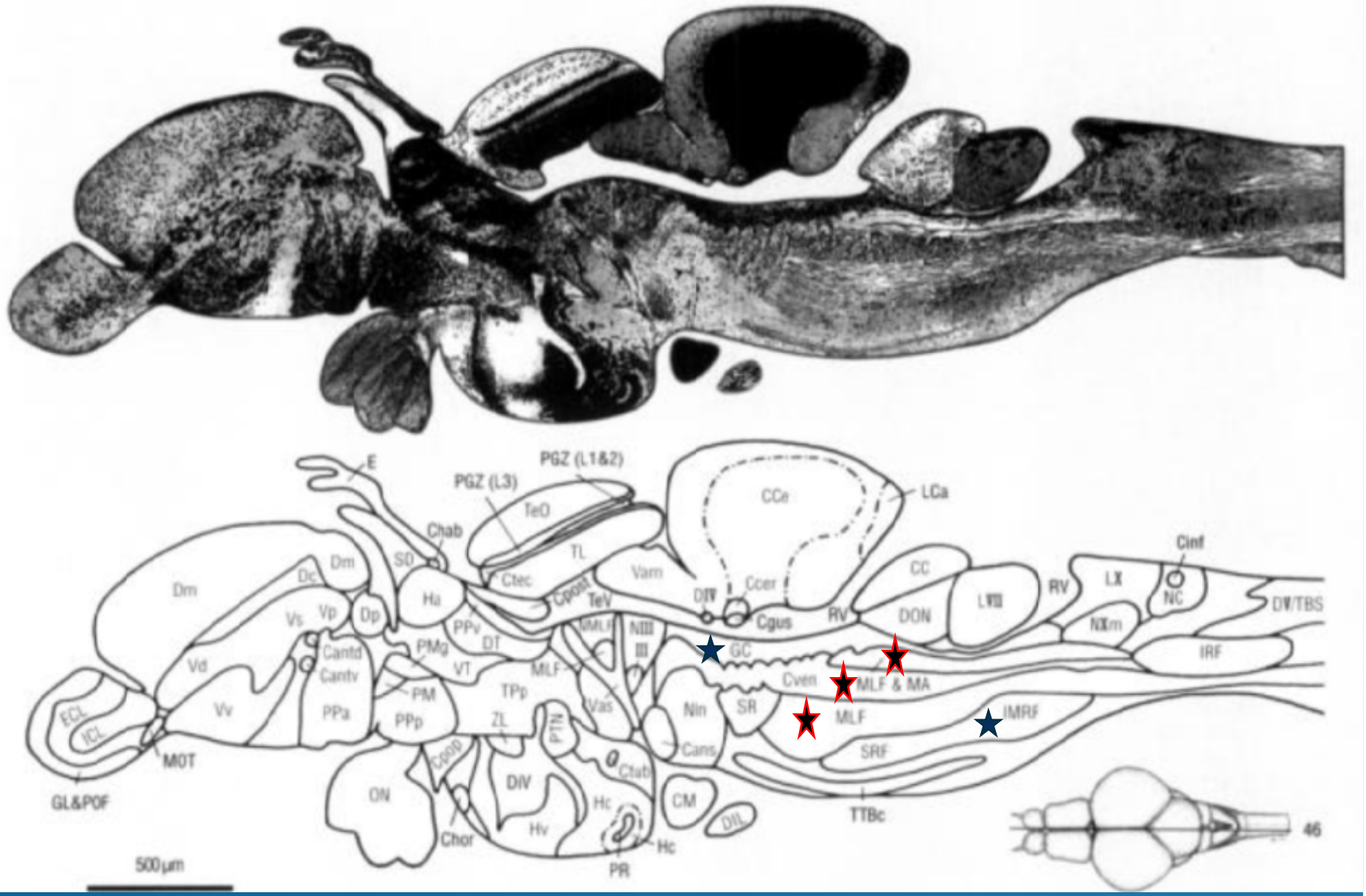


Ramsay, J. M., V. Watral, C. B. Schreck, and M. L. Kent. 2009. *Pseudoloma neurophilia* infections in zebrafish *Danio rerio*: effects of stress on survival, growth, and reproduction. Diseases of Aquatic Organisms 88: 69–84.

CHRONIC INFECTION: IMPACTS ON BEHAVIOR



Dr. Sean Spagnoli (OSU, DVM pathologist)



BEHAVIOR STUDIES

- Infected fish are “hyper vigilant”

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

The common neural parasite *Pseudoloma neurophilia* is associated with altered startle response habituation in adult zebrafish (*Danio rerio*): Implications for the zebrafish as a model organism

Sean Spagnoli[a,1], Lan Xue[b], Michael L. Kent[c,*]

[a] Oregon State University, Departments of Biomedical Sciences, USA
[b] Oregon State University, Departments of Statistics, USA
[c] Oregon State University, Departments of Microbiology, USA

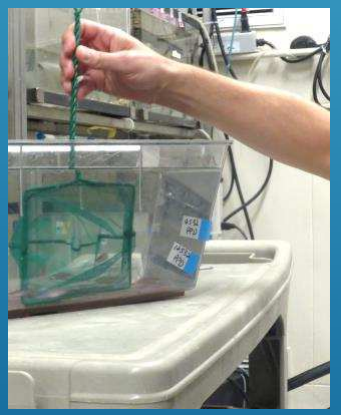
T1-T10 VELOCITY DIFFERENCES AKA, WAS THE SLOPE DIFFERENCE A TANK EFFECT?

Category	Difference
Control	8.193
Ex Negative	7.45
Infected	4.047

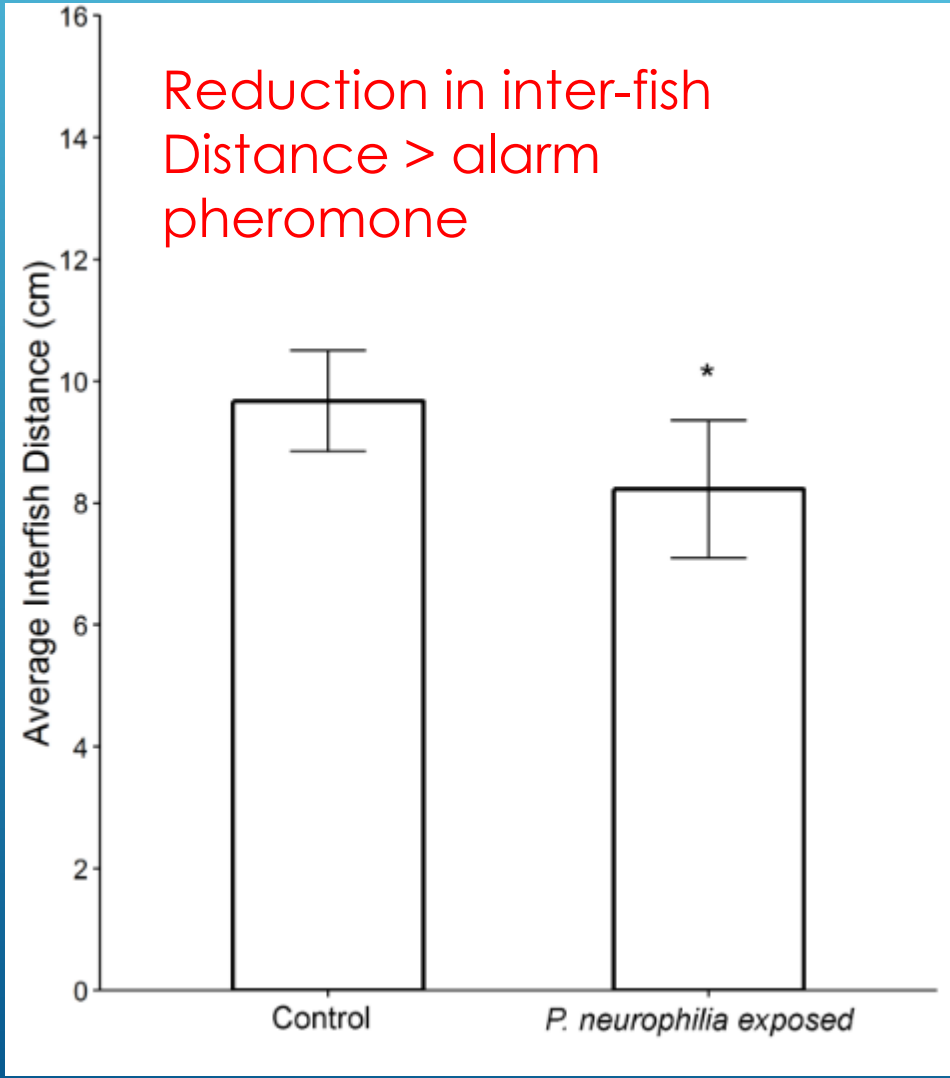
Comparison	p-value
Control vs Ex Negative	0.632
Control and Ex Negative vs Infected	0.0486

CONCLUSION

- Infected fish had a shallower habituation slope and less of a difference between T1 and T10 than control and exposed negative fish.
- *P. neurophilia* infection inhibits habituation to the startle response by approx. half
- Also, subclinical fish avoid capture compared to uninfected.



SHOALING TEST: MEAN INTERFISH DISTANCE POST-EXPOSURE



Exposed shoals had a mean interfish distance approximately 15% LESS than control shoals.

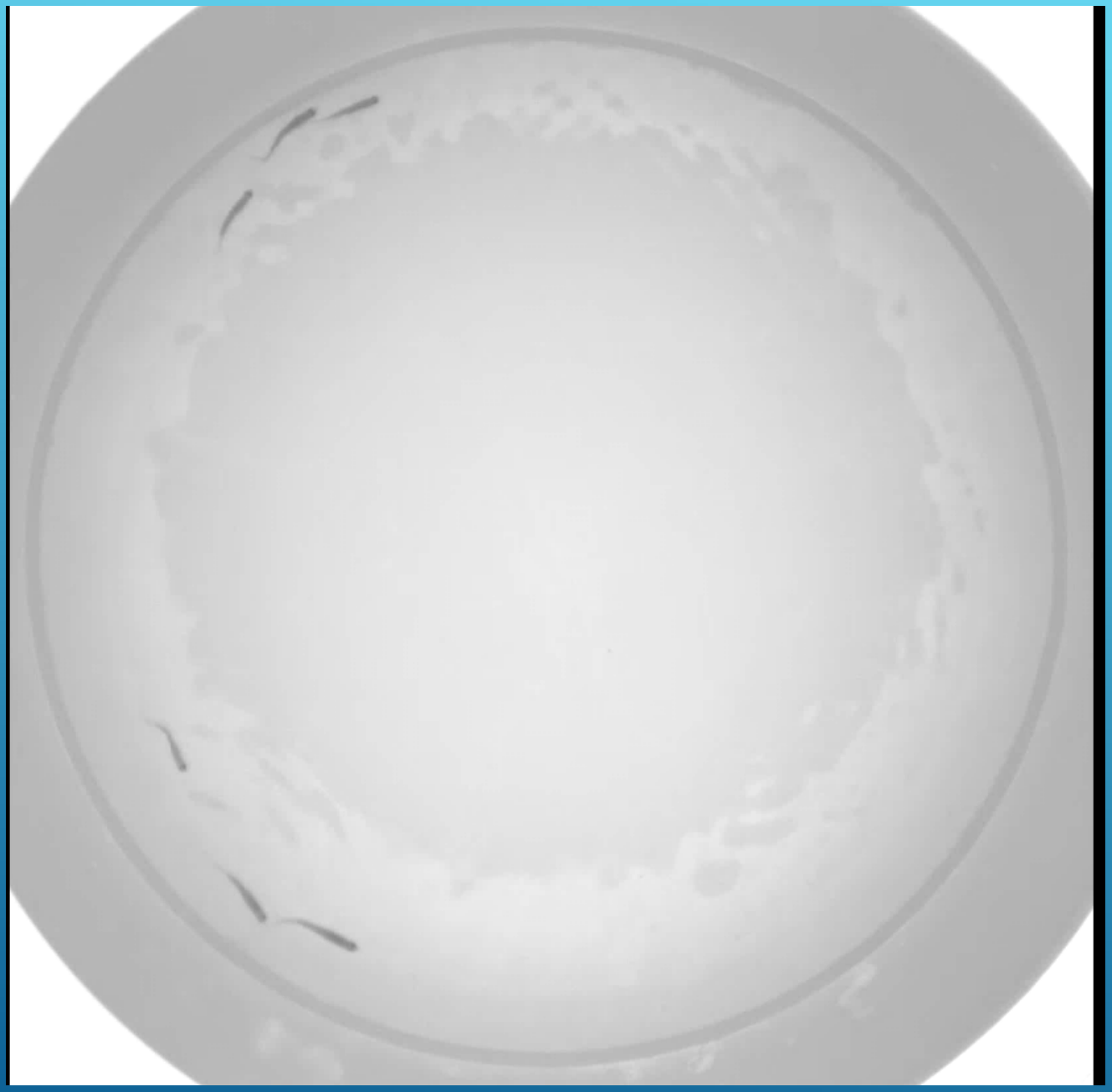
(p=0.026, U-test)

STUDY 3. ONGOING COLLABORATIVE RESEARCH

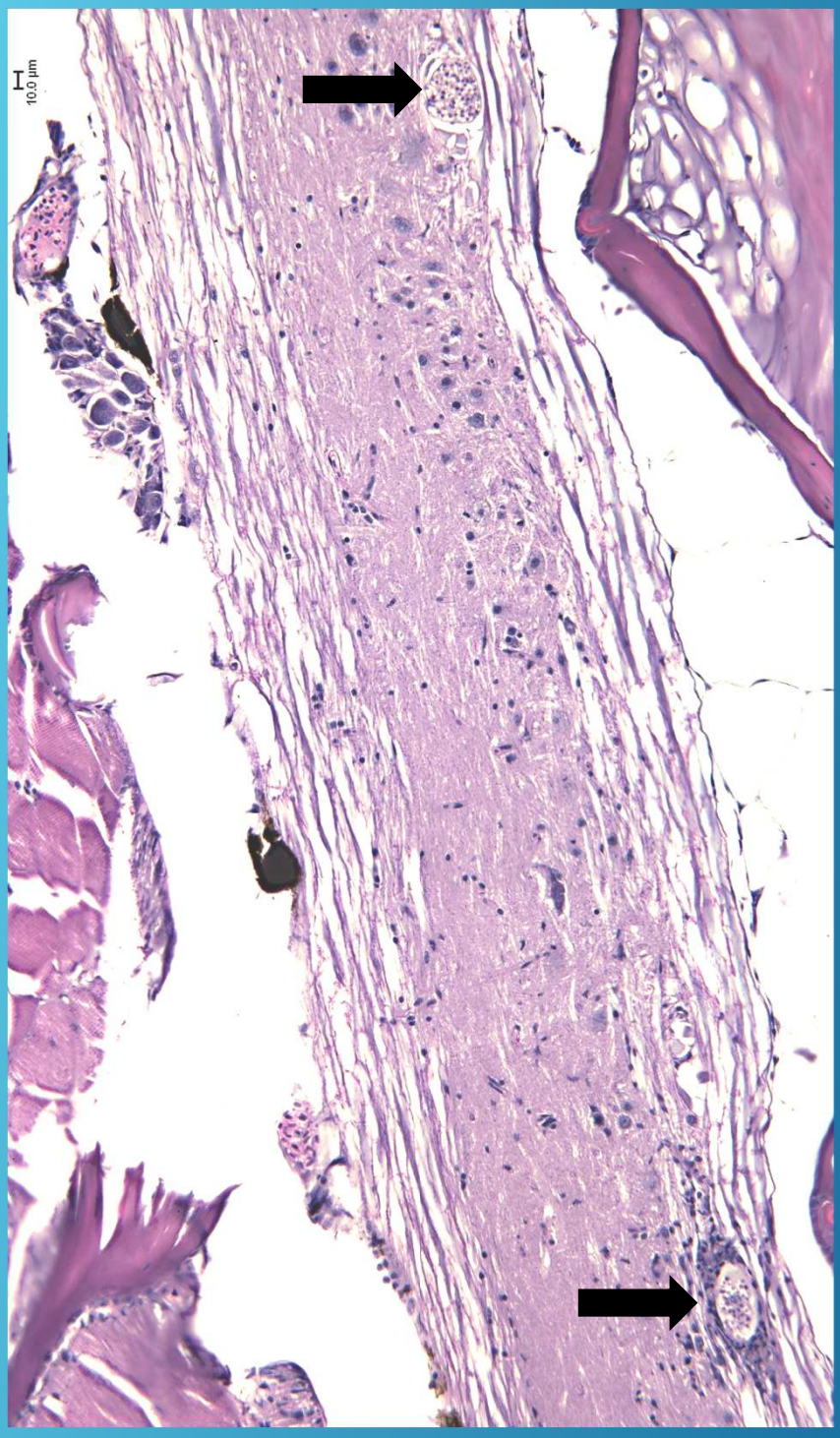
- Kent, Sanders and Spagnoli: OSU
- Polavieja, Hinz, Certal:
  - Champalimaud, Lisbon

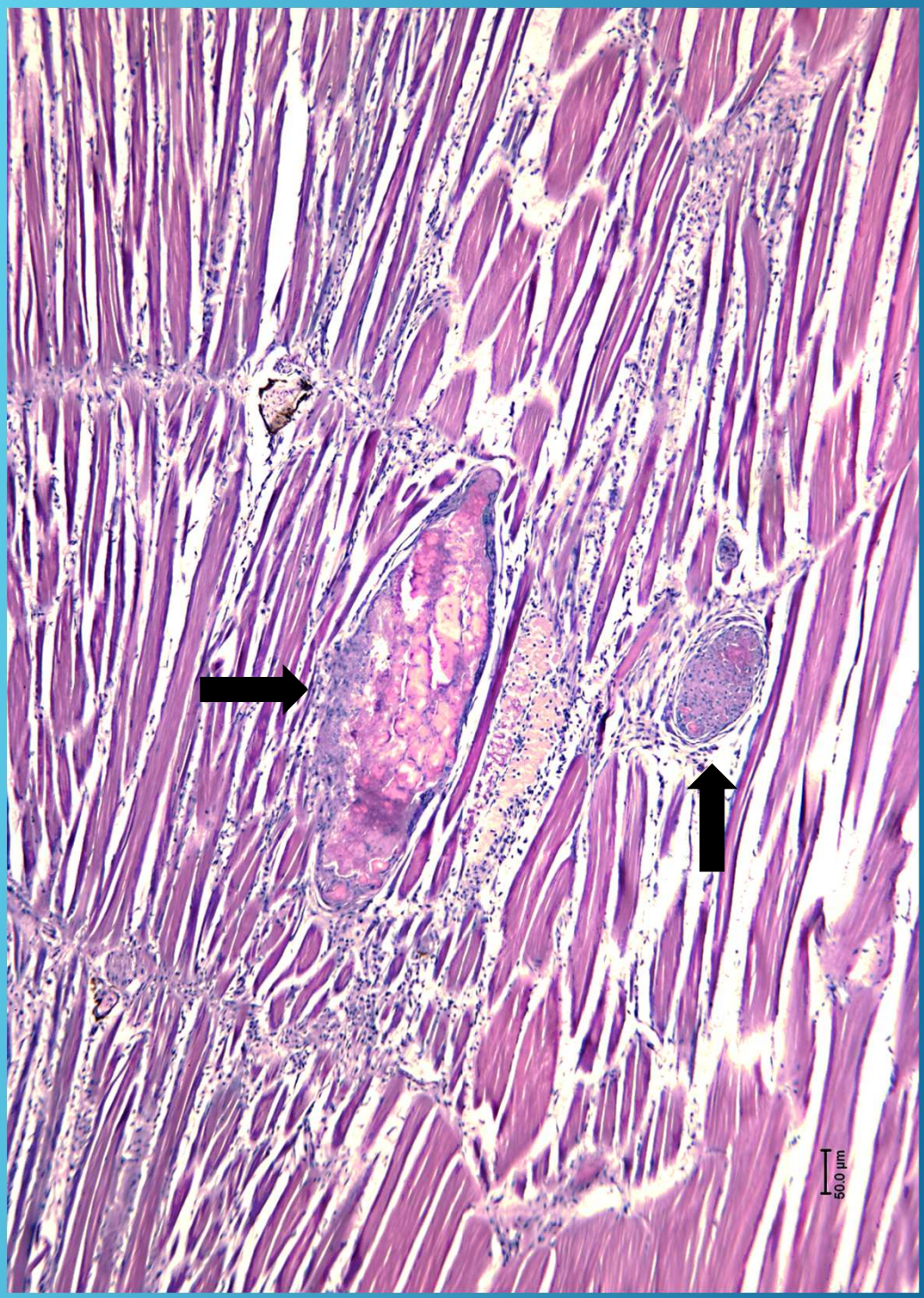
idTracker

A. Pérez-Escudero, J. Vicente-Page, R.C. Hinz, S. Arganda, G.G. de Polavieja, idTracker: Tracking individuals in a group by automatic identification of unmarked animals, *Nature Methods* (2014) (pdf) (web) software at www.idtracker.es)

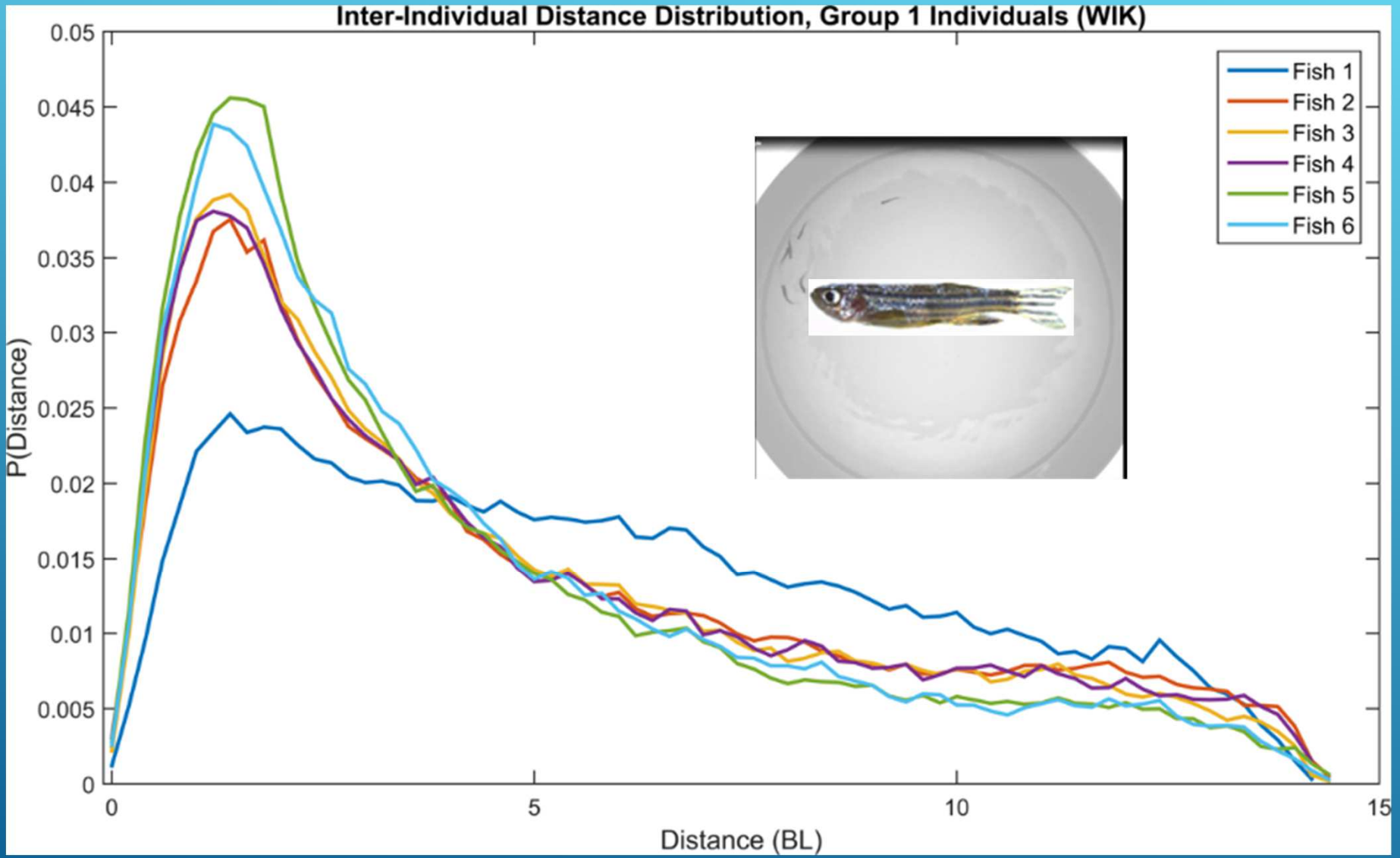


Effects of infection, strains, sex, etc.

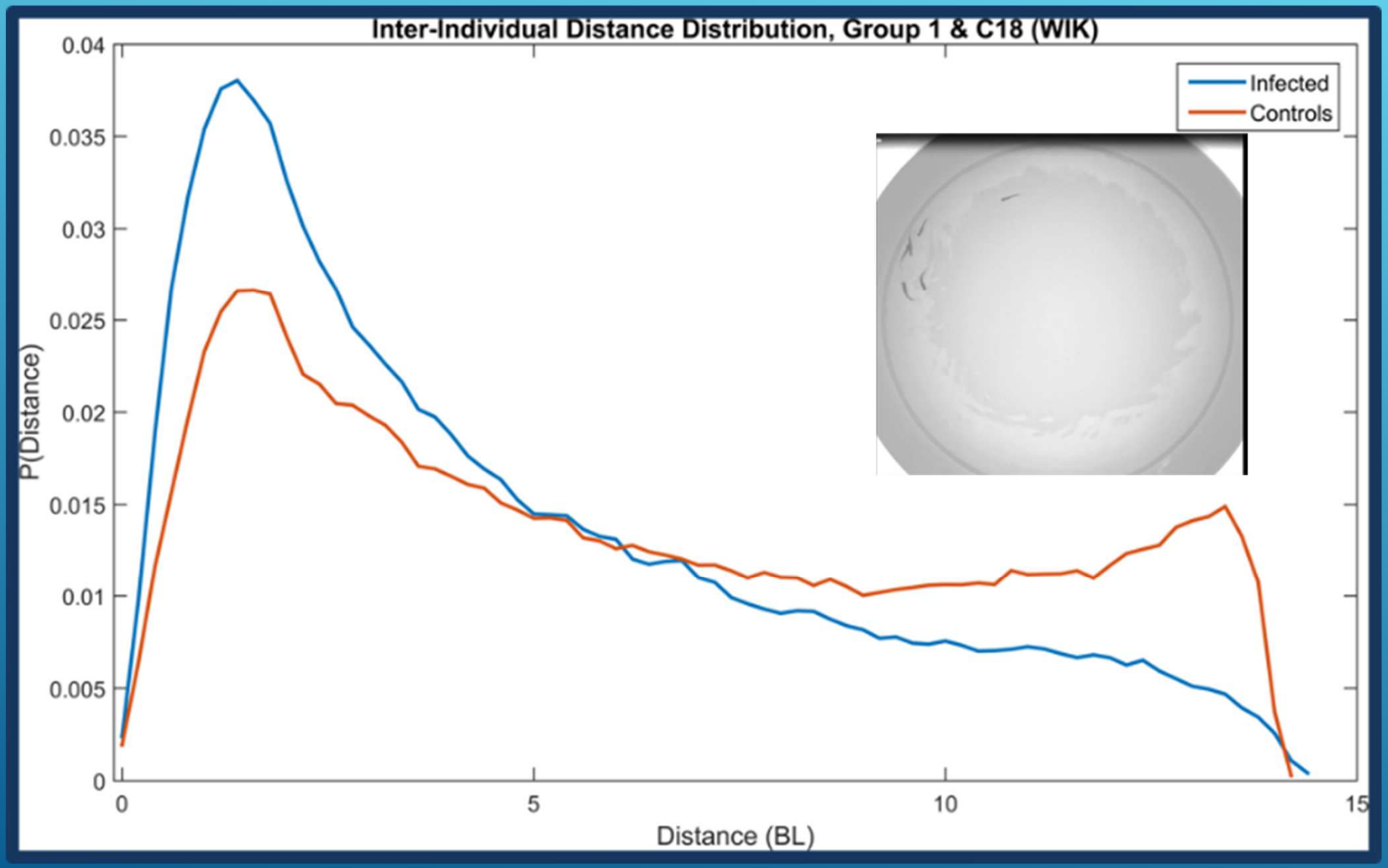

10.0 µm


50.0 µm

Clinically infected fish



Chronically infected fish

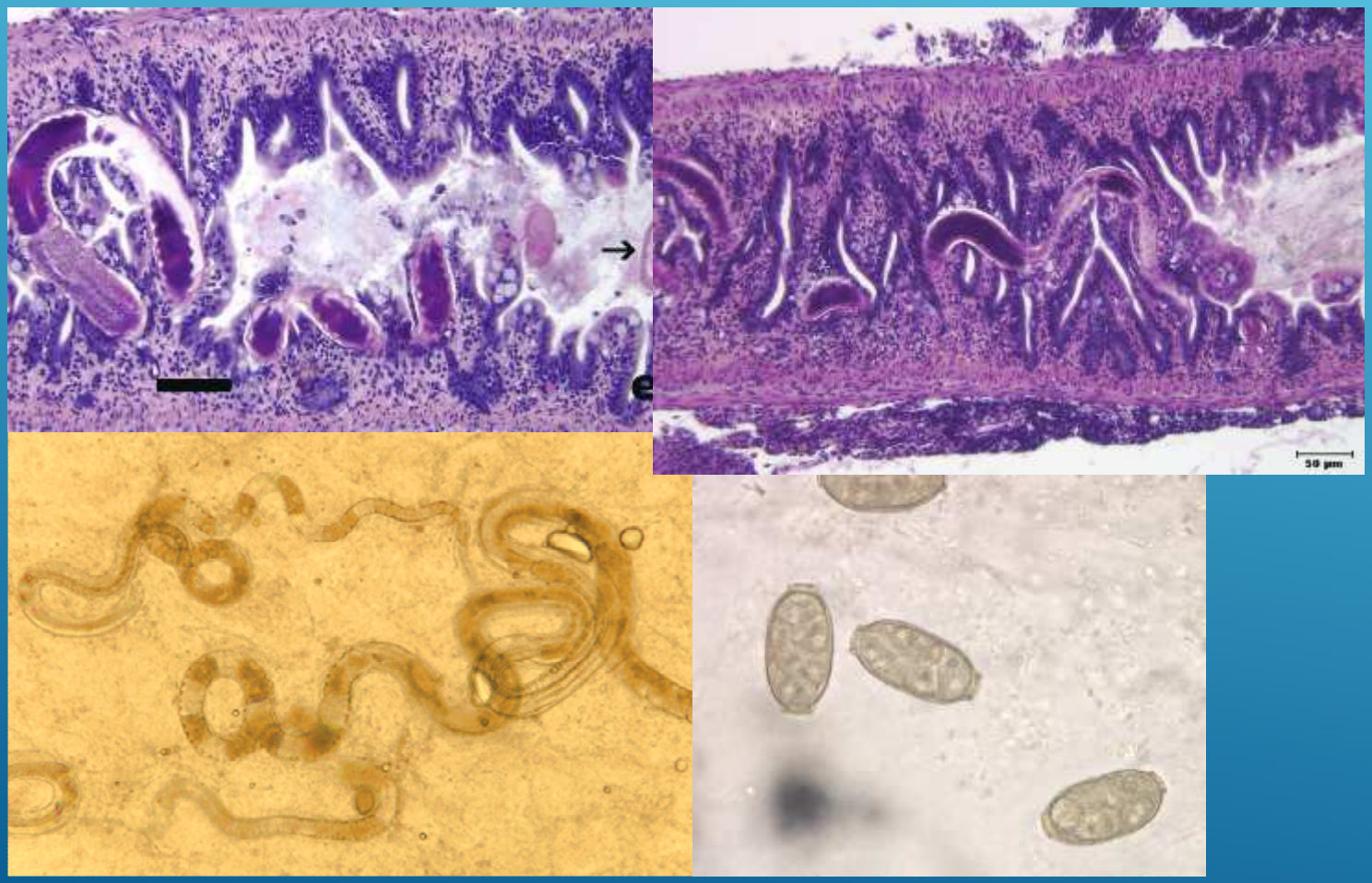


STUDY 3. PRELIMINARY CONCLUSIONS

- Clinical fish have > interfish distance
- Subclinical have < interfish distance
- May offset for mean values compared to controls,
- But probably more variable

COMMON CHRONIC INFECTIONS OF ZEBRAFISH

- *Pseudocapillaria tomentosa*

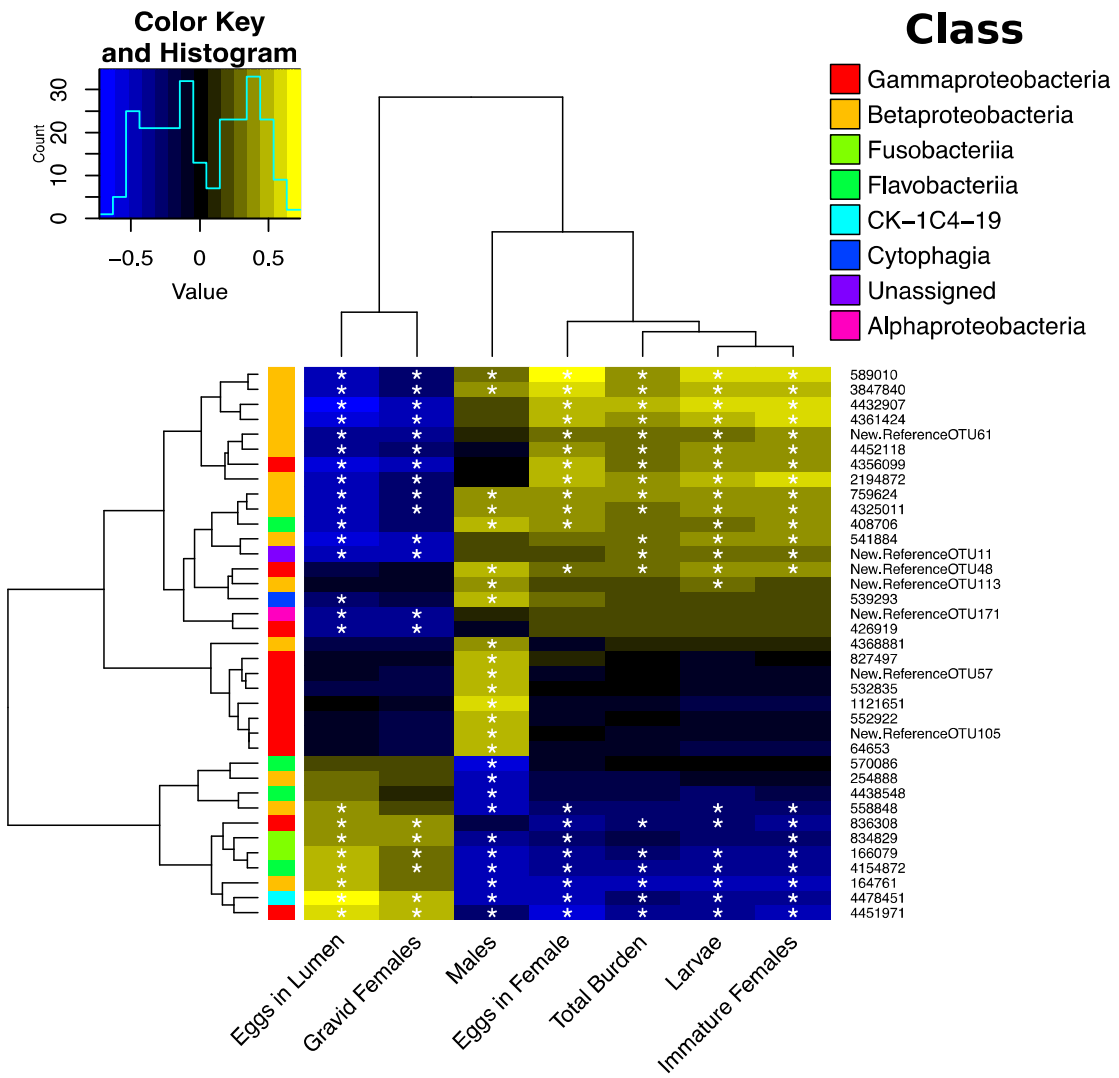


MICROBIOME STUDIES

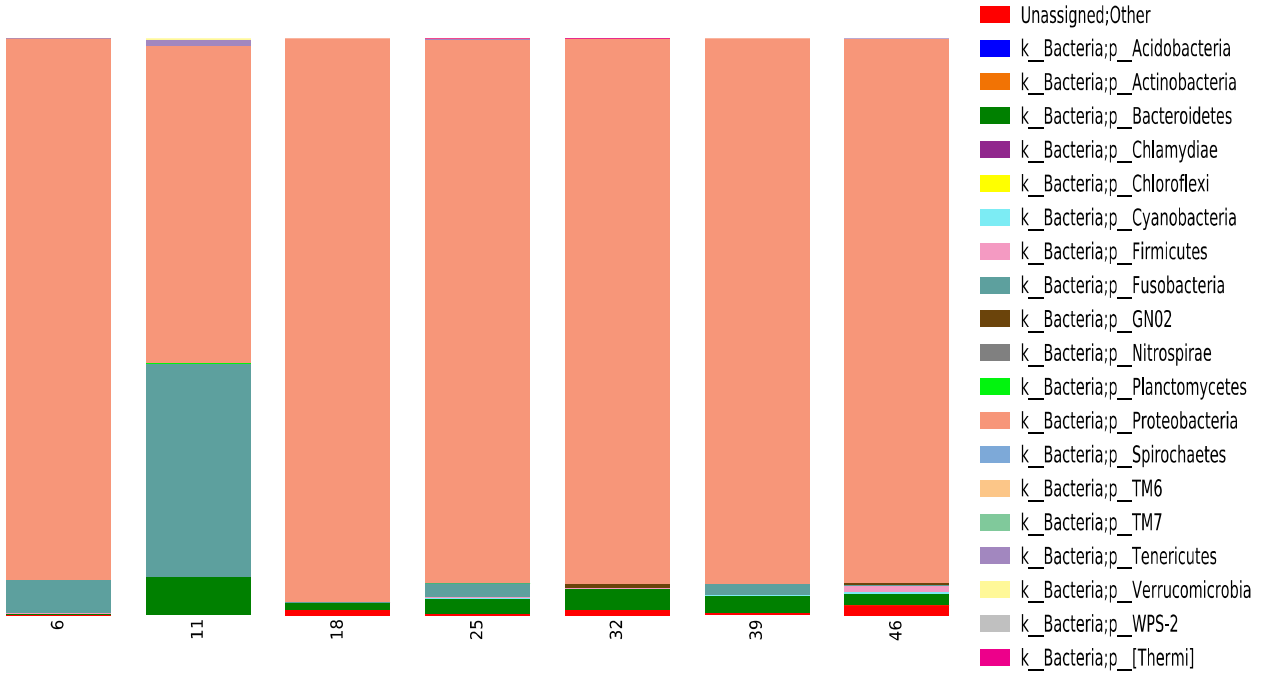
- How does intestinal nematode infection alter the gut microbiome?
  - *P. tomentosa* infection of adult zebrafish

Christopher Gaulke, Thomas Sharpton, Michael Kent

PARASITE BURDEN IS CORRELATED WITH MICROBIAL ABUNDANCE



ABUNDANCE OF MICROBIAL TAXA VARIES WITH LENGTH OF INFECTION



Pseudocapillaria tomentosa

From toxicology/tumor study (Spitzbergen et al. 2000)

Fish exposed to DMBA (7,12-dimethylbenze[*a*]anthracene)

- Of infected fish, 50% had intestinal neoplasms
- Of uninfected fish, 13% had neoplasms
- χ^2 significant (P=0.03)

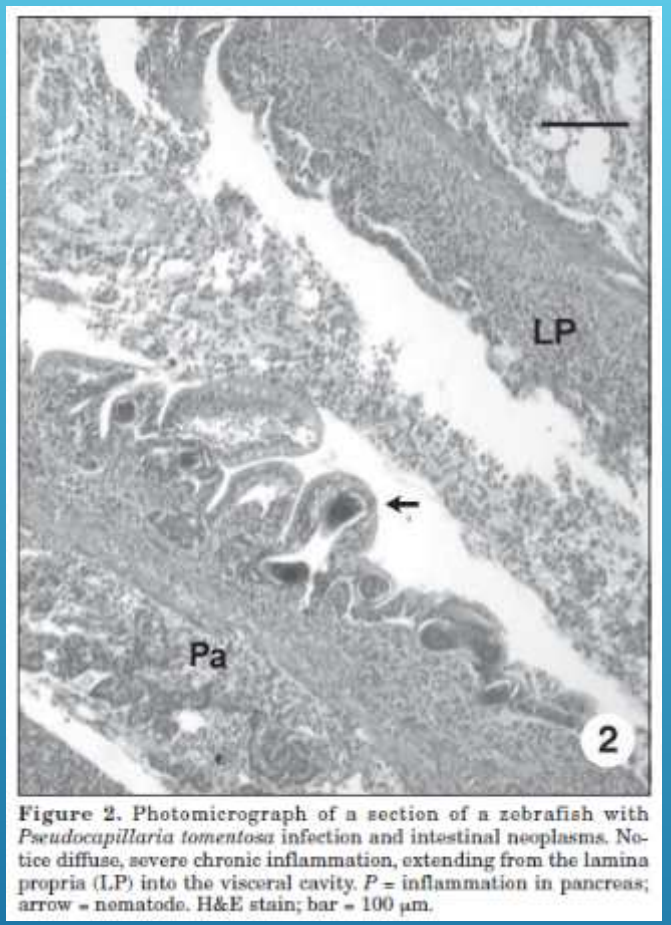
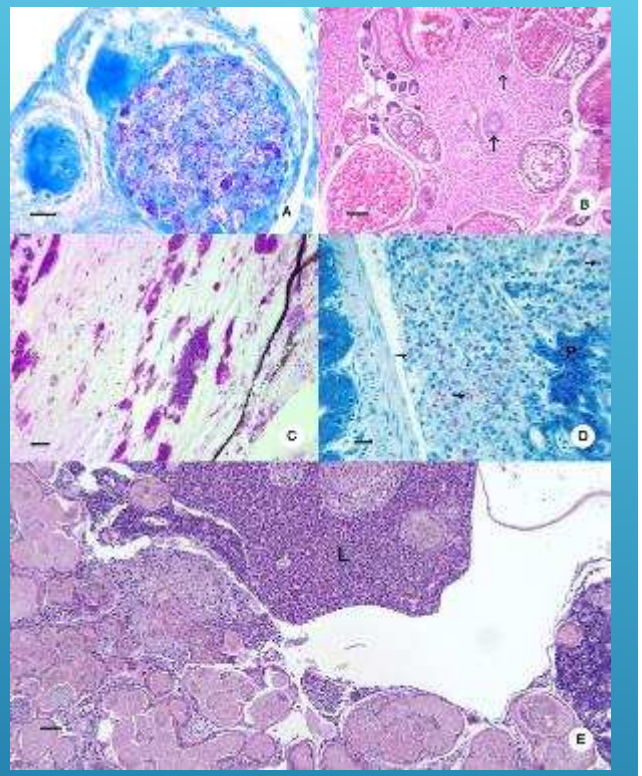


Figure 2. Photomicrograph of a section of a zebrafish with *Pseudocapillaria tomentosa* infection and intestinal neoplasms. Notice diffuse, severe chronic inflammation, extending from the lamina propria (LP) into the visceral cavity. *P* = inflammation in pancreas; arrow = nematode. H&E stain; bar = 100 μm.

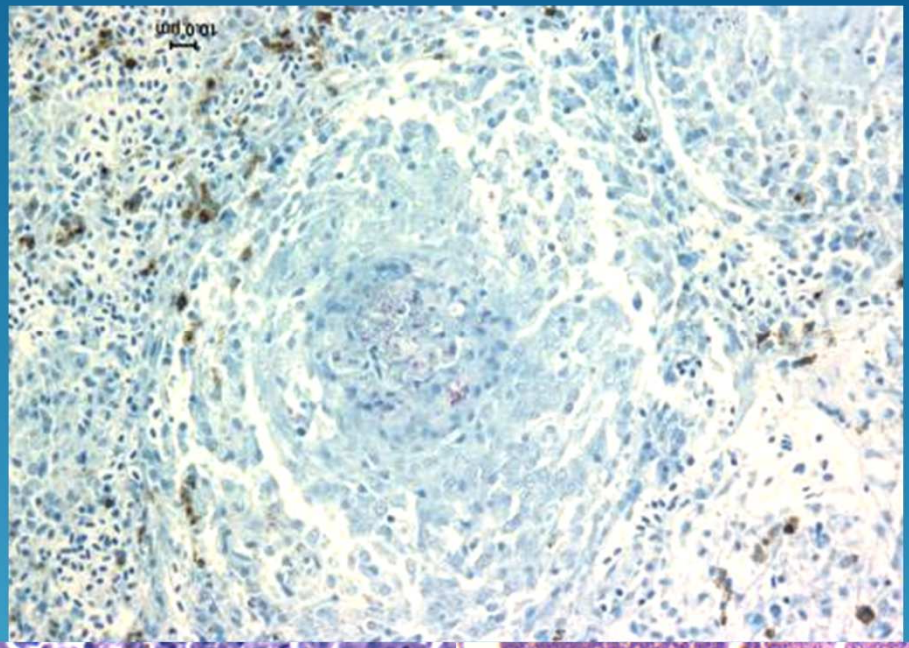
Kent et al. (2002)

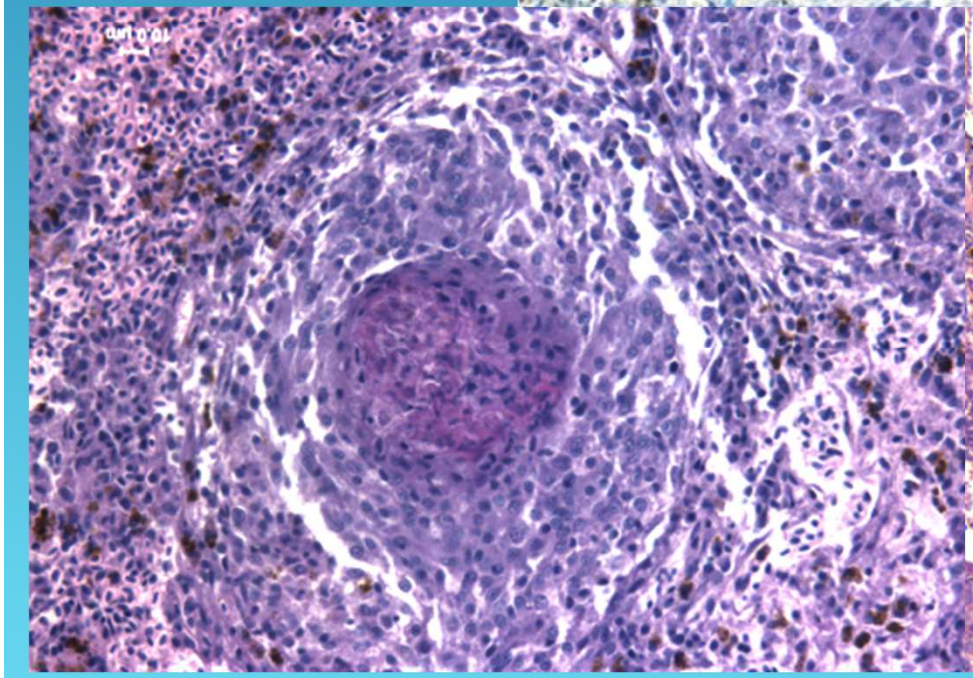
COMMON CHRONIC INFECTIONS OF ZEBRAFISH

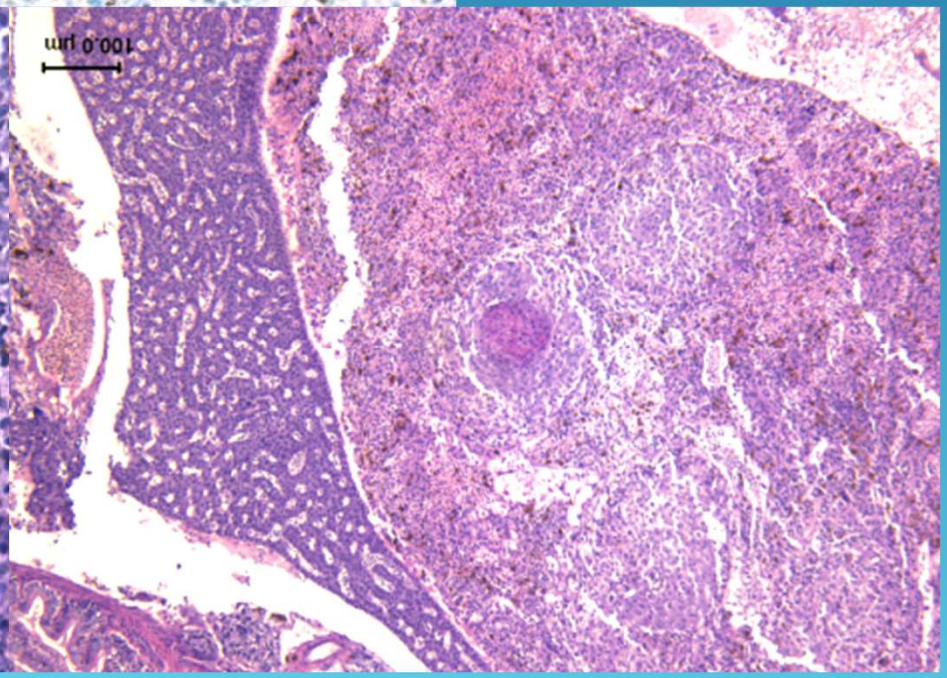
- Mycobacteria
  - *M. marinum*



Mycobacterium chelonae

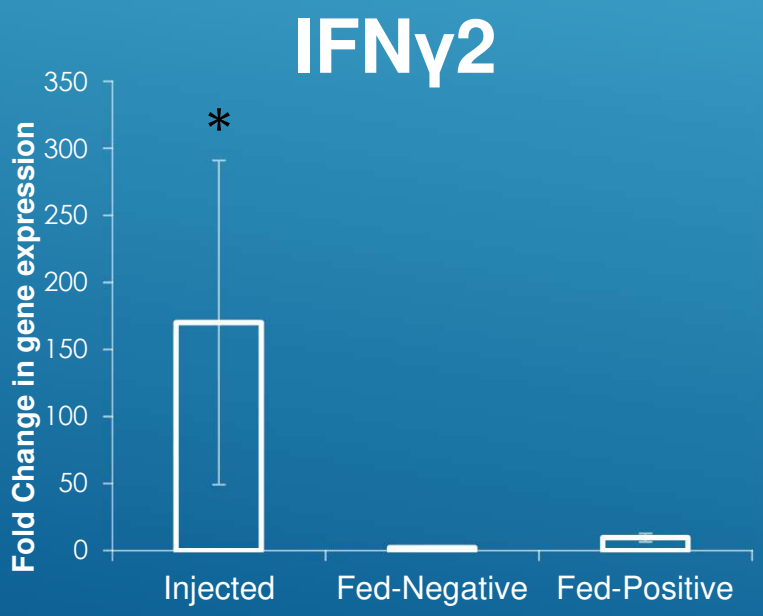

100.0 µm

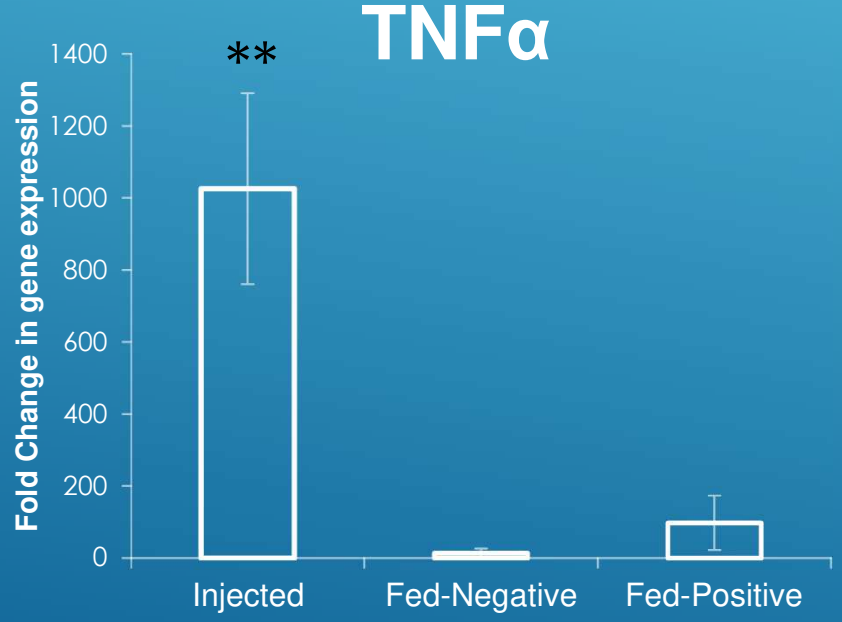

100.0 µm


100.0 µm

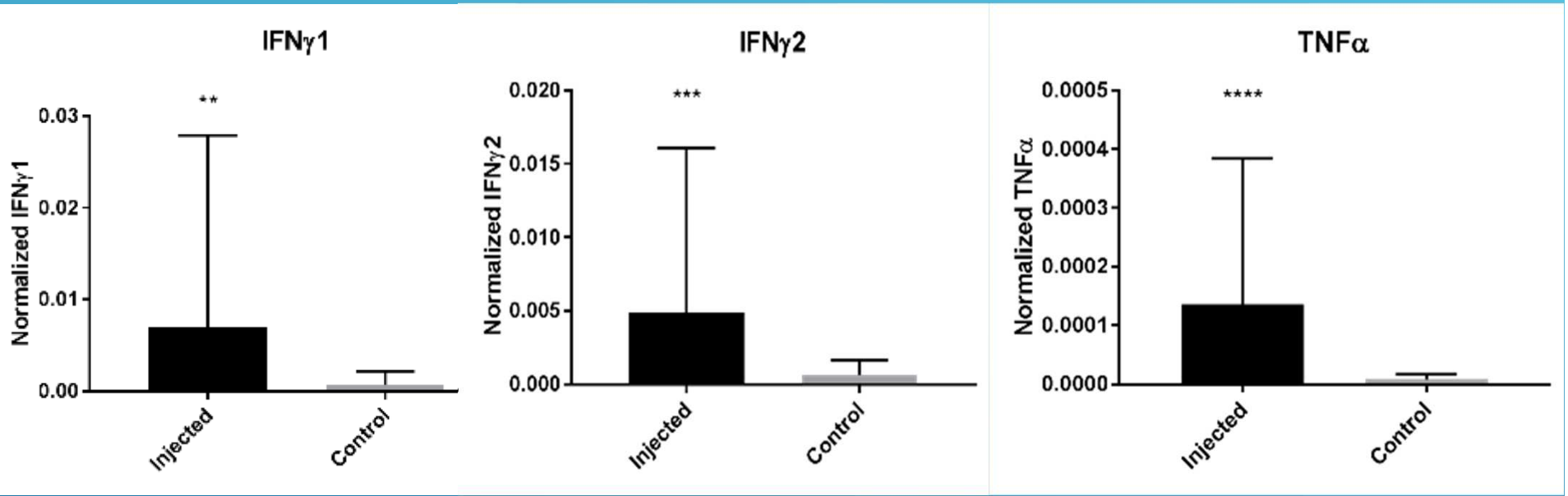
MYCOBACTERIUM SPP.

- *M. marinum, M. haemophilum*
  - Infections generally clinical; high mortalities

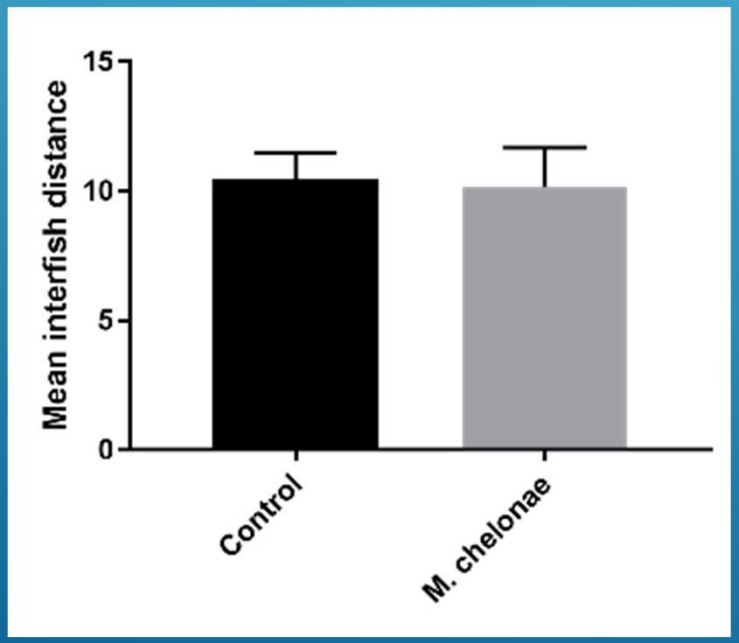


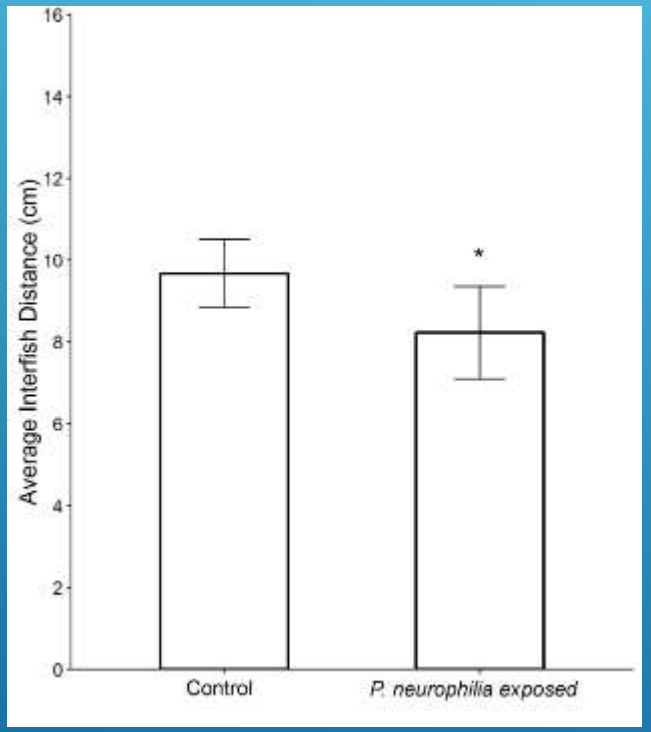


INFLAMMATORY CYTOKINES UPREGULATED IN SUBCLINICAL *M. CHELONAE* INFECTION



M. CHELONAE: NO EFFECT ON SHOALING

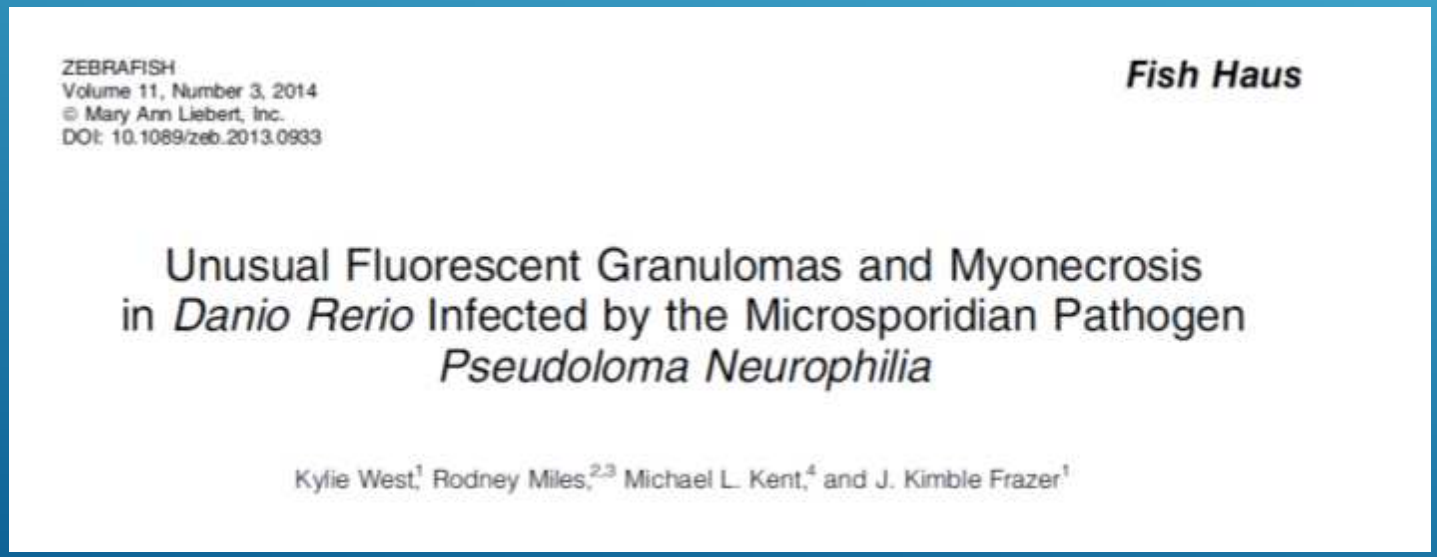




FLUORESCENT NODULES AND *PSEUDOLOMA*

Kimble Frazer and Kylie West

University of Oklahoma



ZEBRAFISH
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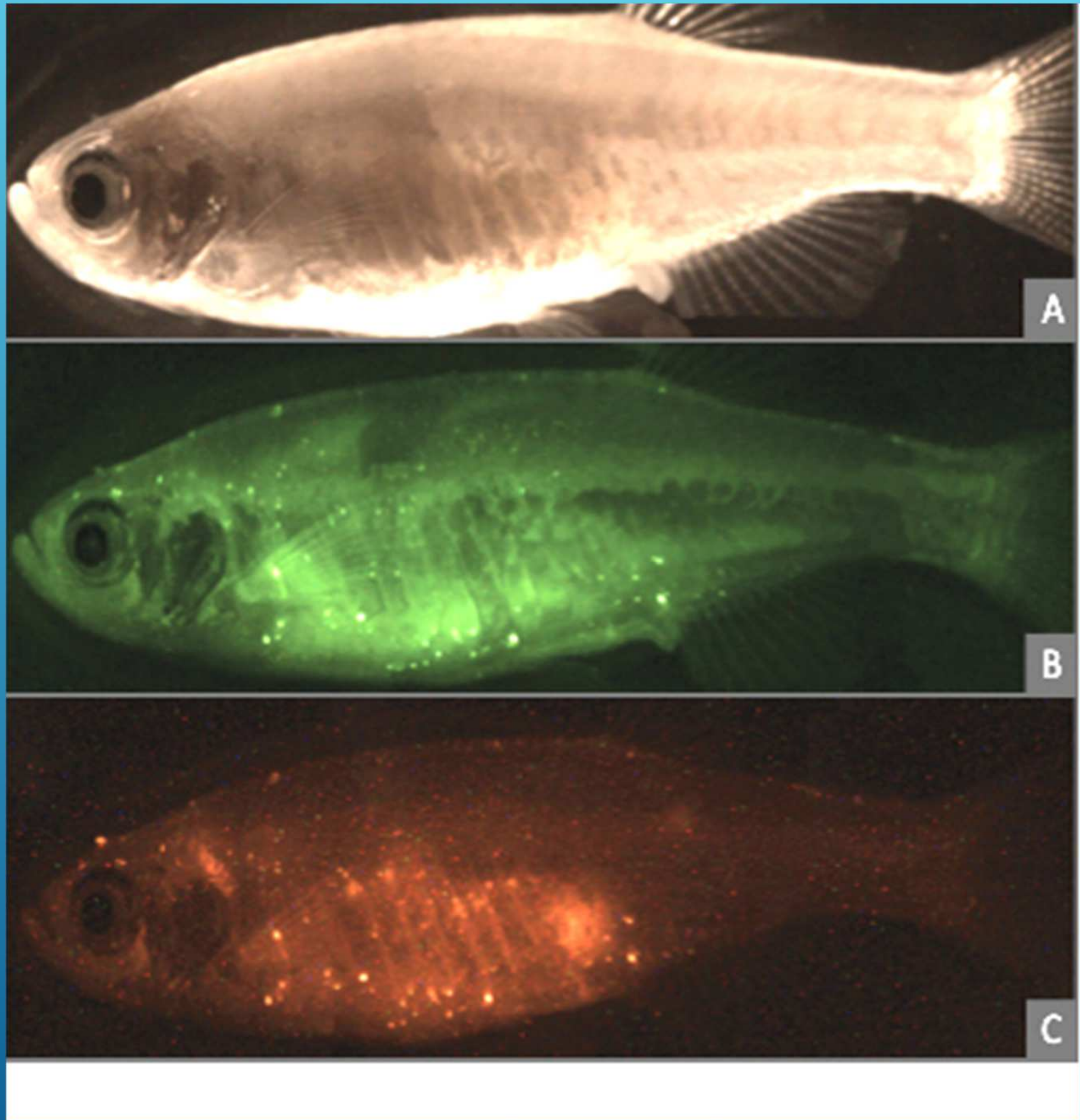
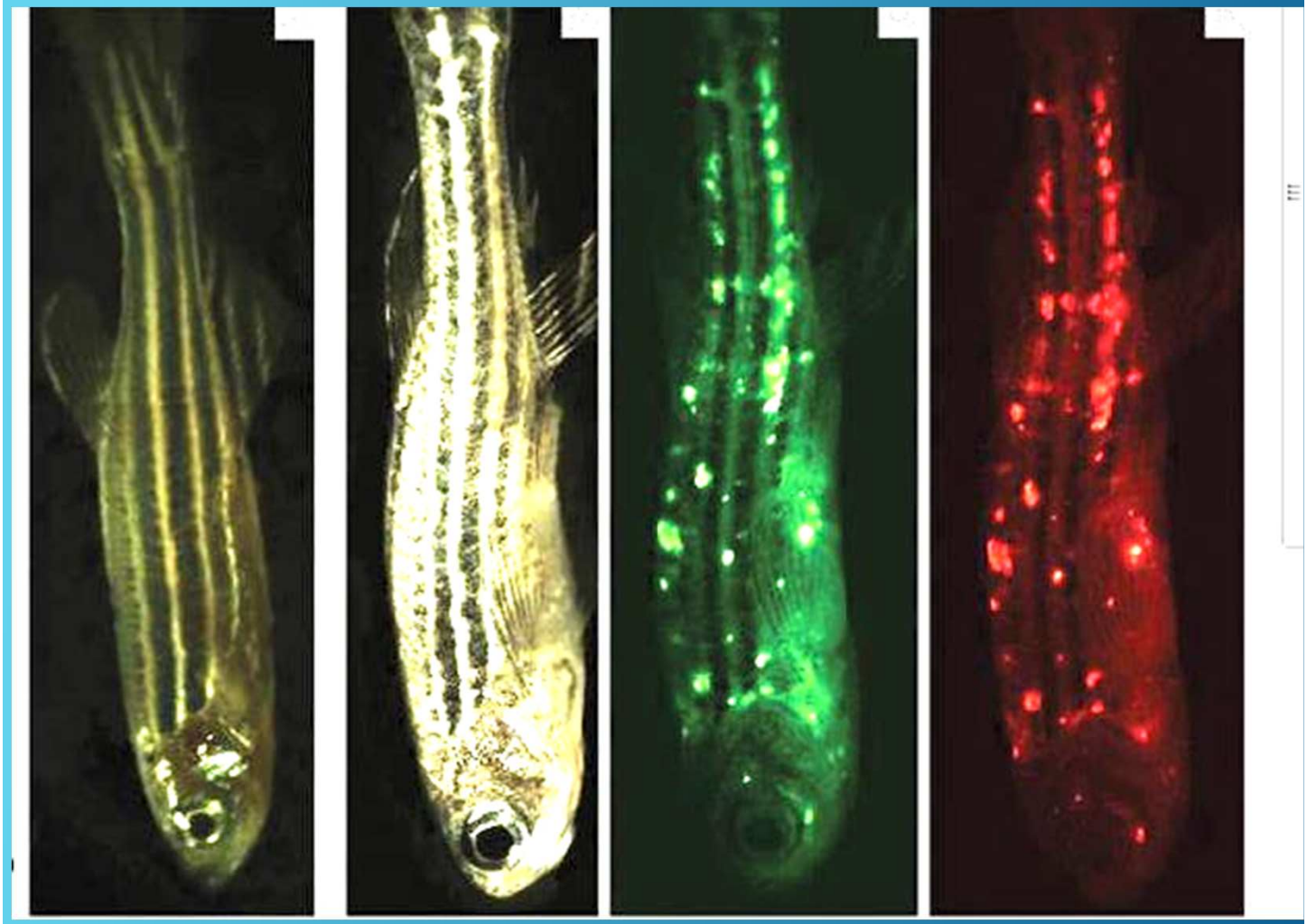
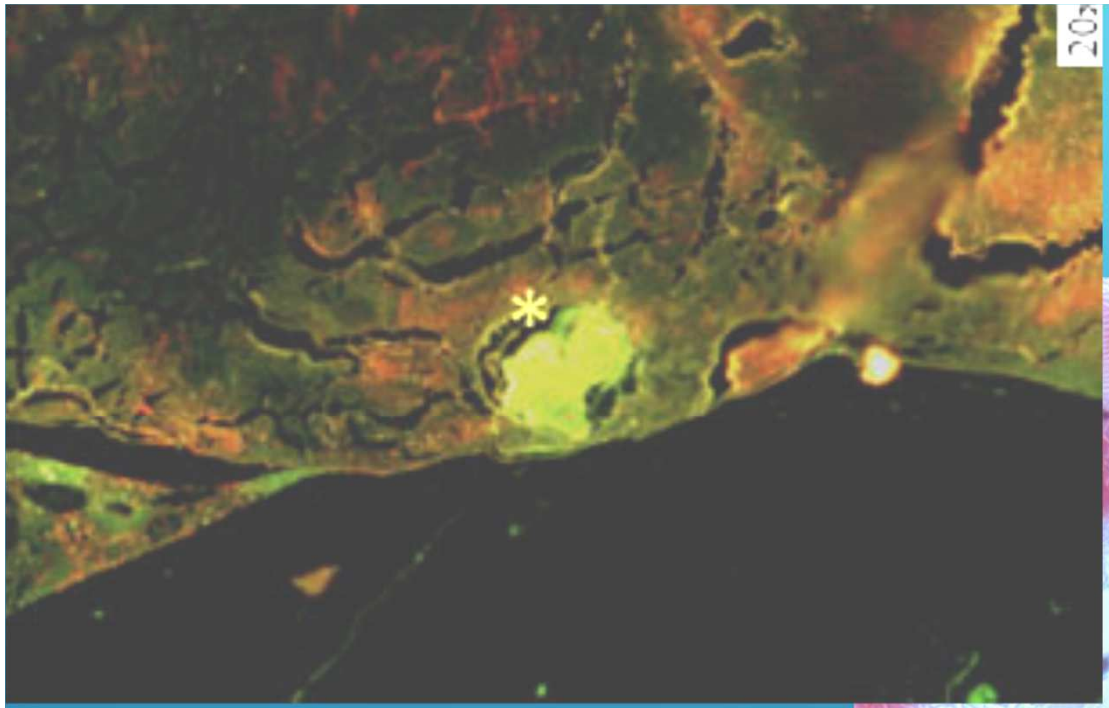
Fish Haus

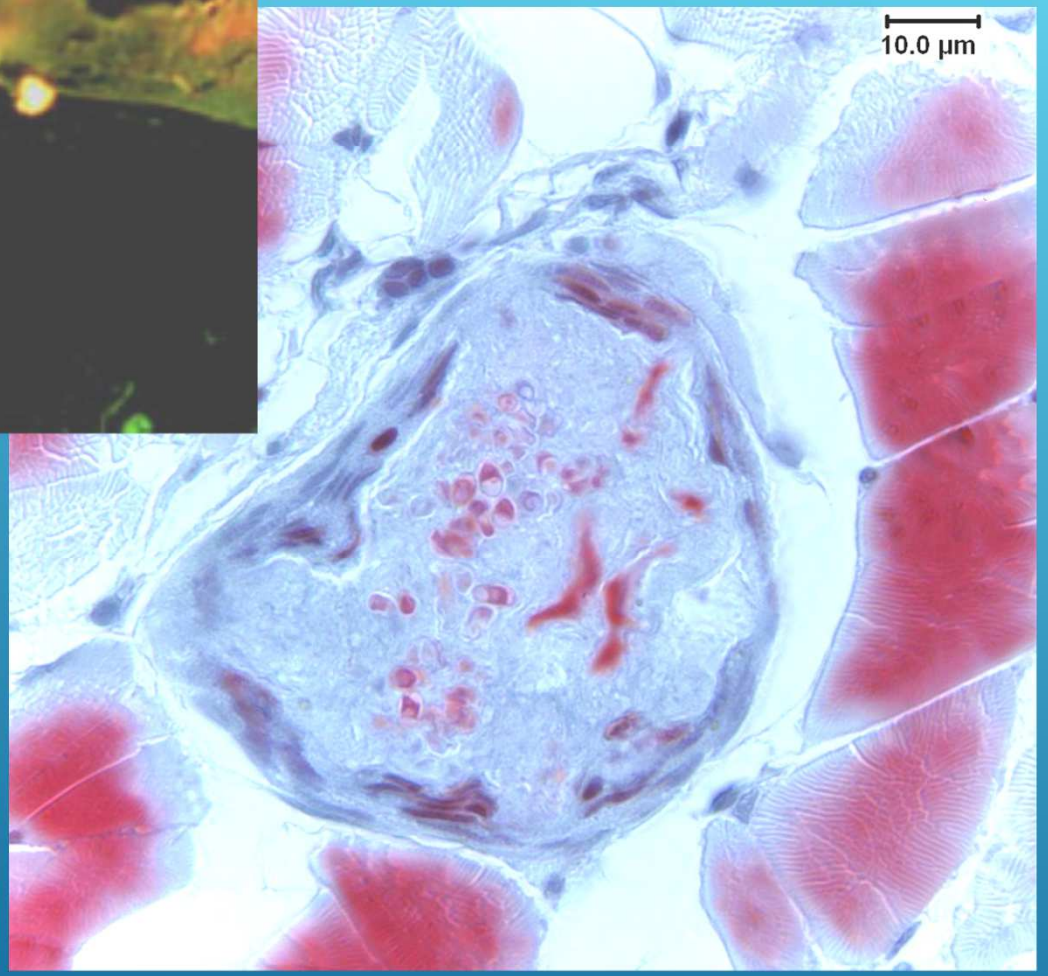
Unusual Fluorescent Granulomas and Myonecrosis in *Danio Rerio* Infected by the Microsporidian Pathogen *Pseudoloma Neurophilia*

Kylie West,[1] Rodney Miles,[2,3] Michael L. Kent,[4] and J. Kimble Frazer[1]


A
B
C



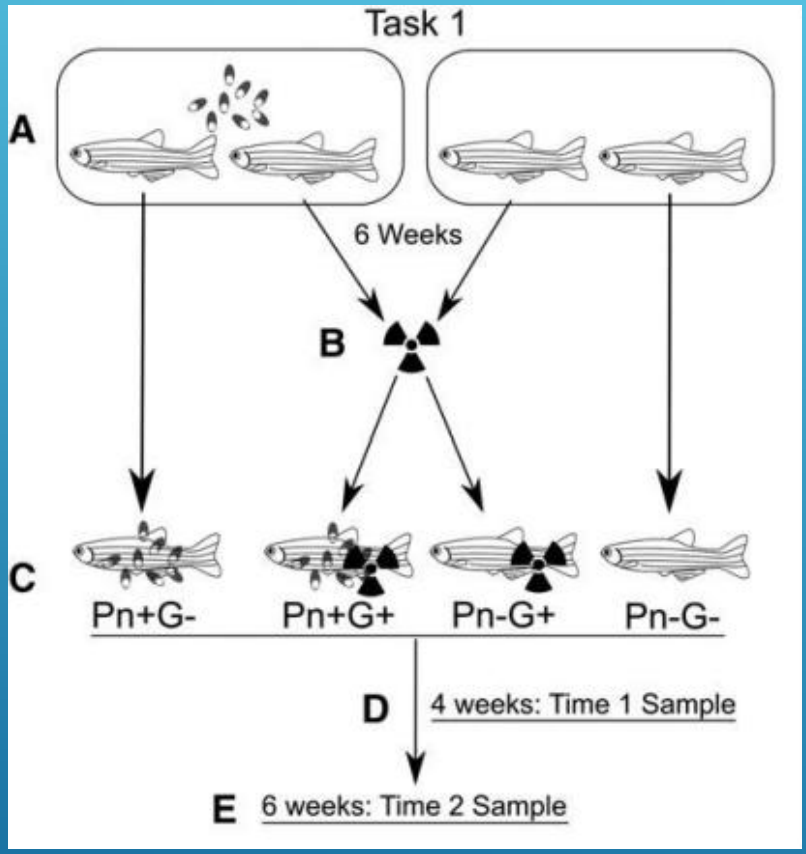

20x

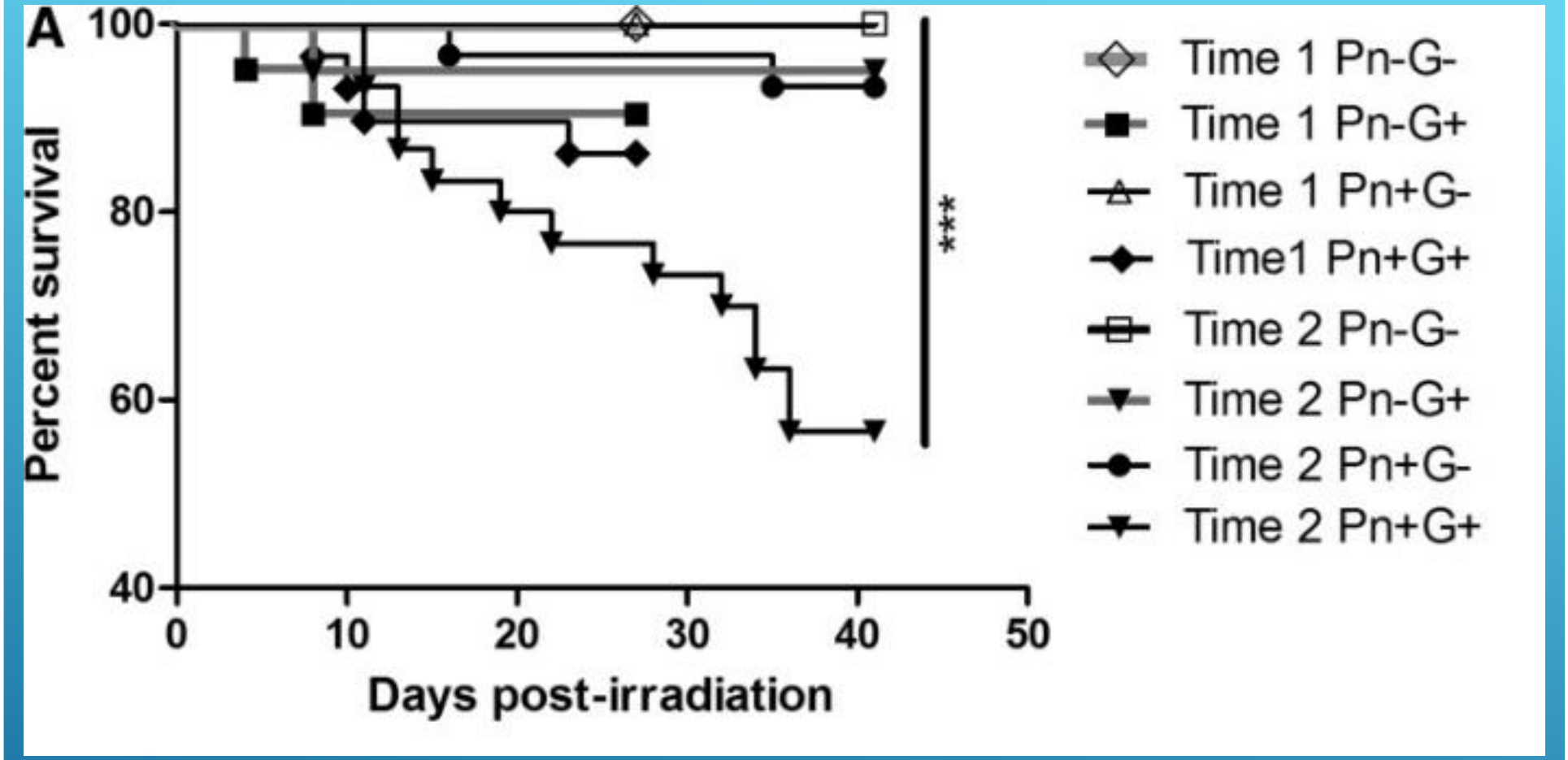

10.0 µm

IMMUNE SUPPRESSION/ABLATION

- Hematopoiesis
- Xenotransplantation

- Gamma irradiation
- Dexamethasone





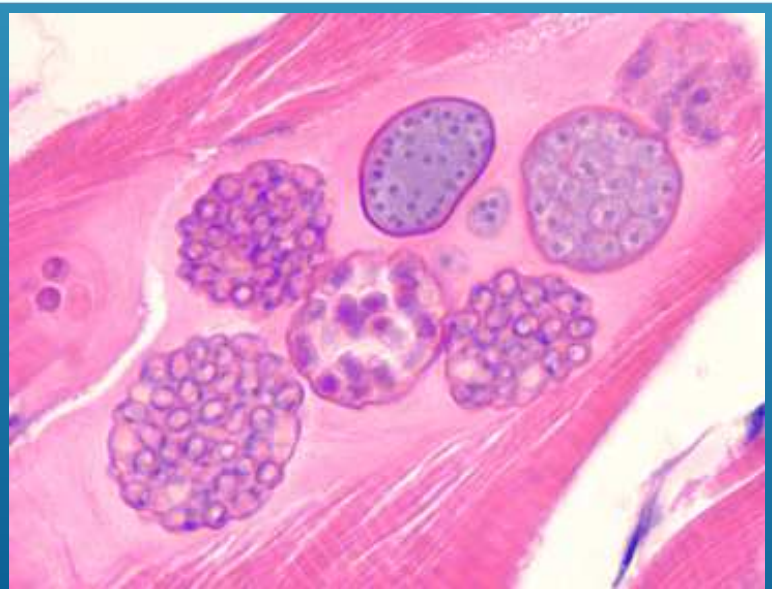
Spagnoli, S., J. Sanders, V. Watral, and M. L. Kent. 2016. *Pseudoloma neurophilia* Infection Combined with Gamma Irradiation Causes Increased Mortality in Adult Zebrafish (*Danio rerio*) Compared to Infection or Irradiation Alone: New Implications for Studies Involving Immunosuppression. Zebrafish 0: 1–8.

Published in final edited form as:
Dis Aquat Organ. 2010 July 26; 91(1): 47–56. doi:10.3354/dao02245.

Pleistophora hyphessobryconis (Microsporidia) infecting zebrafish (*Danio rerio*) in research facilities

Justin L Sanders[1,*], **Christian Lawrence**[2], **Donald K Nichols**[3,4], **Jeffrey F. Brubaker**[4], **Tracy S Peterson**[1], **Katrina N. Murray**[5], and **Michael L Kent**[1]

[1]Department of Microbiology, Oregon State University, Corvallis, Oregon [2]Children's Hospital Boston, Aquatic Resources Program, Boston, Massachusetts [3]US Army Center for Environmental Health Research, Fort Detrick, Maryland [4]US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland [5]Zebrafish International Resource Center, University of Oregon, Eugene, Oregon

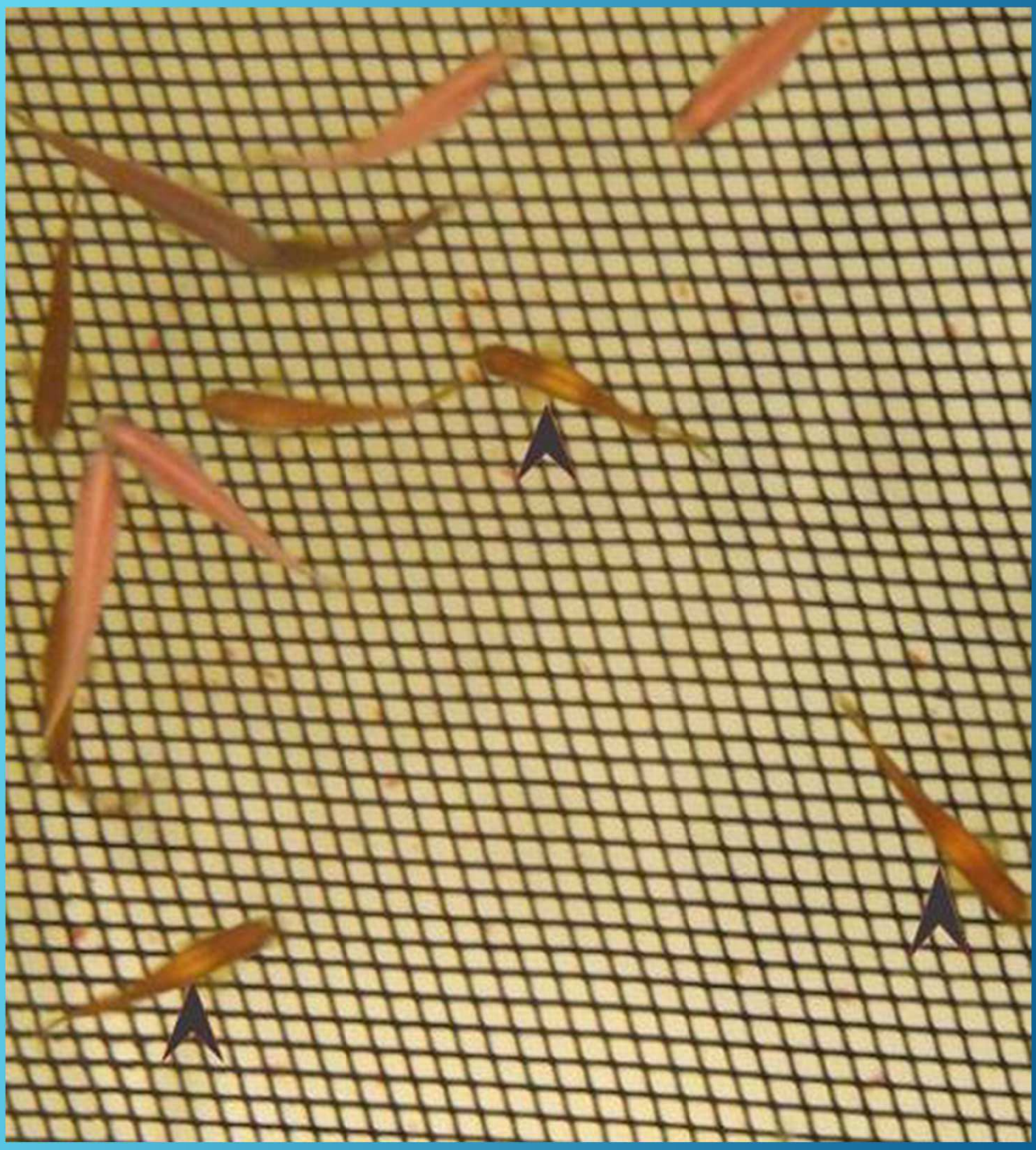


THREE DIAGNOSTIC CASES FROM ZEBRAFISH FACILITIES

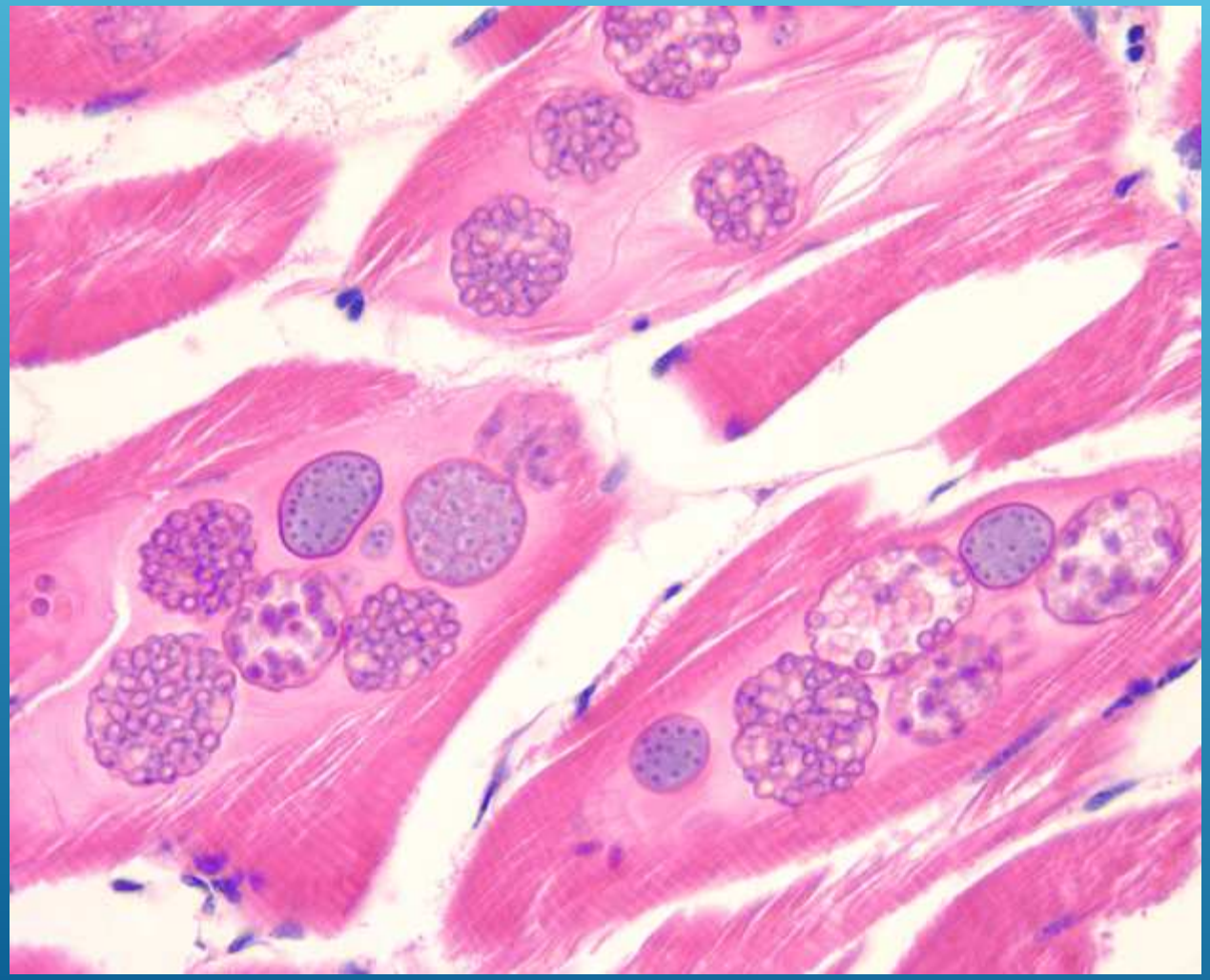
- Case History
  - Lab A) surface disinfected eggs
  - Lab B) unknown history
  - Lab C) surface disinfected eggs
    - Most prevalent in CG1 – isogenic line used for tissue transplant

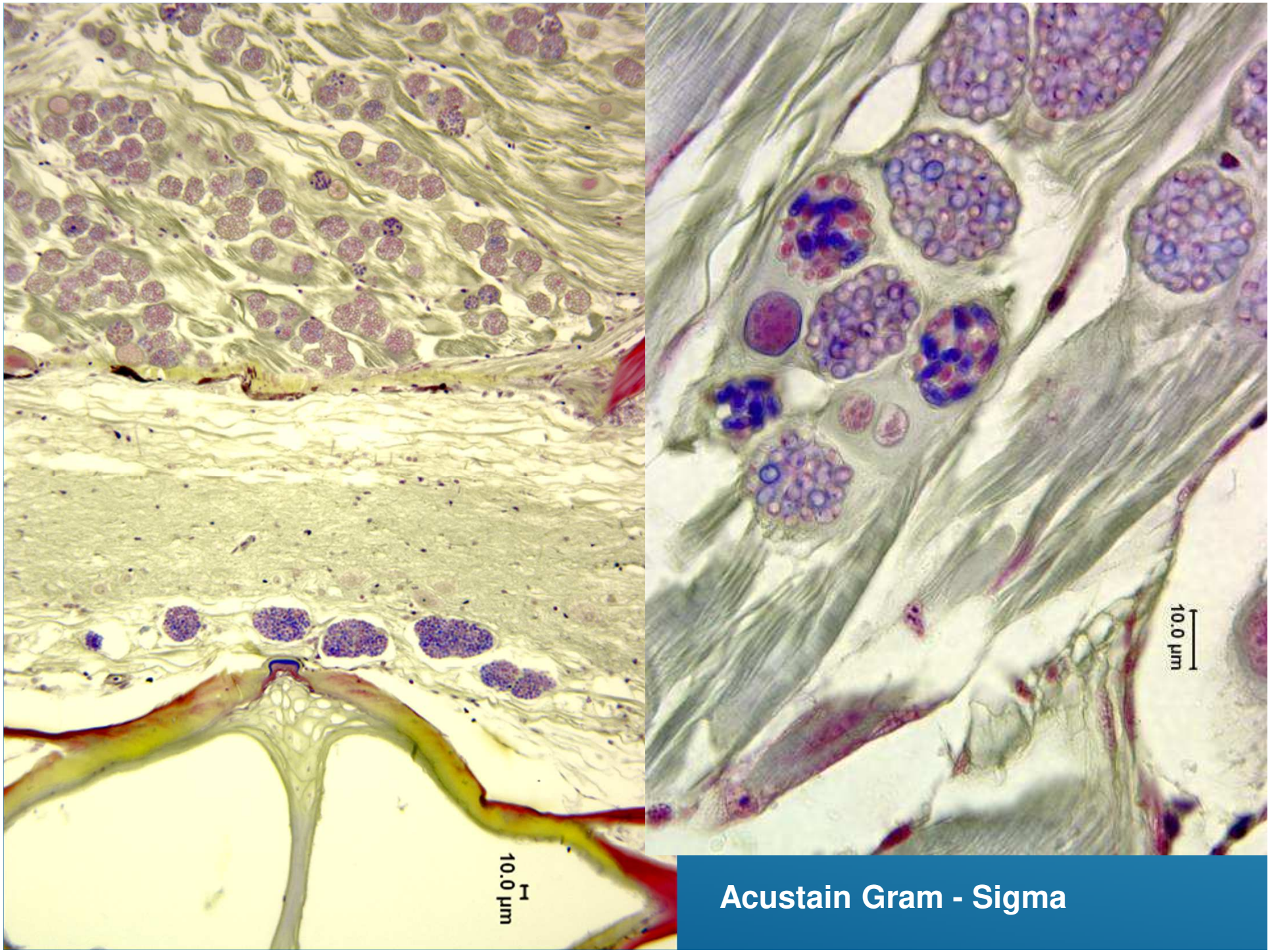
PLEISTOPHORA HYPHESSOBRYCONIS

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

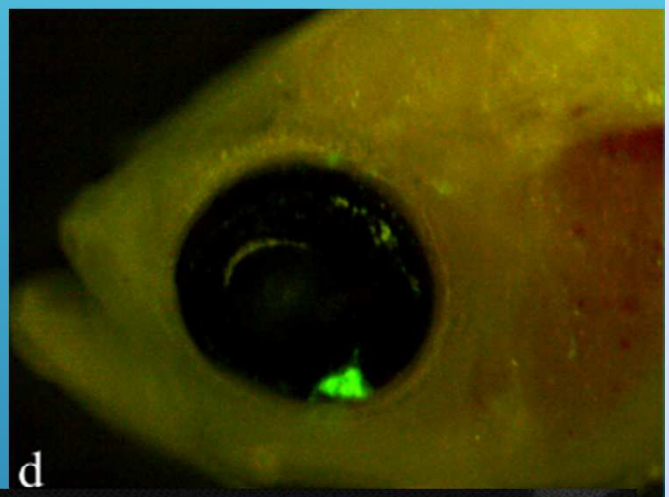


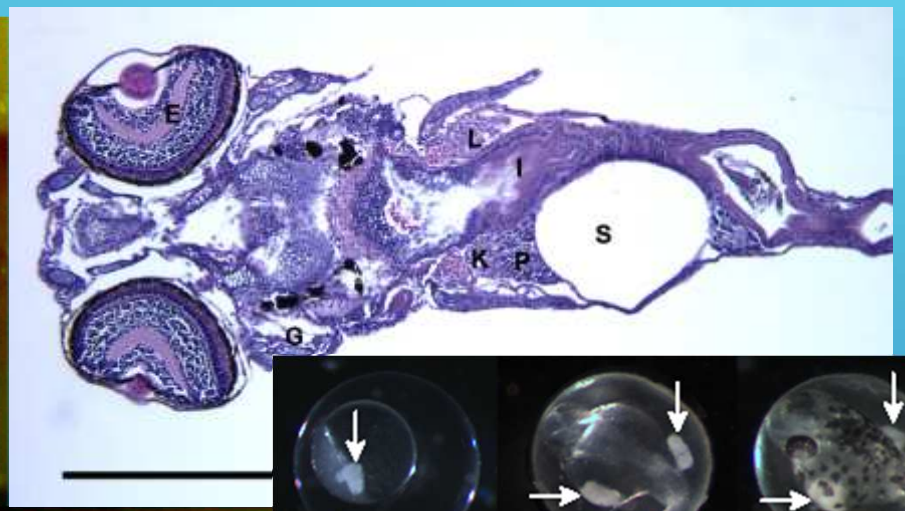
HIGH MAGNIFICATION OF *P.H.* IN MUSCLE WITH DEVELOPMENTAL STAGES

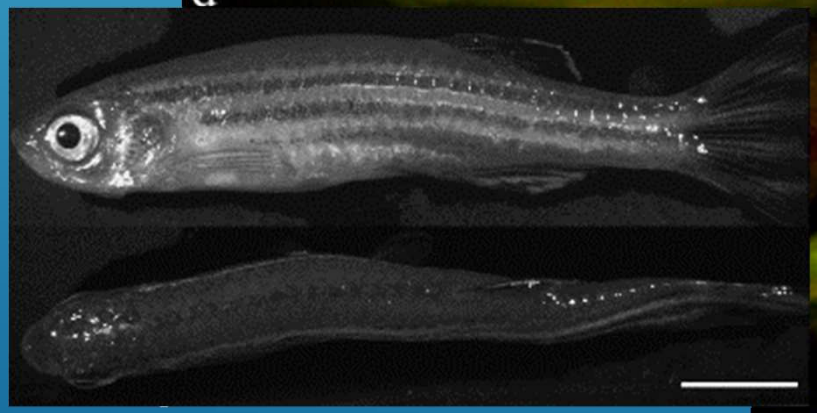


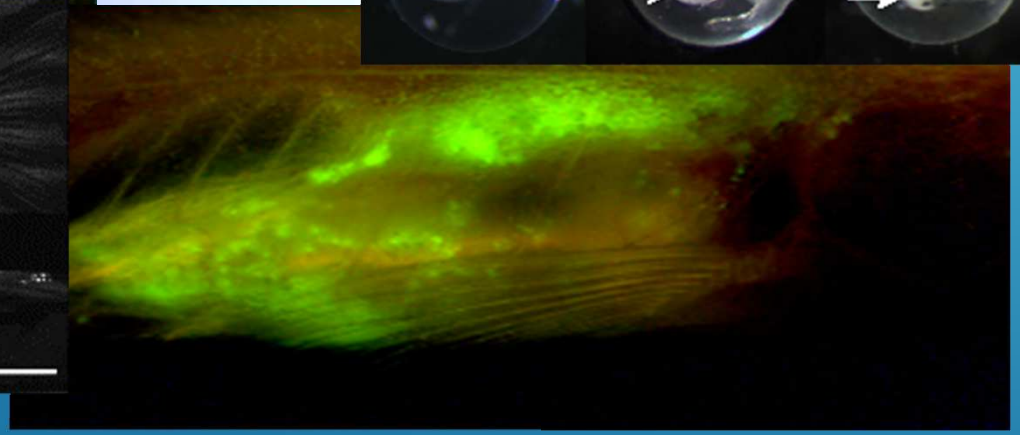


Acustain Gram - Sigma









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