

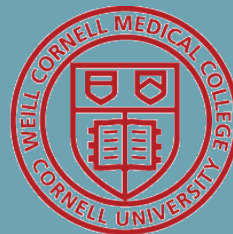
Fish Health and Research Outcomes

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Memorial Sloan Kettering Cancer Center & Weill Cornell Medicine

5th Annual International Zebrafish Husbandry Course

Buguggiate, Italy 2016



Fish Health and Research Outcomes

- Immune status
- Nutritional status
- Stress
- Husbandry
- Infectious diseases:
 - Bacteria
 - Fungi
 - Parasites



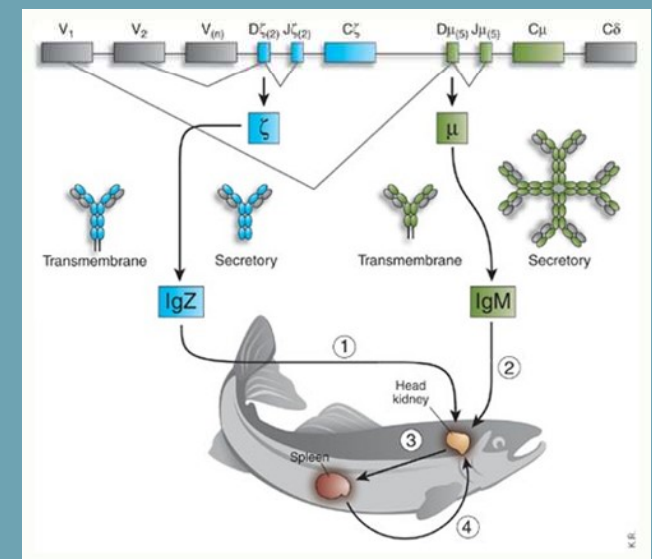
Immune Status -Research Impact

- Many genetic models incompletely characterized
 - Immune alterations
 - Alterations in gene expression

- Infectious disease

- Susceptibility
 - Expression of disease and progression

- Characterization of new models needed



Martin F Flajnik, 2005

Nutrition & Zebrafish

- Optimize health
- Maximize reproductive potential
- Support normal physiologic processes
- Support immune system
- Minimize interference with research

Nutrition -Impact on Research

- Estrogen containing diets
 - Cancer studies
 - Endocrine disrupter research
- Dietary restriction influences cancer development
- Feeding regimen impacts larval rearing and breeding performance

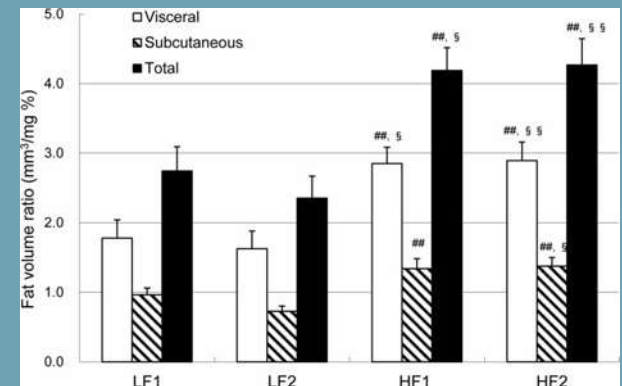
Nutrition- Impact on Research

- Higher spontaneous tumor incidence
 - < 100 ppb nitrosamine in commercial fish meal
- Vitamin C content
 - One commercial diet found deficient



Nutrition – Impact on Research

- Lipid composition
 - Impacts gene expression
 - Impacts body composition & growth rate
 - Impacts fertilization
- Iron content
 - 4 commercial diets found to have > max levels
 - Toxic
 - Impacts cytochrome P450 detoxification system



Meguro, 2015

Live Feed

- Variability in nutrition profile
 - Diet source
 - Culture methods
- Variability in caloric intake
- Unpredictable availability-change of source
- Feeding behavior
 - Stress
 - Social interactions
- Potential exposure to unwanted variables
 - Heavy metals
 - Pesticides
- Infectious organisms
 - *Paramecium* & *Mycobacteria* spp.
 - *Vibrio*, *Mycobacterium*, etc. & Rotifers & *Artemia*
 - *Enterocytozoon hepatopenaei* (EHP) found in *Artemia* *



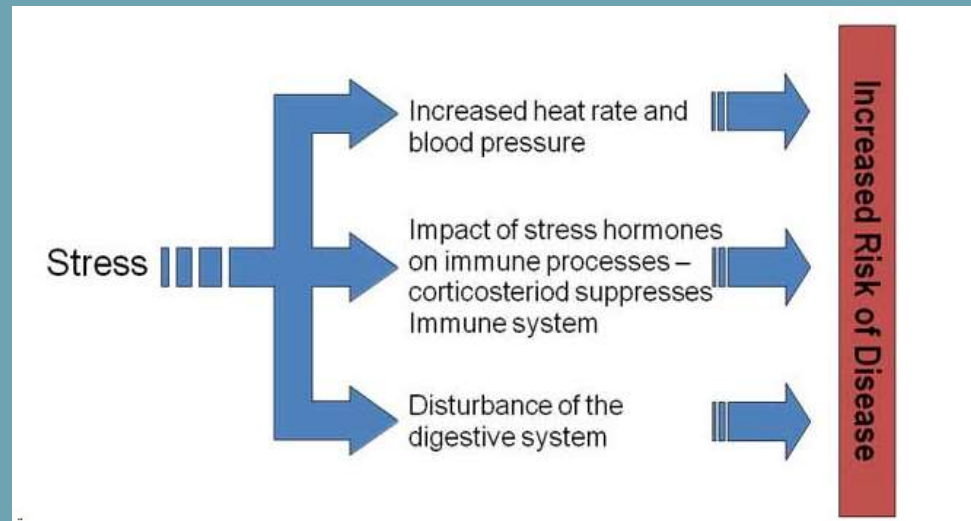
Minimize Variables

- 1960's- 70's- rodent model began to standardize diets
- Zebrafish nutritional requirements largely unknown
- Deserves for focus and research funding



American Society for Nutrition
Excellence in Nutrition Research and Practice

Stress-Impact on Research



- Increased prevalence, mortality and myositis associated with pseudotuberculosis infection
- Increased mortality associated with *M. marinum* infection
- Reduction in growth and reproductive fitness

Stress- Impact on Research

- Elicits changes in tumor development
- In mice: Altered tumor rejection rates, decreased tumor survival and altered tumor latency tumor growth



<https://www.mskcc.org/research-areas/labs/richard-white/overview>

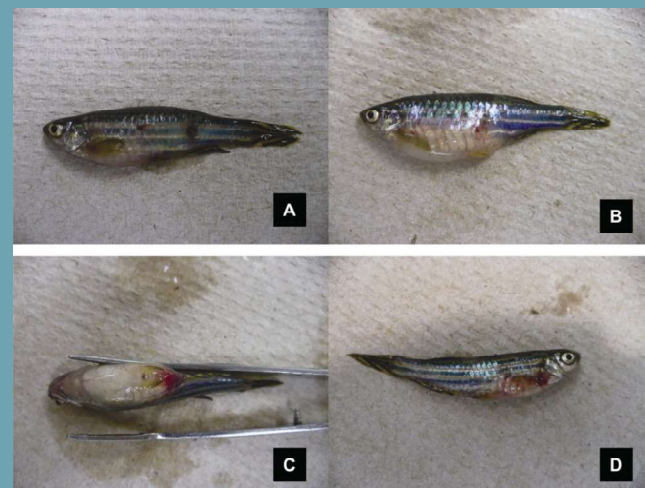
Stress- Reduction

- Provide adequate nutrition
- Stable water quality
- Minimize documented stressors
 - Housing density
 - Handling
- Minimize disease
- Stable environmental parameters
 - Light
 - Temperature
- Environmental enrichment



Infectious Diseases-Obvious Impact

- Morbidity/mortality
- Loss of data
- Requires use of more animals



Infectious Diseases- Impact on Research

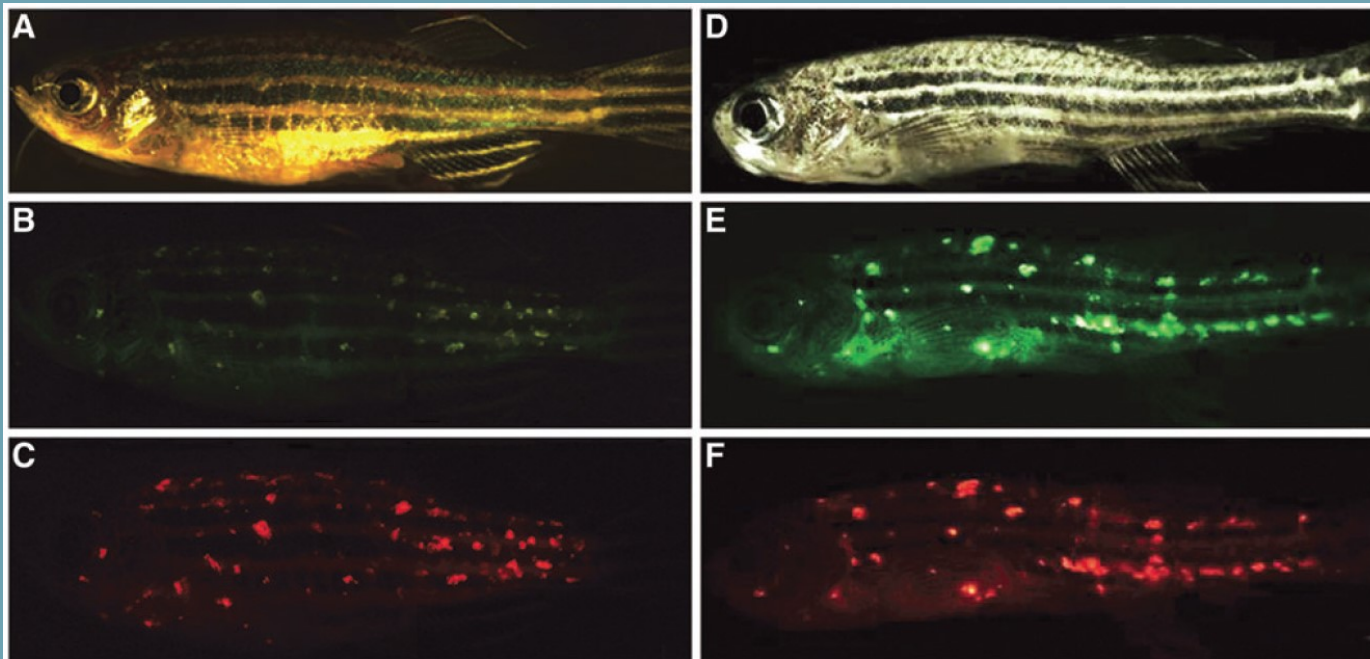
- Alteration in gene expression
- Immunomodulatory effects
- Damage to organs or functions
 - Histologic alterations
 - Altered biotransformation of administered compounds
 - Difficult to interpret research results or lead to misinterpretation

Pseudoloma neurophilia

- Emaciation, skeletal deformities, morbidity, mortality
- Reduced growth
- Decreased fecundity*
- Decreased larval survival*

P. neurophilia- Impact on Research

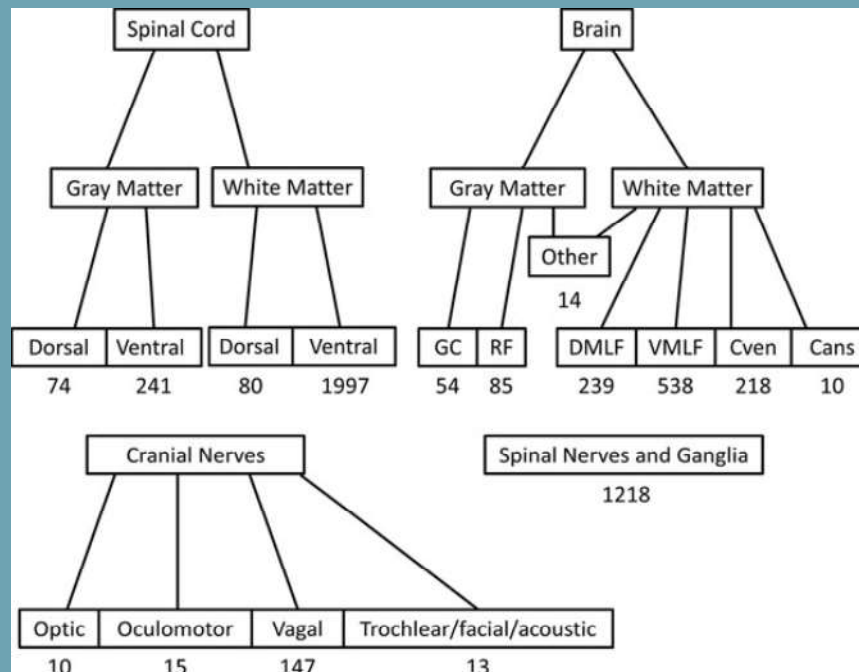
- Intramuscular granuloma development and myonecrosis
 - Interfere with muscular development studies
 - Autofluorescence may interfere with imaging studies



WestKylie, et al. 2014

P. neurophilia- Impact on Research

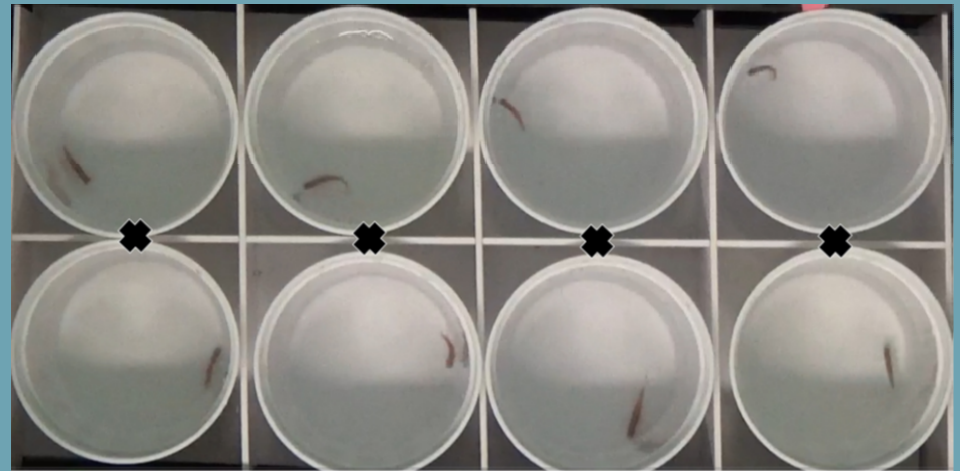
- Infection results in space-occupying or inflammatory lesions in the brain, spinal cord, meninges, or skeletal muscle
- Location, location, location

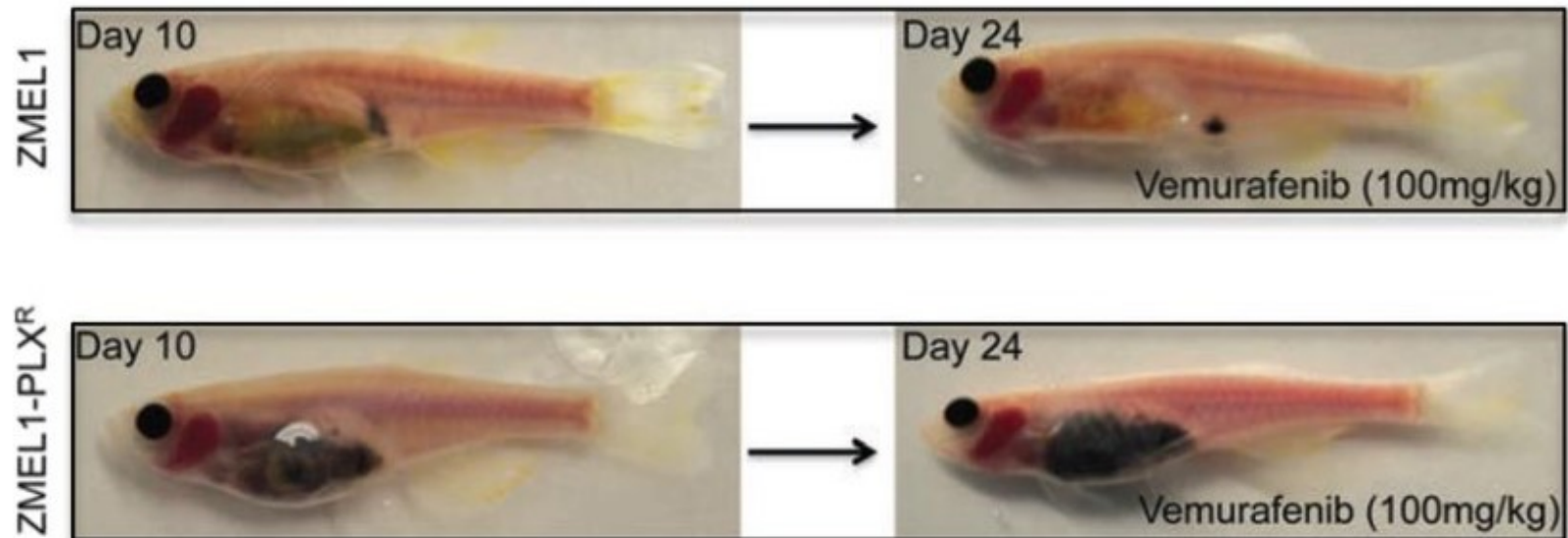
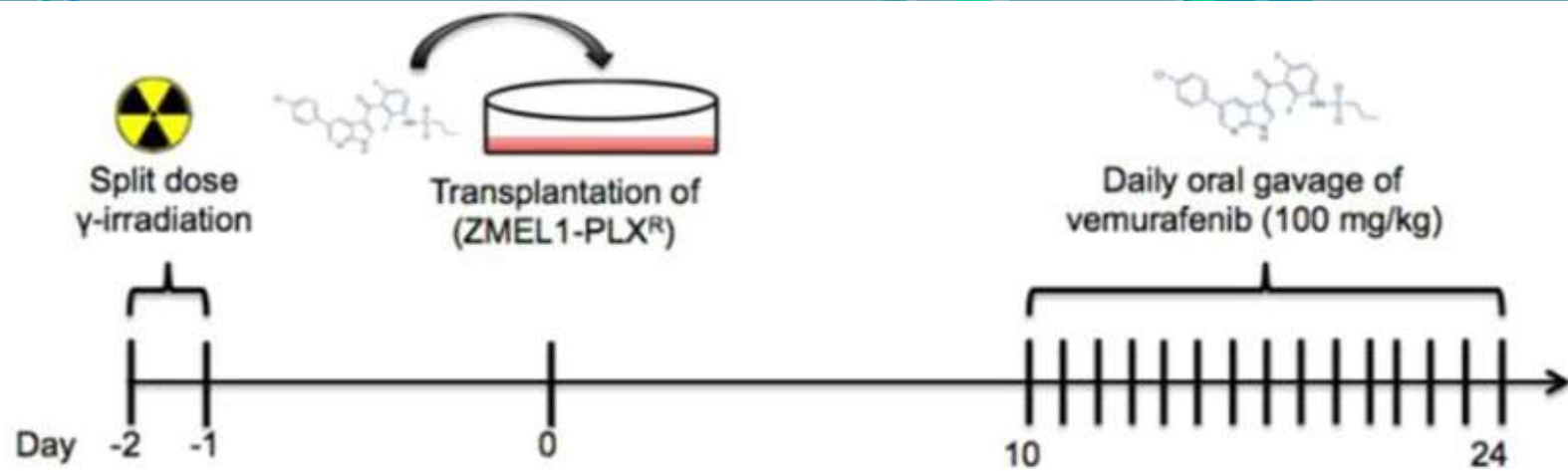


Sean Thomas Spagnoli, et. al 2015

P. neurophilia- Impact on Research

- Tap test to evaluate startle response
- Slower habituation
- Enhanced netting evasion
- Greater variation in response
- **Distinct behavior phenotype**
 - Not associated with severity of infection
- **Altered shoaling behavior**

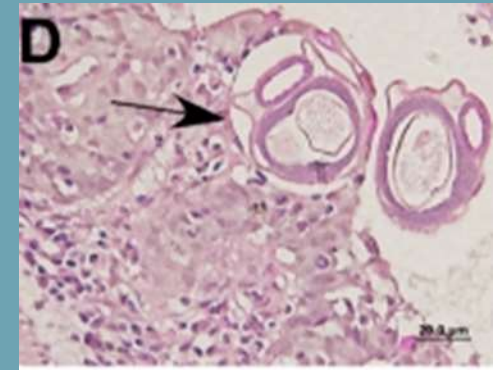




Dang, M. *et al.* 2016

Pseudocapillaria tomentosa

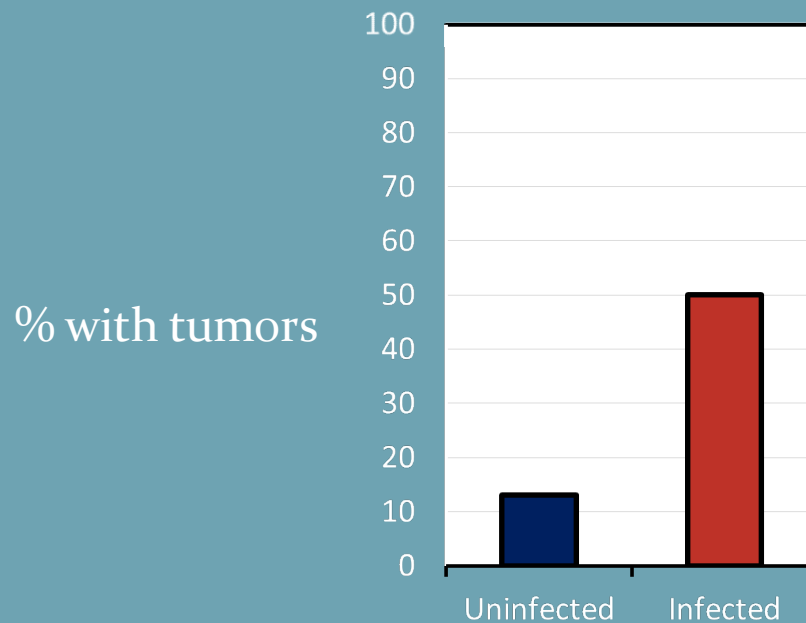
- Infects many tissue types
- Mild or severe locally extensive nonsuppurative inflammation
- Diffuse chronic granulomatous peritonitis
- Alters cytokine responses



Murray, KN and Peterson T, JAVMA, Jan 15, 2015

P. tomentosa- Impact on Research

- Altered results of carcinogenesis studies.
 - 50% of infected had neoplasms vs. 13% of uninfected
 - Nematodes directly associated with lesions



Kent, ML, et al, 2002. *Comp Med*.

Mycobacteriosis- Impact on Research

- *M. marinum*
 - Altered results when exposed to a chemical carcinogen
 - Differences in gene expression
- Down regulation of retinoblastoma gene
- Infection reactivated by gamma **irradiation**

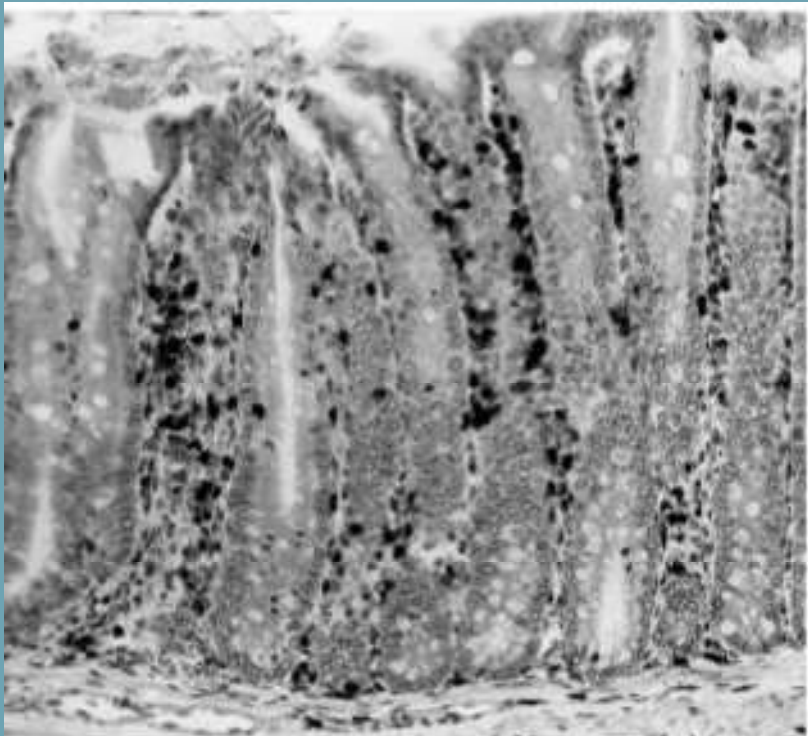


Mycobacteriosis- Impact on Research

- *M. haemophilum*
 - Tumor promoter
 - Neoplasms arise in center of inflammatory lesions in liver/intestines
- May evoke chronic inflammation
 - Alteration of inflammatory mediated pathways
- Granulomatous nephritis and hepatitis
 - Impact on kidney and liver function



Where did my model go?



Kullberg MC, et al., 1998.

Non-Infectious Diseases

Impact on Research

- Egg-associated Inflammation
 - Reduced future viability*
 - Oocyte (follicular) atresia
 - Endocrine Disrupting Compounds
- Toxins/Plasticizers
 - Genetic aberrations
 - Malformations
 - Altered metabolism



Husbandry- Impact on Research

- Water quality has direct impact on health & stress
 - Maintain low levels of nitrogenous waste
 - Know your water source (infectious dz, chemicals)
 - Avoid plasticizers, metals, chemicals
 - Poor water quality results in stress (immune system, dz)
- Poor biosecurity practices results in disease transmission
- Ensure appropriate feeding practices (nutrition)

Fish are not mice but...

SPF

- Standard to use SPF mice
 - SPF salmonids and zebrafish
 - Documentation of disease associated research variables
- Standardized diet
- Standardized husbandry
- Allows for comparison of research data



A standard diet for zebrafish
Option to customize your diets



We have problems....

- Deficiencies in reporting key methodological parameters that can introduce bias
- Publications lacked crucial methodological information that would allow informed judgment about the findings
- How can reviewers adequately identify potential limitations in the experimental design?
 - Limits benefits of findings
- Analysis suggests that inadequate reporting correlates with overstated findings

Research Goals

- Publish clear, complete, science based reports to benefit the scientific community and society
- Study should be reproducible
 - Experimental procedures clearly described
 - Environmental conditions clearly defined
 - Research subjects clearly defined
- Renewed emphasis on complete reporting and transparent study design

ARRIVE Guidelines

- Animal Research: Reporting of In Vivo Experiments

Experimental animals	8	<p>a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range).</p> <p>b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.</p>	
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The ARRIVE guidelines. Originally published in *PLoS Biology*, June 2010¹

Nutrition information missing!

So now what?

- As a researcher:
 - Know what you are working with!
 - Use caution when interpreting data!
 - Report all conditions
- As an Aquatics Professional
 - Know what can impact research values
 - Decrease variability by maintaining stable environment
 - Health monitoring program and open communication

THANK YOU!



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