The Zebrafish Model Has a Problem.

Zebrafish are susceptible to naturally-occurring infectious diseases that can invalidate studies.

- Mortality
- Clinical disease (morbidity)
- Subclinical infections
- Loss of an animal model
- Misinterpreted data (Type I and Type II Errors)
- Repetition of failed experiments
- Inability to replicate experimental results
- Loss of balanced experimental design
- Unexplained variability in experimental data
- More animals required to demonstrate significance
- Lost time and effort

Why should we address this issue now?

- Increasing use of zebrafish and other aquatic spp.
- Institutional investment in infrastructure
- New, expensive-to-create models
- Increasing shipments of zebrafish and embryos
- Centralised facilities:
  - Multiple investigators
  - Multiple species
  - Shared personnel

As the system gets larger and more complex, so do the problems.

Zebrafish Pathogens: Impact on Research

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The Zebrafish Model Has a Problem.

As the system gets larger and more complex, so do the problems.

Development of the zebrafish as a model organism

- Aging
- Behavior
- Growth
- Immunity
- Infection
- Metabolism
- Neoplasia
- Toxicology
Zebrafish Pathogens: Impact on Research

1. Damage due to pathogen virulence factors
   • Energy expenditure
   • Friendly fire
   • Collateral damage
   • Reconstruction (tissue repair and remodeling)
   • Alteration of future immune responses
   • Epigenetic effects

2. Damage due to the host immune response
   • Energy expenditure
   • Friendly fire
   • Collateral damage
   • Reconstruction (tissue repair and remodeling)
   • Alteration of future immune responses
   • Epigenetic effects

Infection can initiate or exacerbate autoimmunity.

Mechanisms by which pathogens may cause autoimmunity:
(a) Molecular mimicry
(b) Epitope spreading
(c) Bystander activation
(d) Cryptic antigens

Examples
• Confounding effect recognized later (zebrafish and other species)
• Experimental infections in zebrafish
• Experimental coinfection studies in other fishes

Impact on research using embryos and larvae
• At least some subclinical infections reduce fecundity of zebrafish.
• Some zebrafish pathogens are transmitted vertically.
• Embryos and larvae are susceptible to infection and mount an immune response to infectious agents.
• Infections alter gene transcription.
• Infections alter cytokine levels.
• Many cytokines have important roles in both the immune response and development.

Pseudoloma neurophilia is vertically transmitted.

• Figure 2. Spores of Pseudoloma neurophilia in developing embryo of zebrafish, Danio rerio.
  • A. Aggregated spores (arrow) in a 4 hpf embryo. Bar = 0.5 mm.
  • B. Two foci of spores (arrows) visible in the same embryo at 24 hpf.
  • C. Spores (arrow) in the same embryo at 48 hpf.
  • D. Differential interference contrast micrograph of spores from an embryo. Bar = 10 mm.
Infection of embryos alters gene transcription.

Annotation of the gene set responsive to Salmonella infection in zebrafish embryos.


The zebrafish innate immune system develops early.

- 22 hpf: Primitive macrophages
- 33 hpf: Primitive neutrophils
- 52 hpf: Neutrophils can migrate and phagocytize bacteria


Infection of embryos alters cytokine levels.

Proinflammatory cytokine mRNA levels of zebrafish embryos 18 hpi with S. pneumoniae.


Altered cytokine levels impact development.

Examples of altered CNS development:


Examples of altered CNS development:


Impact on research using adult zebrafish

- Most zebrafish infections are chronic.
- Many are subclinical.
- Adults have had longer to acquire them.
- Chronic inflammation is problematic for almost any research area.
Subclinical Pseudocapillaria tomentosa infection confounded a zebrafish carcinogenesis experiment.

**Confounded Carcinogenesis study:**
- Uninfected Pseudocapillaria infection
- Only one treatment group was affected
- Worms located within or adjacent to tumors

Significantly different outcome between infected and uninfected fish within the same treatment group.

Subclinical Infections Confound Neoplasia Experiments.

- **Mycobacterium marinum** Infection Acts as a Tumor Promoter in Medaka (Oryzias latipes)
  - \( P = 0.016 \)

Fig. 3. Incidence of proliferative liver lesions in medaka exposed to M. marinum and BaP

Subclinical infections can alter the outcome of subsequent infections.

IHNV Only: 72% Mortality
IPNV + IHNV: 2% Mortality

Questions?

What does confounded data look like?
What does confounded data look like?

Natural infections can alter phenotypes.