Application of a real-time polymerase chain reaction assay for the myxozoan parasite *Henneguya ictaluri* and its year-round prevalence in catfish aquaculture

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Henneguya ictaluri

- Myxozoan parasite
- Causative agent of proliferative gill disease (PGD) in channel catfish
  - Hamburger gill
- 3rd most commonly diagnosed disease at the Aquatic Diagnostic Lab, Stoneville, MS
- Mortalities can approach 100% in severe outbreaks

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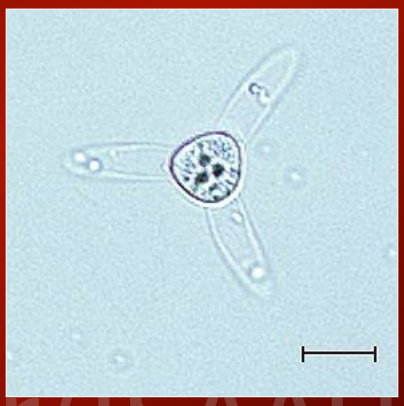
Henneguya ictaluri

- Life cycle involves ubiquitous benthic oligochaete *Dero digitata*
- Exposure to the actinospore stage results in a severe inflammatory response in channel catfish gills causing respiratory distress
- Currently there are no treatments
- Primarily a problem in the spring, with lesser incidence in the fall, when water temps are between 16-25 °C



Bar = 20 µm

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Henneguya ictaluri

- Mostly an issue for fingerlings stocked newly into ponds for grow-out
- Can cause significant losses larger fish, but most severe outbreaks occur in smaller, younger fish
- Different populations of fish in the pond can be affected differently
- Effects age, size and previous exposure to the organism have on outbreak severity is poorly understood
  - Fish appear to be able to acquire resistance
- Once fish are removed from the source of infection they quickly recover

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Problem

- Following an outbreak which results in significant losses, when is it safe to re-stock fish into the pond?
  - Resident fish often show no signs of disease yet pond actinospore concentrations are lethal to newly introduced fish
- During an outbreak, if moving fish is a feasible option how do we identify parasite free environments for which to relocate fish?
- Called for a method to identify approximate actinospore concentrations in a pond prior to stocking

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Current practices

- Fish Health Program at NWAC utilizes sentinel fish (Wise et al. 2004; 2008)
  - Two separate week long exposures
  - Quantify damage by calculating the percent filaments exhibiting at least one chondrocytic lesion or “break”
  - Does it get better or worse between exposures?
  - Helps determine dynamics of a given outbreak.
  - Outbreaks do not tend to relapse
    - Once an outbreak is done, its done for that season

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Disadvantages . . .

- Takes a minimum of one week to determine status of pond
- Takes a minimum of two weeks to determine the dynamics of the outbreak
- Requires equipment, source of PGD free fish, and is fairly labor intensive
- Is inconclusive if cage system fails
  - Lose cage/fish
  - All fish die before sampling

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Alternatives

- Using QPCR can we measure the actinospores directly from pond water samples?
  - Eliminates need for sentinel fish
  - Significantly less labor intensive than sentinel fish exposures
  - Much more rapid than sentinal fish exposures

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How do you get from this . . .

to this?

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Water sampling, DNA isolation and PCR

- Sorvall RC-6 Ultracentrifuge
  - A series of centrifugation steps concentrates >500 mls of pond water and sediment into 1.5 ml tubes
- Mo Bio Soil DNA Isolation Kits
  - Powersoil
  - Ultraclean
- Bovine Serum Albumin added to PCR reaction
  - 1 µg/µl final reaction concentration
- ABI's Environmental Taqman Mastermix 2.0

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Pond trials

- 20 replicate pond trials
- Sentinel fish were held in pond for seven days
- Water was sampled from two different geographic locations within the pond in coordination with the placement and collection of sentinel fish
- % mortality was correlated to actinospore equivalents per liter of pond water

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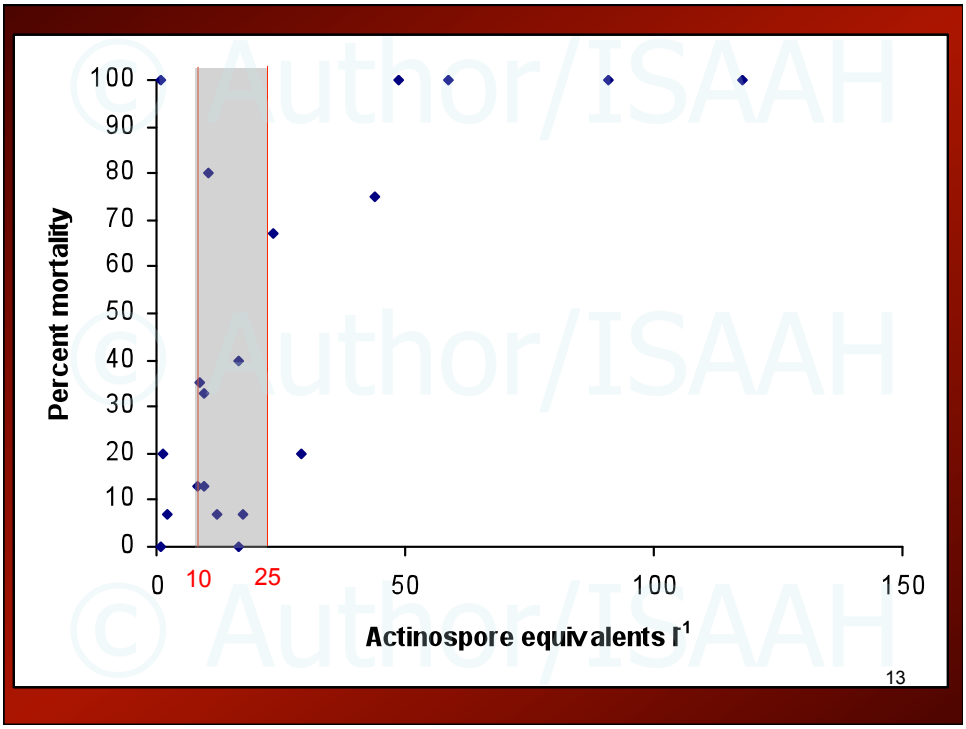

Conclusion . . .

The *H. ictaluri* specific QPCR assay can detect *H. ictaluri* in environmental samples and has tremendous potential in both research and production settings.

The ability to quantify *H. ictaluri* DNA from fish tissue and environmental samples provides an invaluable tool in diagnostics, epidemiological studies, treatment evaluations and management of this disease.

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Year-round prevalence of *H. ictaluri*

- In the spring of 2009 a producer was experiencing significant PGD related mortalities on his operation
- Once monthly we sampled pond water from 24 ponds on his operation
  - (May 2009-May 2010)

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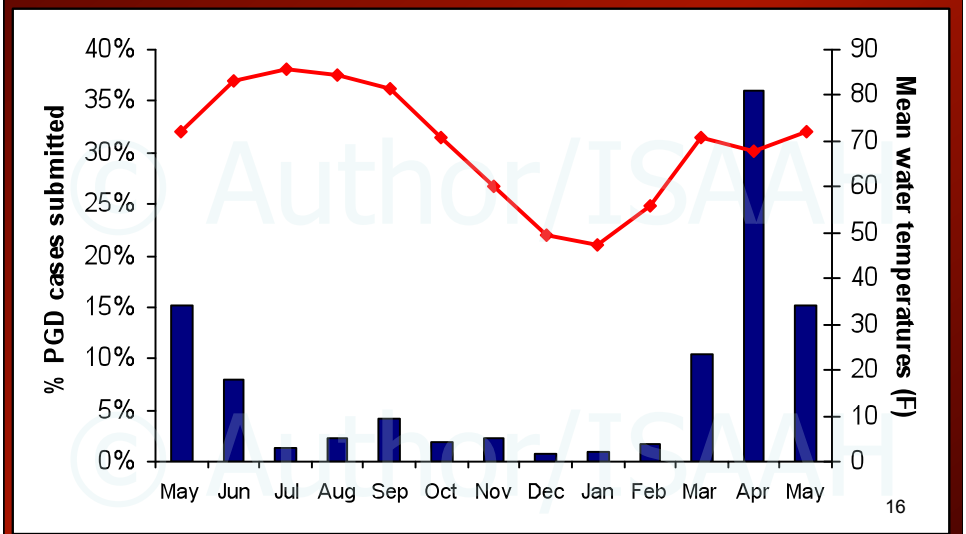
Percent of PGD cases submitted by month (2007-2009)



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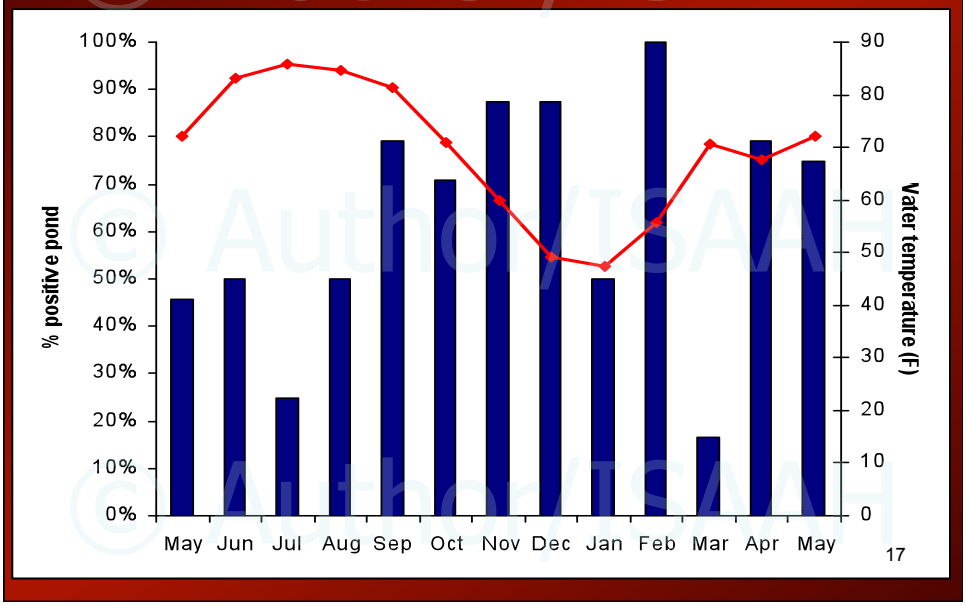
% Positive Ponds (by month)



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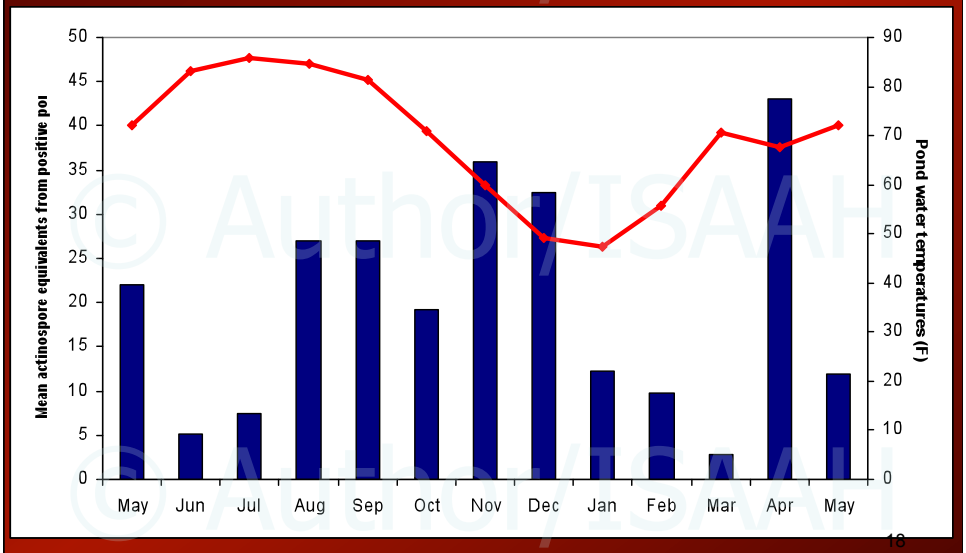
Mean actinospore equivalents from positive ponds



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Conclusions

- *H. ictaluri* is present in catfish ponds year round, but does not always cause disease
- *H. ictaluri* is present in numbers that would result in significant mortality throughout the year and not only in the spring
- Previous exposure to the organism may provide protection against re-exposure

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New questions . . .

- What effect does age, size, and or previous exposure have on PGD severity
  - Takes a larger dose of parasites to affect larger fish, which are normally older, and in the pond, more likely to have seen H. ictaluri before
- What effect does temperature have on PGD?
  - If PGD is actually an immune response to H. ictaluri, in an immune suppressed state (cold temperatures) do fish get PGD?
- If previous exposure during an immune suppressed state provides protection, would fall stocking reduce incidence of PGD in the spring?

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