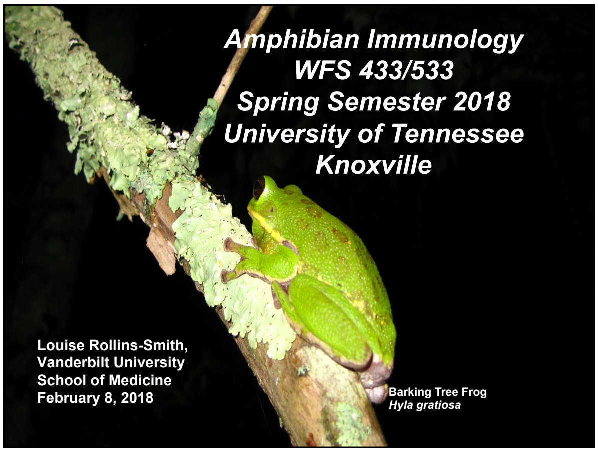
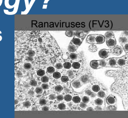


Quiz Questions

1. Do amphibians have a thymus and what does the organ do?
2. Do tadpoles have an immune system?
3. Do amphibians have IgM antibodies?
4. What are antigen presenting cells?
5. What are two important pathogens of amphibians?

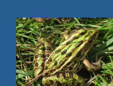
Why is it important to learn amphibian immunology?

- We need to understand how amphibians are protected from infectious diseases.
  - Chytridiomycosis
  - Ranaviruses



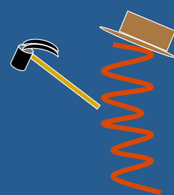
- We need to understand some factors that affect immunity.
  - Temperature
  - Environmental chemicals

- We need to understand immune system changes from egg to tadpole to adult.



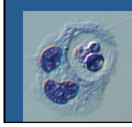
The innate immune system

- Ready to respond immediately
  - Phagocytic cells (macrophages, neutrophils, and dendritic cells)
  - Complement (protease cascade to assist antibodies and macrophages)
  - Antimicrobial peptides (AMPs)
  - Natural killer cells (recognize virus-infected cells)

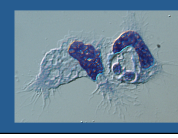


The Innate Immune System

- Preformed components (cells and defensive secretions)
- Essentially the same magnitude of response during re-exposure
- Includes anatomical barriers, cells and secretions



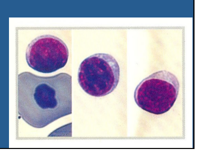
Frog neutrophil has engulfed fungal cells



Frog macrophage has engulfed fungal cells

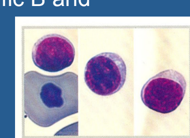
The adaptive immune system

- Requires time to develop
- Slow to respond but highly specific and regulated.
- Made up of lymphocyte-mediated defenses (B lymphocytes and T lymphocytes).
- Central to the cooperation of all subsets of lymphocytes is the major histocompatibility complex (MHC).

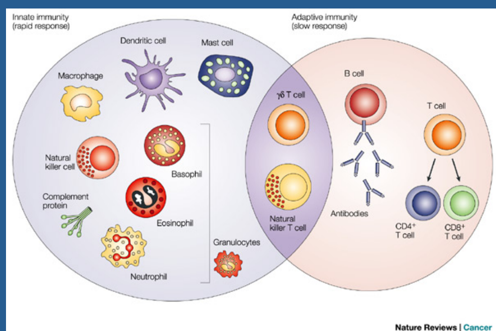


The adaptive Immune System

- Expansion of pathogen-specific B and T cell populations
- Memory responses develop
- Involves a much more efficient secondary response during re-exposure

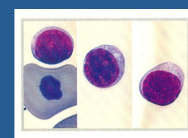


Innate and adaptive arms of the vertebrate immune system



Amphibian immune system: overview

- The immune system of amphibians is very similar to that of all other vertebrate groups.

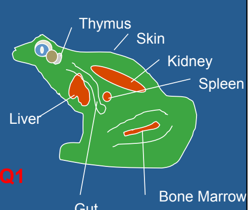


- The cells, the organs, and the critical effector molecules (antibodies, complement, cytokines and chemokines) are very similar to those found in fish, reptiles, birds and mammals.

Amphibian immune system: overview

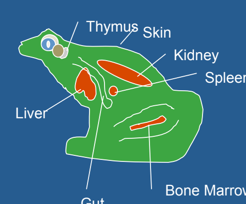
- Amphibians lack true lymph nodes, but in other respects, the immune system of amphibians is very much like that of mammals.
- Primary lymphoid organs that produce the immune cells are:
  - Thymus—site of T lymphocyte development
  - Bone Marrow or liver and spleen that produces B lymphocytes and all other lymphoid cells

Q1



Amphibian immune system: overview

- The only major secondary lymphoid organ for amplification of an immune response is the spleen.
- The kidney can also produce lymphocytes in some species.
- Bone marrow is present in some adult anuran species (frogs and toads) where it is involved in development of lymphocytes (white blood cells) and granulocytes.



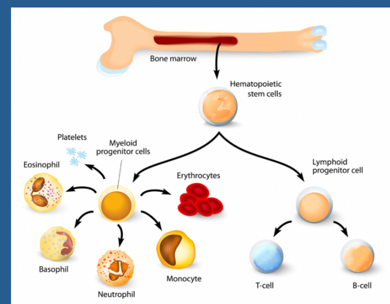
Other immune organs

Mucosal surface associated lymphoid tissues are present in all vertebrates including amphibians

Spleen and thymus are present in all vertebrates from sharks on up

Lymph node-like structures first seen in birds

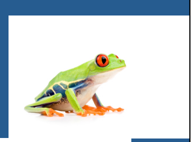
Bone marrow: the source of blood cell progenitors in mammals



http://www.medicalnewstoday.com/articles/285666.php

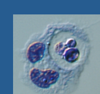
Amphibians: may or may not have a hematmopoietic (blood forming) bone marrow.

They can use the liver for development of immune cells



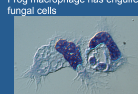
Cells of the immune system

Innate immune cells
Macrophages
Neutrophils
Dendritic cells



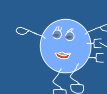
Frog neutrophil has engulfed fungal cells

Frog macrophage has engulfed fungal cells

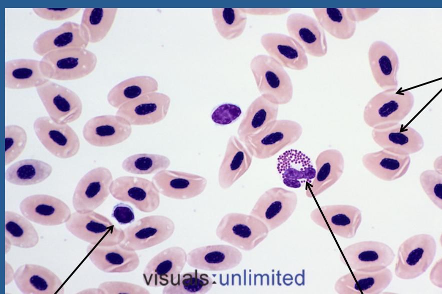


Adaptive Immune cells
T cells
- Cytotoxic T cells
- T helper cells
- Regulatory T cells

B cells → antibodies

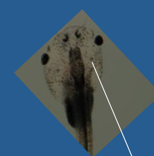


Cells of the Immune System



Leopard Frog blood (*Rana pipiens*)

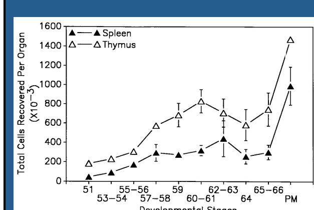
Tadpole Immune System

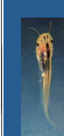


Q2

Development of the immune system

- Amphibians have two immune systems, tadpole and adult
- The tadpole immune system is reorganized at metamorphosis.





Redrawn from Rollins-Smith, L.A. et al. 1984. Immunology 52: 491-500;

- Tadpoles have an immature immune system which undergoes significant change at metamorphosis.
- Thus metamorphosis is a time of increased vulnerability to pathogens.

Tradeoffs at metamorphosis

- There may be a trade-off between the need for additional energy and immune defense at metamorphosis resulting in the possibility of increased disease.

Metamorphosis is associated with increased disease and mortality

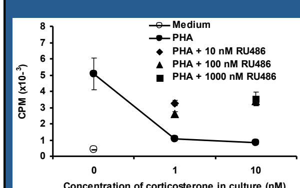
- Infected tadpoles die from chytridiomycosis soon after metamorphosis
  - *Mixophyes fasciolatus* in Australia (Lee Berger)
  - *Rana muscosa/Rana sierrae* in California (Cherie Briggs)
  - *Aletes obstetricans* in Switzerland and Spain (Ursina Tobler, Beni Schmidt, and Jamie Bosch)
  - *Rana luteiventris* metamorphs in Idaho have highest incidence of Bd (Bree Rosenblum)

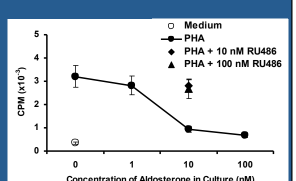
- Late stage tadpoles and metamorphs of *Rana sylvatica* and *Rana pipiens* are more susceptible to ranavirus infections than adults at the same locations (Greer et al. 2005)

Stress hormones can affect immune defenses

Stress: Corticosteroid hormones

- Both glucocorticoid and mineralocorticoid hormones inhibit lymphocytes at low concentrations
- These concentrations are found at metamorphosis and during explosive breeding when frogs are fasting.





Other factors can lead to stress and affect immune defenses

- Natural stressors include:
  - Metamorphosis
  - Predation
  - Decreased food resources
  - Disturbance of the skin that affects transport of sodium and potassium

Environmental Factors and Immunity

- Environmental chemicals can impair development and function of the immune system.
- Low temperatures can impair function of the immune system.

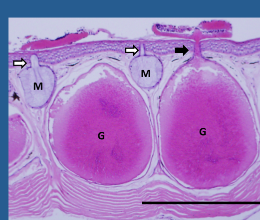
Environmental Factors and Immunity

- Endocrine disruptors such as perchlorate or atrazine can impair development of reproduction or the immune system.
- Stress that elevates corticosteroid hormones can impair immune function.
- Immune function in tadpoles may be impaired under conditions of poor nutrition or disease.

Amphibian barrier defenses

- Keratinized Skin
  - Adults (dermis and epidermis)
  - Tadpoles (mouthparts only)
- Mucus glands
  - Mucus protects from mechanical damage
  - Inhibits pathogen colonization
  - May assist with osmoregulation

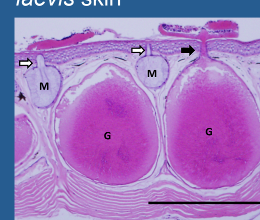
Mucus (M) and granular glands (G) in *Xenopus laevis* skin



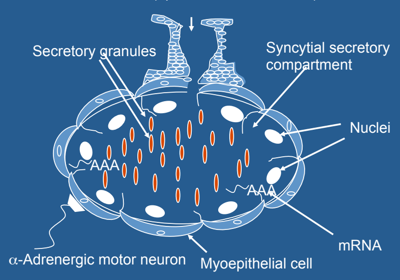
Amphibian barrier defenses

- Granular glands (Serous or Poison glands)
  - Sequester toxins
  - Make and store antimicrobial peptides
  - Peptides protect against bacterial and fungal infections

Mucus (M) and granular glands (G) in *Xenopus laevis* skin



Granular glands produce and store antimicrobial peptides (AMPs)



Granular Gland modified after Dockray and Hopkins 1975

What do antimicrobial peptides do?

- Kill bacteria
  - Gram positive
  - Gram negative
- Kill viruses
- Kill fungi
- Kill protozoa

E. coli bacteria in presence of AMPs

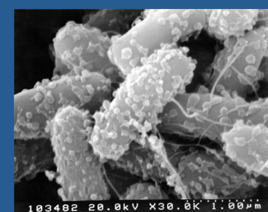
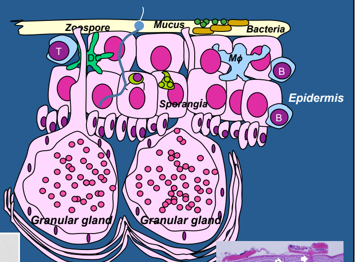


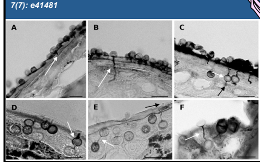
Image: Robert Hancock

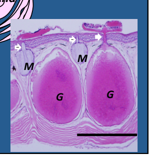
Amphibian barrier defenses

Mucus:
- ***Antimicrobial peptides***
- ***Lysozyme***
- ***Antibodies***
- ***Protective bacteria and their products***



Van Rooij P. et al. 2012 PLoS One 7(7): e41481





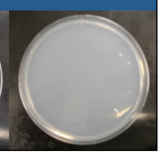
Gammill W. et al. 2012. Dev. Comp. Immunol. 37: 19-27

Amphibian barrier defenses

- Gut defenses
  - Mucus
  - Lysozymes
  - Antibodies
  - Protective Microbes

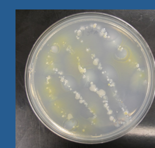
What is the role of symbiotic microorganisms in the skin?

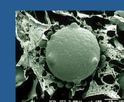
- Multiple species of bacteria inhabit the skin mucous of both anuran and urodele amphibians.
- Some of these species produce antimicrobial metabolites or compete with pathogens for resources.



Natural flora: the skin microbiome

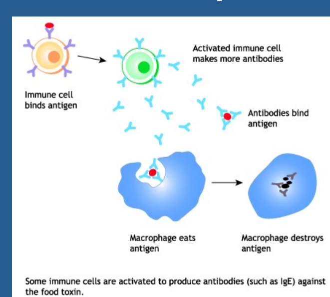
- Different amphibian species have different microbial communities
- Microbiome offers protection against colonizing bacterial and fungal pathogens
  - *Batrachochytrium dendrobatidis* (Chytrid fungus)
  - Opportunistic pathogens: *Aeromonas hydrophila* and *Chryseomonas luteola*
- Many amphibians eat their skin; possibly a cycle is established between the skin and the gut microbiome





Brain Break or Coffee Time!

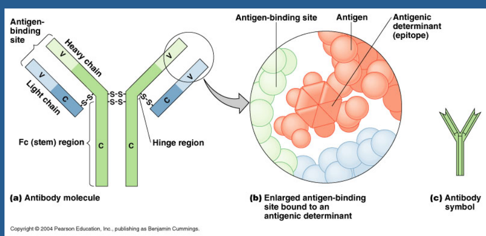
Immunoglobulins and the immune response



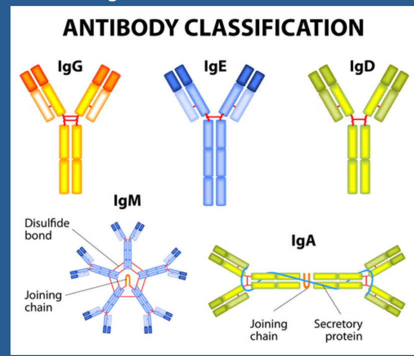
http://www.hhvc.com/blog/harnessing-the-human-immune-system-on-a-single-cell-level/

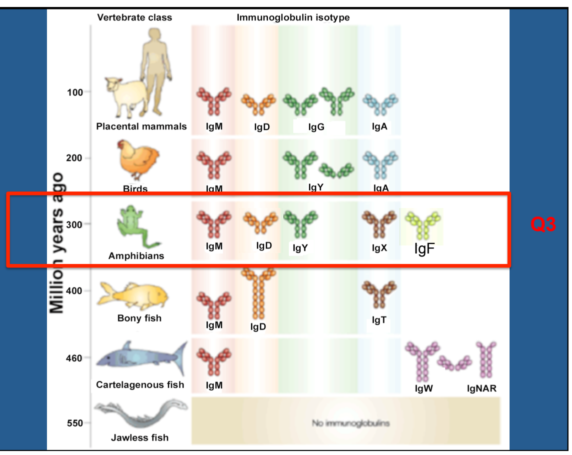
Antibodies and Immunity

- Antigens are substances that cause the production of antibodies.
- Each antigen "fits" with a specific antibody like a lock and key.
- Amphibians can make millions of different antibodies.



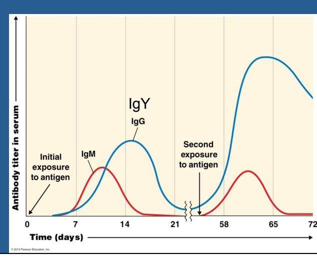
Antibody classes in humans





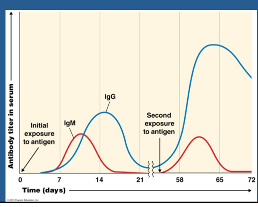
Primary and Secondary Antibody Responses

- **In a primary response, IgM antibodies** are produced in response to first exposure.
- Primary response is slow to develop (7-14 days), and antibody titers (quantities) are not high.
- The IgM response switches to an IgG response in humans in areas of lymph nodes and spleen called **B cell follicles.**
- **In amphibians, the IgM response switches to IgY**



Primary and Secondary Antibody Responses

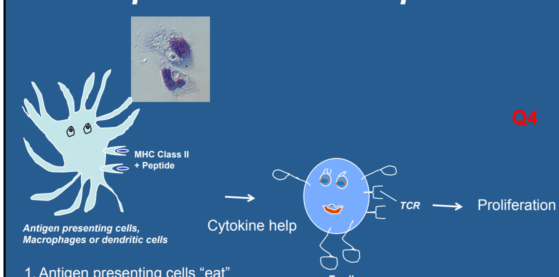
- In a secondary response to a repeat exposure to the antigen, memory B cells produce predominantly IgY antibodies **in amphibains.**
- Response is more rapid.
- Antibody titers (quantities) are higher.
- Antibodies bind better (greater affinity).



Amphibian B cell responses

- Amphibian (*Xenopus*) B cells expand in the spleen instead of lymph nodes as in mammals because amphibians lack lymph nodes.
- Increased Antibody specificity poor in frogs in comparison with mammals
- Protective Abs are generated after re-exposure to pathogens

Amphibian T cell responses



1. Antigen presenting cells “eat” antigens, digest them into “peptides” and present them to T cells.
2. APCs also provide cytokine help.
3. T cells proliferate and become effector cells.

Amphibian T cell immunity

- Amphibians (*Xenopus*) appear to possess all major T cell receptor components
- Amphibians have helper T cells (CD4+) and effector T cells including cytotoxic T cells (CD8+)
- Tadpoles exhibit low-to-no MHC class I and limited MHC class II expression
- Tadpoles can reject foreign skin (allogeneic response) after tadpole stage 55 in *Xenopus*)

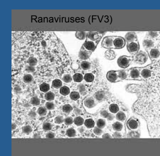
Immune Response to Amphibian Pathogens

- Do frogs and toads get a cold?
- Amphibians are susceptible to several types of pathogens that can be fatal.
- Major pathogens are ranaviruses and chytrid fungi.

Q5

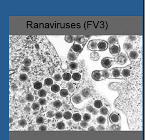
Immune Response to Ranaviruses

- Disease agents linked to amphibian declines include
  - Ranaviruses



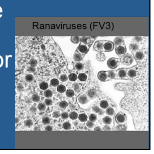
Ranaviruses and immunity

- *Ranavirus* are DNA viruses in the family iridoviridae that infect, fish, amphibians, and reptiles.
- Ranaviruses have been associated with amphibian mortality events in the western United States, the eastern United States (including Tennessee), in Venezuela , in Saskatchewan and Manitoba Canada, in Australia, and in the United Kingdom.



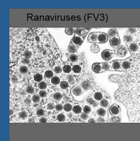
Ranaviruses and immunity

- Pathological findings include hemorrhagic and necrotic lesions within internal organs (liver, spleen kidney), gastrointestinal ulceration, skin ulceration and erosion, and necrosis within hematopoietic cells.
- Diseases caused by ranaviruses were listed as internationally notifiable diseases by the World Organization for Animal Health (OIE) in 2008.



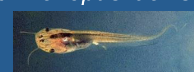
Ranaviruses and immunity

- Transmission occurs by exposure to contaminated water or soil, contact with infected individuals, or ingestion of infected tissue through predation.
- Viruses infect tadpoles and adults of many species, although adults are more resistant.
- Outbreaks are usually localized and limited.



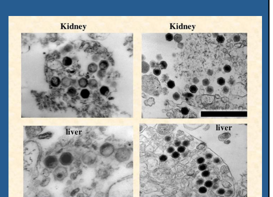
Ranaviruses and immunity

- Most of the work to understand the immune responses to ranaviruses has been done by a limited group of immunologists.
- Jacques Robert and students have made great progress.
- Most of the immune studies have been done with *Xenopus laevis*.




Ranaviruses and immunity

- Healthy adult *Xenopus* can resist fairly high doses of ranaviruses [frog virus 3 (FV3)] with limited pathology and clearance after about 20 days.
- FV3 mainly targets the kidney but can be found in the liver as well.
- Macrophages may harbor viruses for long periods after hosts have cleared the infections.



Ranaviruses and immunity

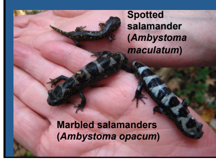
- In adults, antibody (IgY) responses develop and a second infection results in a more rapid clearance demonstrating that B cells are involved in clearance of the virus.
-
- Adult frogs sublethally irradiated to impair lymphocytes become much more susceptible to FV3 showing that adaptive immunity is necessary for recovery.

Ranaviruses and immunity

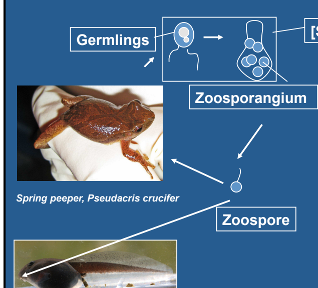
- Depleting T cells (CD8 cytotoxic subset) results in greater susceptibility to FV3 showing that T lymphocytes are necessary for protection from FV3.
- Tadpoles are more susceptible to FV3 than adults showing that their immature immune system is less able to clear the infection.

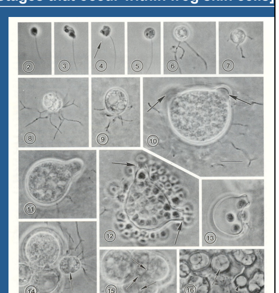
Chytrid fungi and immunity

- My lab has focused our research on *Batrachochytrium dendrobatidis (Bd)*, which causes the skin disease chytridiomycosis.
- Until recently, *Bd* was the only known chytrid pathogenic to vertebrates.
- In 2013 a new species, *Batrachochytrium salmandrivorans* (*Bsal*) was described.
  - Likely originated in Asia
  - Lethal to European salamanders



Chytrid fungi and immunity

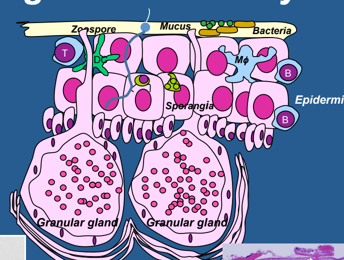




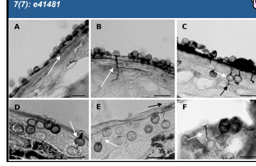
Duration of life cycle is 4-5 days at 22ºC

J.E. Longcore et al. 1999. Mycologia 91:219

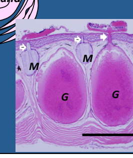
Chytrid fungi and immunity



Van Rooij P. et al. 2012 PLoS One 7(7): e41481

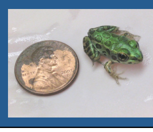


Gammill W. et al. 2012. *Dev. Comp. Immunol.* 37: 19-27



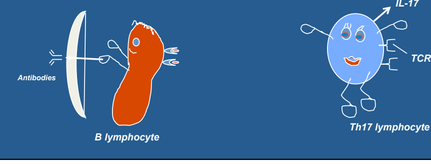
Chytrid fungi and immunity

- Frogs constitutively release low amounts of AMPs that inhibit *Bd*.
- AMPs are effective inhibitors of *Bd* at these low constitutive concentrations but degrade within two hours, protecting the integrity of the skin and commensal bacteria.
- Depletion of AMP responses increases susceptibility to *Bd* in juvenile frogs.

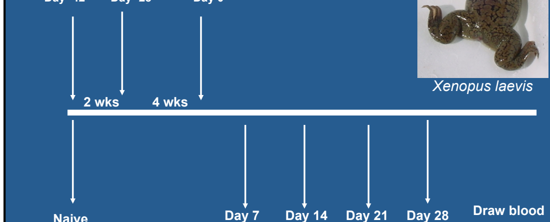


Chytrid fungi and immunity

- What is the role of the adaptive immune system in control of *Bd*?
- Is there a role for B lymphocytes?
- What is the role for T lymphocytes?
- Does *Bd* evade adaptive immune defenses?



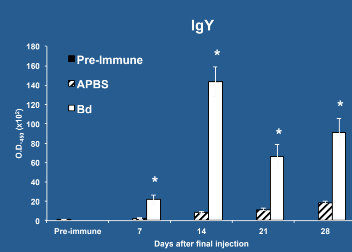
Immunization Protocol



Xenopus laevis

*Freshly killed *Bd* (5 x 10^5/10µl/gbw) injected into the peritoneum
Controls injected with 10 µl/gbw of amphibian phosphate buffered saline (APBS)

Immunization generates robust antibody responses in Xenopus laevis



Peak Antibody titers 1/800 to1/6400

Chytrid fungi and immunity

- Collectively, these studies showed that both innate defenses (antimicrobial peptides) and conventional lymphocyte-mediated immune responses help to protect frogs from lethal *Bd* infections.

- However, many species are not well protected?

Chytrid fungi and immunity

- ***Bd*** **releases heat-resistant factors that inhibit T and B cells by induction of cell death.**

- **Among the factors released by *Bd* are small metabolites, methylthioadenosine, kynurenine, and spermidine which can inhibit lymphocytes.**

Dendrosophus ebraccatus

Chytrid fungi and immunity

- **Taken together, these results suggest that *Bd* has evolved strategies to resist immune surveillance in order to survive in amphibian skin.**

Litoria xanthomera
Image D. Woodhams

Questions?

I have lots of questions too!

***Hyla cinerea* (green treefrog)**

Louise.rollins-smith@vanderbilt.edu